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First Molecular Identification and Diversity of Endophytic Fungi from Native Plants in the Cyrene Region, Libya

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التعريف الجُزيئي الأول والتنوع للفطريات الداخلية من النباتات المحلية في منطقة شحات (قورينائية)، ليبيا

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Abstract
Endophytic fungi live inside plant tissues without causing symptoms. They play important roles in boosting host growth and helping plants handle stress. This study is the first Molecular Identification endophytic fungi from native plants in the Cyrene region of Libya at the molecular level. We collected 68 fungal isolates from six plant species after thorough surface sterilization. We selected six representative isolates for molecular analysis based on their physical differences. We amplified and sequenced the Internal Transcribed Spacer (ITS) region of the ribosomal DNA for identification. The identified fungi and their GenBank accession numbers are as follows: Aspergillus niger (PQ178951), Fusarium sp. (PQ178952), Penicillium nagliovense (PQ178953), Fusarium chlamydosporum (PQ178954), Penicillium chrysogenum (PQ178955), and Stagonosporopsis lupini (PQ182696). BLASTn analysis indicated 99-100% sequence similarity to reference strains. The presence of genera like Fusarium and Penicillium suggests strong ecological adaptation. This study provides essential DNA barcode data for fungal endophytes in this unexplored region. The results highlight the region's rich fungal diversity and suggest that these isolates could be useful for future biotechnological research, such as the production of bioactive compounds for industrial and pharmaceutical uses.

Keywords: Biodiversity, DNA barcoding, Endophytes, ITS sequencing, Phylogeny, Cyrene, Libya.

الملخص
تعيش الفطريات الداخلية داخل أنسجة النبات دون أن تسبب أعراضًا مرضية ظاهرة. وتلعب أدوارًا هامة في تعزيز نمو النباتات المضيفة ومساعدتها على التعامل مع الضغوط البيئية. تُعد هذه الدراسة الأولى من نوعها التي تقوم بالتوصيف الجزيئي للفطريات الداخلية المعزولة من نباتات محلية في منطقة شحات (قورينائية) في ليبيا. تم جمع 68 عزلة فطرية من ستة أنواع نباتية بعد تعقيم سطحي دقيق. ثم تم اختيار ست عزلات ممثلة للتحليل الجزيئي بناءً على اختلافاتها المظهرية. لجأنا لتضخيم وتسلسل المنطقة الداخلية للمنسوخ (ITS) للحمض النووي الريبوسومي من أجل التعريف. الفطريات المحددة وأرقام الانضمام الخاصة بها في بنك الجينات (GenBank) هي على النحو التالي: Aspergillus niger (PQ178951)، Fusarium sp. (PQ178952)، Penicillium nagliovense (PQ178953)، Fusarium chlamydosporum (PQ178954)، Penicillium chrysogenum (PQ178955)، و Stagonosporopsis lupini (PQ182696). أظهر تحليل BLASTn تشابهًا في التسلسل بنسبة 99-100٪ مع السلالات المرجعية في قواعد البيانات. يشير وجود أجناس

مثل Fusarium و Penicillium إلى قدرة تكيف بيئي قوية. توفر هذه الدراسة بيانات أساسية للرموز الشريطية DNA لفطريات المناطق الداخلية في هذه المنطقة غير المُستَكشَفة سابقًا. تُسلط النتائج الضوء على التنوع الفطري الغني للمنطقة، وتشير إلى أن هذه العزلات يمكن أن تكون مفيدة لأبحاث التكنولوجيا الحيوية المستقبلية، مثل إنتاج المركبات النشطة بيولوجيًا للاستخدامات الصناعية والصيدلانية.

الكلمات المفتاحية : التنوع البيولوجي، الرموز الشريطية DNA، الفطريات الداخلية، تسلسل المنطقة ITS، التطور السلالي، شحات (قورينائية)، ليبيا.

Introduction

Endophytic fungi are integral components of plant microbiomes, residing intra-and intercellularly within healthy plant tissues without inducing visible disease symptoms. These fungi enhance host plant performance by promoting growth, conferring stress tolerance, and providing disease resistance, rendering them a focal point for ecological and biotechnological research (Barnett and Hunter, 1998). Proper identification and classification remain challenging due to the similarities in appearance among different groups. Molecular techniques, particularly the sequencing of the internal transcribed spacer (ITS) region of ribosomal DNA, have become the main method for identifying fungi (Schoch et al., 2012). Global surveys have revealed extensive diversity among endophytic fungi, with genera such as *Aspergillus*, *Penicillium*, and *Fusarium* frequently reported across diverse host plants and ecosystems. For instance, studies in tropical forests and agricultural systems have underscored the prevalence and ecological significance of these fungi. In adjacent regions like Egypt, endophytic *Penicillium* isolates from medicinal plants have demonstrated notable antibacterial activity (e.g., Fouda *et al.*, 2015; Elnahal *et al.*, 2022). Similarly, investigations in arid environments have identified distinct fungal assemblages adapted to extreme conditions, with unique enzymatic capabilities influenced by environmental stressors (e.g., Sun *et al.*, 2021, Kuchkarova *et al.*, 2024).

Despite the advancement of knowledge worldwide, the Mediterranean Basin—particularly its arid and semi-arid zones, including Libya—remains underexplored. Libya encompasses diverse Mediterranean ecosystems, yet the diversity of its endophytic fungi associated with native plants is virtually undocumented. The Cyrene region (Shahat), a historically significant area characterized by unique flora and a distinctive Mediterranean climate, exemplifies a critical research gap. To date, no studies have employed molecular techniques to characterize endophytic fungi from native plants in this region.

This study addresses this gap by pioneering the molecular identification of endophytic fungi isolated from native plants in Libya's Cyrene region. The overall purpose of this study is to: (1) explore and select the native plant hosts, isolate and purify endophytic fungi from selected native hosts, (2) correctly identify isolates to genus or species level via integrative approaches of morphological and molecular tools, (3) assess their genetic diversity and (4) document and conserve them using short-and long-term techniques. This preliminary research will establish a foundational catalog of endophytic fungi from an underrepresented region, providing a basis for subsequent ecological investigations for the discovery of biotechnologically valuable compounds.

Materials and Methods

Study Area and Sample Collection

This research took place in the coastal and mountainous regions of Shahat, historically known as Cyrene, in northeastern Libya. This area is recognized for its typical Mediterranean climate. In April 2024, healthy aerial parts, including stems and leaves, were collected from six common native plant species: *Scolymus hispanicus*, *Nepeta scordotis*, *Parietaria judaica*, *Quercus coccifera*, *Ceratonia siliqua*, and *Rhamnus lycioides*. A total of 30 samples were collected, with five individual plants from each species. The plant materials were placed in sterile zip-lock bags, transported to the laboratory in an insulated cooler, and stored at 4°C until they were processed within 24 to 48 hours.

Surface Sterilization and Isolation of Fungal Endophytes

To isolate true endophytic fungi and eliminate epiphytic and surface contaminants, all plant segments were cut into 0.5 cm pieces and underwent surface sterilization using a slightly modified method based on Schulz et al. (2002). The sterilization procedure proceeded as follows:

1. Immersed in 70% ethanol for 2 minutes;
2. Immersed in a 2% sodium hypochlorite (NaOCl) solution for 4 minutes;
3. Immersed in 70% ethanol for 30 seconds.

The plant segments were then rinsed three times in sterile distilled water to remove any remaining sterilant. We verified the effectiveness of the surface sterilization by plating 100 µL of the final rinse water onto potato dextrose agar (PDA) plates, which were incubated at 27°C for 5 days. The absence of microbial growth on these control plates confirmed that the surface disinfection was successful.

Next, the sterilized segments were aseptically transferred to Water Agar (WA) plates with chloramphenicol (50 µg/mL) to prevent bacterial contamination. We placed five segments on each plate, sealed them with Parafilm®, and incubated in the dark at 27°C for 1 to 2 weeks until we observed fungal growth.

Purification and Morphological Characterization

The fungal hyphae that emerged from the plant segments were transferred onto new PDA plates to obtain pure cultures. "We were able to achieve purification using the hyphal-tip method."

We classified the fungal isolates based on their macro-morphological traits, including colony color, texture, growth rate, and exudate production, along with their micro-morphological features, such as hyphal structure and conidiophore morphology viewed under light microscopy. We referenced identification keys from Watanabe (2010) and Seifert et al. (2011). In total, we obtained 68 pure fungal isolates, which were preserved for further molecular analysis.

DNA Extraction, PCR Amplification, and Sequencing

We extracted genomic DNA from 7-day-old fungal mycelia, using about 100 mg of fresh weight grown on PDA, following the CTAB-based method from Brandfass and Karlovsky (2008). The concentration and purity of the extracted DNA were checked using a NanoDrop spectrophotometer. We selected samples with an A260/A280 ratio between 1.8 and 2.0 for PCR.

We amplified the Internal Transcribed Spacer (ITS) region using universal fungal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). The PCR reaction mixture, totaling 25 µL, included 12.5 µL of 2X PCR Master Mix (with Taq DNA polymerase, dNTPs, and $MgCl_2$), 1 µL of each primer (10 µM), 1 µL of template DNA (approximately 20-50 ng), and 9.5 µL of nuclease-free water. We carried out the amplification in a thermal cycler with the following conditions: initial denaturation at 94°C for 5 minutes; followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute, ending with a final extension at 72°C for 10 minutes. We separated the PCR products on a 1.5% agarose gel stained with ethidium bromide, visualized them under UV light, and used a 100 bp DNA ladder to confirm the expected amplicon size of approximately 500-700 bp.

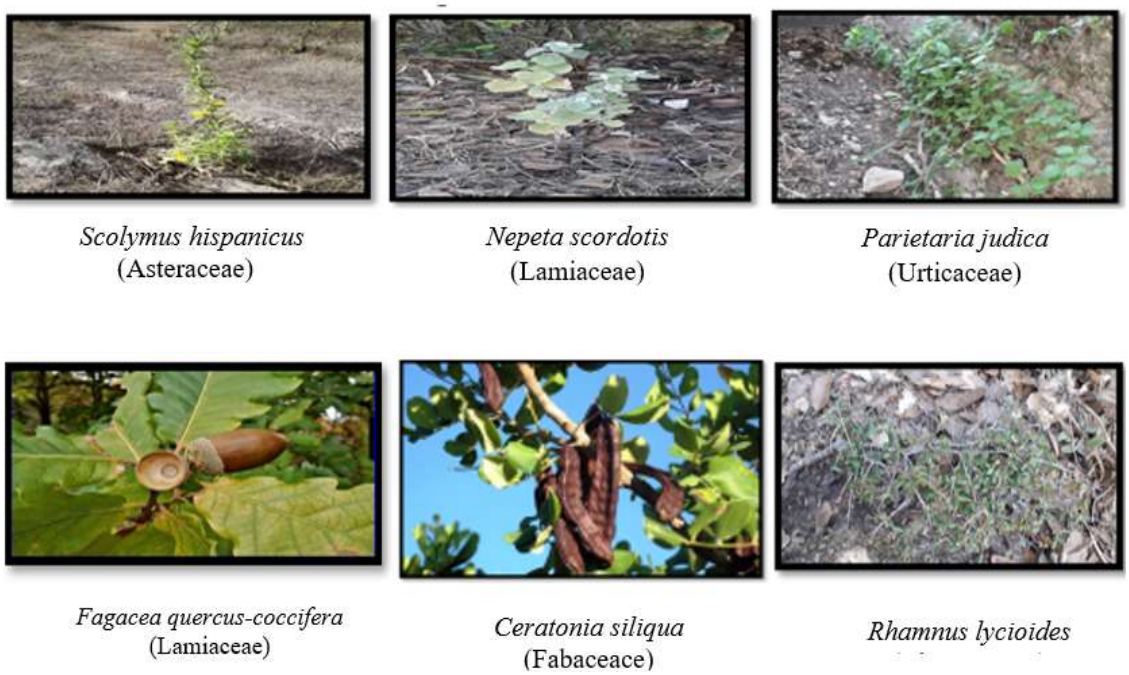

Figure 1. Collection of six different native plants

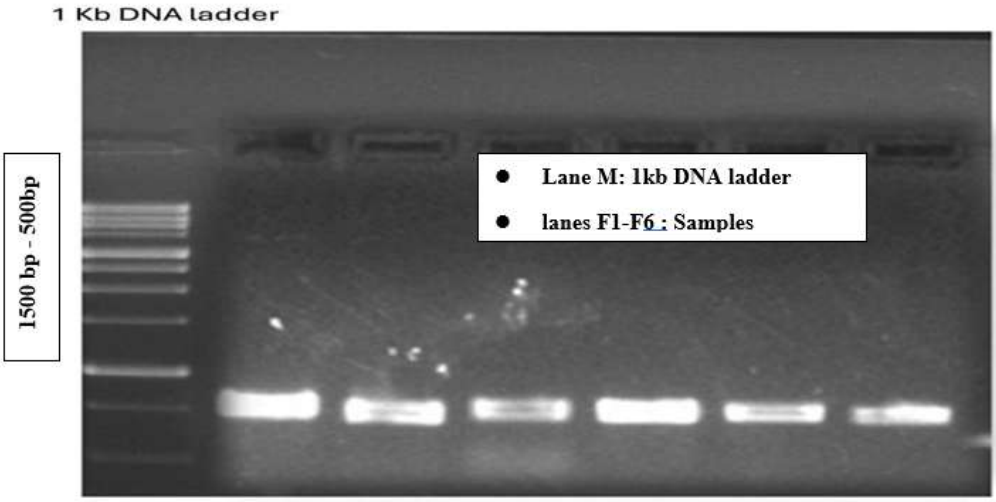

Figure 2.PCR Amplification of the ITS Region .

Figure 2, shows the PCR amplification of the internal transcribed spacer (ITS) region using primers ITS1 and ITS4. Lane M represents the 1 kb DNA ladder. Lanes F1 to F6 correspond to the fungal isolates from various plant samples. Clear and distinct amplicons of about 600 bp appeared in all samples, properly matched with the DNA ladder. This confirms successful amplification of the ITS region.

Extraction of crude Metabolites from selected fungal endophyte

We extracted secondary metabolites from the selected endophytic fungi using a process similar to what was described earlier. Ethyl acetate served as the solvent for extracting the filtrates obtained from fungal growth. We separated the culture medium and mycelium by filtration. Then, we extracted the filtrates three times with an equal volume of ethyl acetate. We repeated this extraction process for three to four cycles. Once the extraction was complete, we evaporated the organic extracts under reduced pressure to obtain solid residues. These residues were used to evaluate antibacterial activity.

Biological activity of crude metabolites from selected endophyte

We examined the antibacterial activity of the secondary metabolites by adding fungal extracts to cultures of the target bacteria. We measured the inhibition zone, or determined the Minimum Inhibitory Concentration (MIC), using the Disk Diffusion Method *(Walsh et al.*, 2016).

Microorganisms Used for Antibacterial Activity Testing: We obtained the bacteria used in this study from the National Collection of Industrial Microorganisms (NCL) in Pune, India. We prepared bacterial suspensions and adjusted them according to the McFarland 0.5 standard, which is approximately 1.5×10^8 CFU/mL (CLSI 2023).

Bacteria Tested: Gram-positive bacteria:

Staphylococcus aureus (Identification number PV606376), Gram-negative bacteria: *Klebsiella pneumoniae* (Identification number PV606419).

Antibacterial Activity Testing

We screened the antibacterial activity of the isolated endophytic fungi against both pathogenic and non-pathogenic bacteria using the Disc Diffusion Method.

Preparation of Test Sample for Antibacterial Activity Testing:: We dissolved the extract in Dimethyl Sulfoxide (DMSO) to prepare stock solutions at a concentration of 3 mg/100 mL

Disc Diffusion Method: We impregnated sterile filter paper discs (6 mm diameter) with a specific amount (e.g., 20 µL) of fungal extract. We placed the discs on the prepared agar plates, which included the fungal extract-treated discs and control discs (e.g., containing only gentamicin). We incubated the plates at 37°C for 18-24 hours. After incubation, we measured the inhibition zones around the discs to assess antibacterial activity.

Results

Fungal Isolation and Morphological Grouping

We collected 68 endophytic fungal isolates from six native plant species. By examining macro- and micro-morphological traits such as colony color, texture, conidiophore structure, and conidial shape, we grouped the isolates into six distinct morphological types. Figure 3 shows Isolate F1 (*Aspergillus niger*). It has thick black colonies on top and brown on the bottom, with conidia visible under the microscope. F2 isolate (*Stagonosporopsis*

sp.) has yellow-brown colonies on top and dark centers, with segmented hyphae visible under the microscope. F3 isolate (*Fusarium* sp.) shows pinkish-white, cottony growths, dark centers below, and noticeable crescent-shaped conidia. Isolate F4 (*Penicillium nagliovnes*) has colonies that are pink to red on top and creamy below, with branched conidiophores visible microscopically. F5 (*Fusarium chlamydosporum*) has a deep pink to brown appearance, and its microscopic structures show chlamydospores characteristic of the species. F6 (*Penicillium chrysogenum*) features bluish-green colonies on top and grayish-white colonies below, with branched, regular conidiophores seen under the microscope.

Our results showed that the endophytic fungi isolated from plant tissues belonged to different genera, including *Aspergillus*, *Fusarium*, *Penicillium*, and the *Stagonosporopsis* isolate, all of which are common endophytes in plants. The isolates displayed significant variation in colony color and growth pattern when grown on PDA medium. This variation reflects differences in genetic makeup and environmental factors that affect fungal growth (Klich 2002). Microscopic examinations revealed fine details of conidiophores and hyphae, which helped with the initial identification of fungal species based on classical taxonomic keys (Watanabe, 2010). *Penicillium* species (F4 and F6), known for their green or pink colonies, are common endophytic fungi and may have antimicrobial or plant growth-promoting effects (Frisvad and Samson, 2004). In contrast, the isolation of *Stagonosporopsis* (F2) is notable since it is often linked to leaf diseases. However, it can also act as a harmless endophyte in certain circumstances (Chen et al., 2025). *Fusarium* isolates (F3 and F5) were found to produce crescent-shaped conidia and sometimes chlamydospores, which is a typical growth pattern for species that are part of symbiotic or parasitic relationships with plants (Summerell et al., 2003).

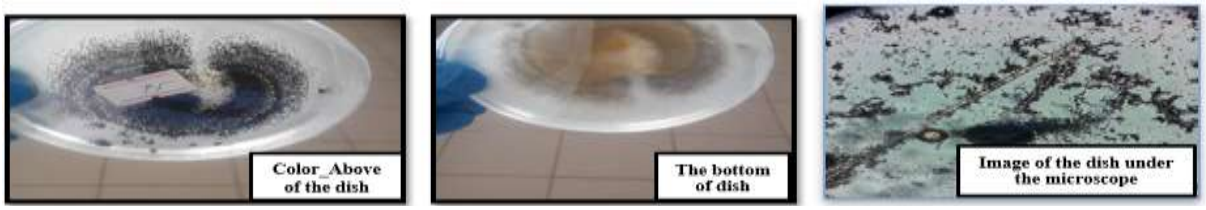

Plate 1. *Aspergillus niger* (F1) grown on PDA. Showing colony characters (front and. reverse sides), and microscopic features .

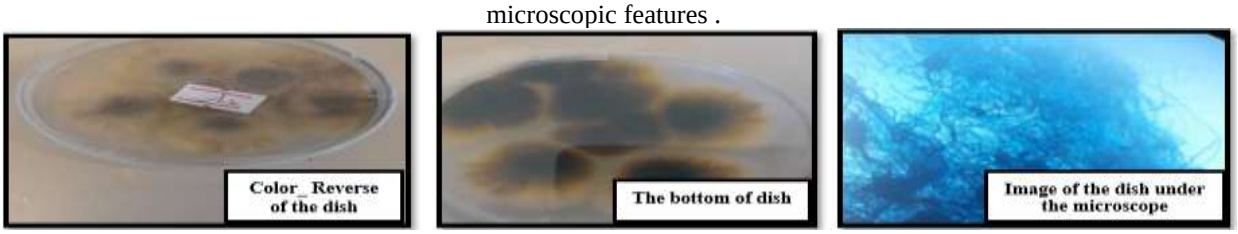

Plate 2. *stagonosporopsis* (F2) grown on PDA. Showing colony characters (front and. reverse sides), and microscopic features.

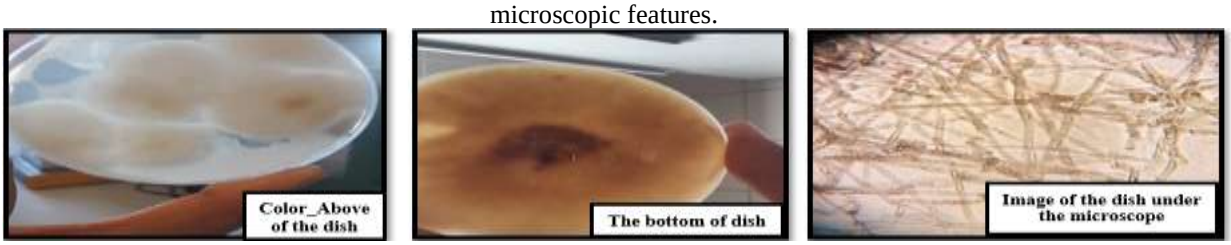

Plate 3.*Fusarium* sp. (F3) grown on PDA. Showing colony characters (front and. reverse sides), and microscopic features.

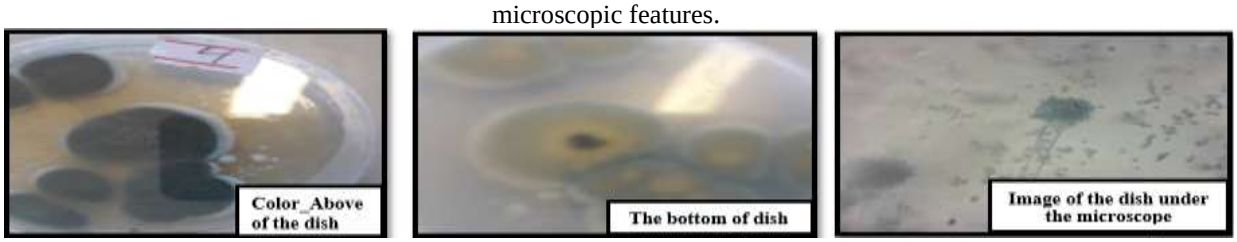

Plate 4. *Penicillium nagliovnes* (F4) grown on PDA. Showing colony characters (front and. reverse sides), and microscopic features

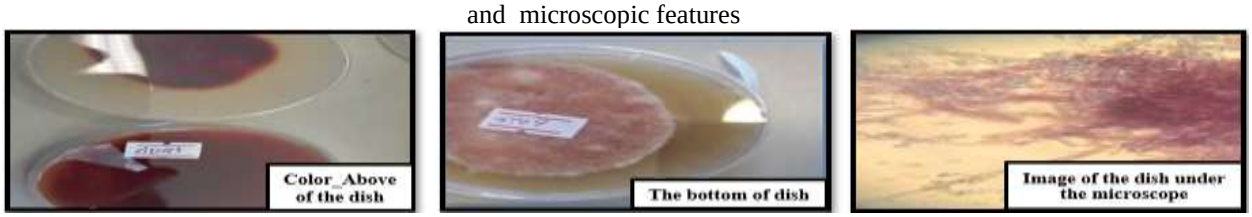

Plate 5. *Fusarium Chlamydosporum* (F5)grown on PDA. Showing colony characters (front and. reverse sides), and microscopic features

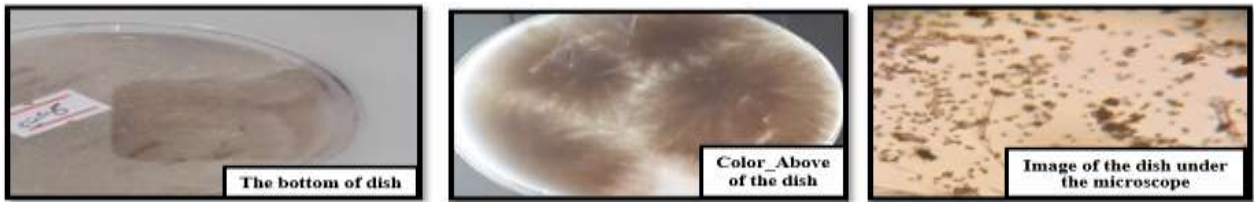

Plate 5. *Penicillium chrysogenum* (F6) grown on PDA. Showing colony characters (front and. reverse sides), and microscopic features.

Figure 3. Morphological characteristics of six isolates of endophytic fungi isolated from plant tissues, after cultivation on potato dextrose agar (PDA) medium.

The images show the appearance of the colonies from the top and bottom, in addition to the microscopic characteristics of the fungal structures under the microscope. The Maximum Likelihood phylogenetic tree, built from the ITS sequences and closely related reference sequences, strongly supported the identifications (Figure 3). The tree showed clear clustering of the isolates with their respective type strains. Bootstrap values exceeded 85% for all major clades We selected one representative isolate from each group for molecular identification, labeled as F1 - F6. We successfully amplified the ITS region and produced clear PCR products of about 500 to 700 base pairs for all six selected isolates (Figure 2). Sanger sequencing, along with BLASTn analysis against the NCBI GenBank database, confirmed the identity of the isolates with high sequence similarity, between 99% and 100%.

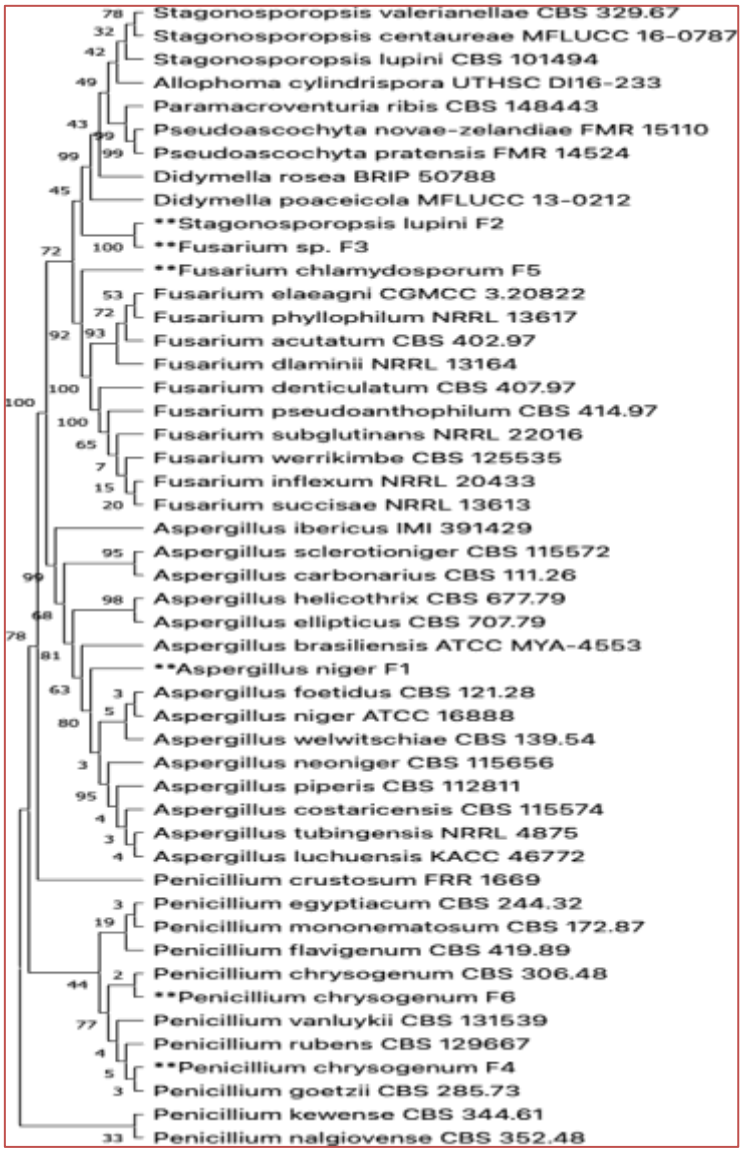

Figure 3. Phylogenetic tree showing the relationship of the six endophytic fungal isolates (F1-F6) and their closest reference strains. The tree was built using the Maximum Likelihood method based on ITS sequences. Numbers at the branches show bootstrap support values greater than 85%. The scale bar indicates genetic distance.

Table 1. Molecular identification of endophytic fungal strains isolated from native plants in the Cyrene Region.

Isolate Code	Closest Identified Species (GenBank Match)	Host Plant	Gen Bank Accession No.	Percent Identity (%)
F1	*Aspergillus niger*	*Ceratonia siliqua*	PQ178951	100
F2	*Stagonosporopsis lupini*	*Quercus coccifera*	PQ182696	99.8
F3	*Fusarium* sp.	*Scolymus hispanicus*	PQ178952	99.5
F4	*Penicillium nagliovense*	*Rhamnus lycioides*	PQ178953	99.9
F5	*Fusarium chlamydosporum*	*Myrtus communis*	PQ178954	100
F6	*Penicillium chrysogenum*	*Olea europaea*	PQ178955	99.7

Table 2 shows clear differences in the inhibitory activity among the tested endophytic fungi at various extract concentrations (P-value=0.000). Overall, higher concentrations tended to produce larger inhibition zones. However, the response varied based on the fungal species and the bacterial strain. *K. pneumoniae* generally showed more sensitivity to some extracts than *S. aureus*, especially at higher concentration.

Discussion

This study presents the first brief report of endophytic fungi found in native plants in the Cyrene region of Libya. We identified a diverse community of endophytic fungi, mainly from the genera *Aspergillus, Fusarium, Penicillium* and *Stagonosporopsi*s, primarily based on morphological and sequencing of ITS region only due to constraint of facility and funding. However, the identity of the endophytic fungi require further confirmation using gold standard of multigene sequencing and phylogenetic analysis. Molecular methods highlight the effectiveness of technique for initial classification of fungal endophytes in arid and Mediterranean environments (Rodriguez *et al.*,2011, Schoch *et al*.,2012). The presence of *Fusarium* species (Isolates F3 and F5) in our study matches their reputation as common and widespread endophytes in various plant hosts around the world (O'Donnell *et al.*, 2015, Gakuubi *et al.*, 2021). Their frequent isolation indicates that they may have adapted well and formed a complex symbiotic relationship with the local plants in Cyrene. *Fusarium* species can act as pathogens, saprobes, or beneficial endophytes, depending on the host and environmental conditions.

Table 2. Antibacterial activity of crude metabolite extracted from endophytic fungi against *Staphylococcus aureus* (PV606376) and *Klebsiella pneumoniae* (PV606419) at different concentrations (mean ± standard deviation).

		ST. D ± Mean (mm)			
Concentration %		**25**	**50**	**75**	**100**
Fungus	**Bacteria**				
A. niger	**S.***aureus*	0.50 ± 4.50	0. 50 ± 4.50	1.00 ± 6.00	1.00 ± 7.00
	K.*pneumoniae*	0. 75 ± 5.23	0. 50 ± 5.50	0.100 ± 7.00	1.00 ± 9.00
***Stagonosporopsis* sp.**	**S.***aureus*	0.00 ± 0. 00	1.50 ± 7.50	1.51 ± 8.40	0. 50 ±9.50
	K.*pneumoniae*	2.89 ± 1.67	0. 29 ± 5.33	0.00 ± 7.00	0. 58 ±7.67
***Fusarium* sp.**	**S.***aureus*	0.00 ± 0.00	2.89 ± 1.67	1.25 ± 5.73	0.100 ± 0.700
	K.*pneumoniae*	0. 29 ±5.33	0.00 ± 6.00	1.25 ± 7.27	2.00 ± 9.00
P. naguforme	**S.***aureus*	0.50 ± 5.50	0.29 ± 7.33	0.25 ± 5.77	1.26 ± 0.783
	K.*pneumoniae*	0.0± 0.500	0.00 ± 0.600	0.25 ± 6.77	0.29 ± 8.83
F. chlamydosporum	**S.***aureus*	0.25 ± 4.77	0.50 ± 6.00	0.29 ± 7.67	0.50 ± .8.50
	K.*pneumoniae*	0.50 ± 4.50	0.29 ± 6.17	0.76 ± 6.83	0.50 ± 9.50
P. chrysogenum	**S.***aureus*	1.04 ±4.83	1.04 ± 7.17	1.04 ± 8.17	0.50 ± 8.50
	K.*pneumoniae*	0.058 ± 5.33	0.00 ±6.00	0.29 ±6.17	0.50 ± 7.50
P-Value = 0.03					

In the table2, The inhibitory effects of the endophytic fungal extracts on *S.aureus* varied among species. A. *niger* and P.*chrysogenum* showed moderate activity, mainly at higher concentrations. This suggests a dose-dependent response related to how fungal secondary metabolites behave (Gakuubi et al., 2021). Klebsiella pneumoniae was particularly sensitive to several fungal extracts, especially from Fusarium sp., F. *chlamydosporum*, and *Aspergillus*

niger. Stronger inhibition occurred at concentrations of 75% or higher. This might arise from the vulnerability of Gram-negative membranes to certain fungal metabolites like terpenoids and polyketides (Ayob & Sim, 2011).
Extracts from *Stagonosporopsis* showed weak inhibition of *Staphylococcus aureus* but had a more significant effect on K. *pneumoniae*. This could be due to small, diffusible secondary metabolites that are more effective against Gram-negative bacteria (Nischitha & Shivanna, 2015). Both *Fusarium* sp. and F. *chlamydosporum* significantly inhibited K. *pneumoniae* at higher concentrations. This is likely because of bioactive compounds like fusaric acid and trichothecenes (Wang & Xu, 2012). Their limited effect on S. aureus may be related to the thicker peptidoglycan layer found in Gram-positive bacteria (Nischitha & Shivanna, 2015).
P.*chrysogenum* consistently inhibited S. aureus, which is consistent with its known β-lactam-type metabolites. However, its weaker impact on K. *pneumoniae* supports the idea that Gram-negative bacteria are less susceptible due to their protective outer membrane (Keller, 2019).
The statistical significance of the results ($P = 0.03$) shows that differences in antibacterial activity result from the species of endophytic fungi and extract concentration, rather than random variation. This highlights the biological relevance of the findings (Field, 2018). F. chlamydosporum, known for attacking nematodes and helping control pests, may play a role in plant defense in this ecosystem (Manzanilla-López et al., 2011).
We also isolated *Penicillium* species (P. *nagliovense* F4 and P. *chrysogenum* F6), which is consistent with findings from other studies in North Africa and arid regions. The common occurrence of *Fusarium* and *Penicillium* genera matches data from desert plants, which often host unique fungal communities with specific adaptations. However, the specific species we identified (e.g., P. *nagliovense*, S. *lupini*) differ, highlighting the unique fungal profile of the Cyrene region and the role of local plant species and microclimates in shaping endophyte communities.
A key strength of this study is the in-depth phylogenetic tree based on the ITS region, which allowed for clear species identification for most isolates, supported by strong bootstrap values. While multi-locus sequence analysis (like using tef1-α, rpb2) is often recommended for detailed classification in complex genera like *Fusarium* and *Aspergillus*, the ITS region continues to serve as a reliable fungal barcode for genus-level and often species-level identification in diversity surveys (Schoch et al., 2012; Samson et al., 2014). Our research effectively establishes a foundational molecular inventory for the region.

Conclusion

This study represents the first molecular characterization of endophytic fungi associated with native plants in the Cyrene region (Shahat) of northeastern Libya. Through ITS rDNA sequencing and phylogenetic tree , we identified six fungal taxa belonging to four genera: *Aspergillus niger*, *Fusarium chlamydosporum*, *Fusarium* sp., *Penicillium chrysogenum*, *Penicillium nagliovense*, and *Stagonosporopsis lupini.* The corresponding sequences were deposited in GenBank, providing the first DNA barcode records for endophytic fungi from this region and confirming the usefulness of the ITS region as a reliable barcode for fungal identification (Schoch et al., 2012; Samson et al., 2014).
The isolated endophytes showed moderate diversity, with a community dominated by genera of recognized ecological and biotechnological importance. The prevalence of *Fusarium* and *Penicillium* species indicates strong adaptation to the Mediterranean climate of the Cyrene region. Notably, several identified taxa have well-documented functional traits: *Fusarium chlamydosporum* is known for its biocontrol potential, *Penicillium chrysogenum* for producing antibiotics, and *Aspergillus niger* for various industrial enzyme applications (Gakuubi et al., 2021; Keller, 2019). These findings emphasize the promising biotechnological value of the isolated strains.
This research fills a critical gap in understanding fungal endophyte diversity in North Africa and serves as a foundational reference for the region. Future studies should prioritize: (1) broader sampling across additional geographical areas and host plants to capture endophytic diversity more comprehensively; (2) multi-locus sequencing methods to clarify taxonomic complexities within genera like *Fusarium*; and (3) functional tests to assess the isolates' ability to produce bioactive compounds for potential applications in agriculture, pharmaceuticals, and industry.
In conclusion, this study provides essential baseline information for understanding the endophytic mycobiome of Libya's Mediterranean ecosystems and highlights its untapped biotechnological potential. Conserving native plant hosts and their associated fungal communities will be crucial for preserving and sustainably utilizing valuable microbial resources.

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Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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