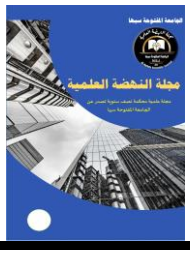
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Endophytic Fungi Associated with the Common Reed, *Phragmites australis* (Cav.) Trin. ex Steud, in Libya

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Abstract

Endophytic fungi live inside plant tissues without triggering disease, and in many plant–fungus partnerships they help their hosts resist pathogens, herbivory and abiotic stress such as salinity or heavy-metal exposure. The common reed, *Phragmites australis* (Cav.) Trin. ex Steud., is widespread across Libya's coastal and wetland habitats, yet its endophytic mycobiota had never been examined. Leaf, rhizome and root tissue were sampled from five reed plants growing in the Al-Shaab area of Tripoli Sea Port, Tripoli, Libya, in June 2024, surface-sterilized, and cultured on chloramphenicol-amended potato dextrose agar (PDA) to recover culturable fungi. Thirteen taxa were identified morphologically. Below-ground tissues carried more taxa than leaves: thirteen were recovered from rhizomes and ten from roots, against eight from leaves. Six taxa (*Acremoniumsp*., *Alternariasp*., *Aspergillus niger*, *Aspergillussp*., and *Cladosporiumsp*., plus an unidentified yeast morphotype) turned up in all three tissue types, indicating that they represent the core endophytic community. ACochran's Q test indicated no significant differences in taxon occurrence among the three compartments ($Q = 5.43$, $df = 2$, $p = 0.066$); pairwise similarity, measured with the Sørensen and Jaccard indices, was highest between rhizomes and roots and lowest between leaves and roots. This is the first record of the endophytic fungal community of *P. australis* from Libya, and it provides the first baseline dataset for future molecular identification and functional studies of reed-associated endophytic fungi in North African wetlands.

Keywords: endophytic fungi; *Phragmites australis*; culturablemycobiota; Libya; wetland reeds; tissue-specific colonization

الفطريات الداخلية المرتبطة بالقصب الشائع، *Phragmites australis* (Cav.) Trin. ex Steud، في ليبيا

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الملخص

تعيش الفطريات الداخلية داخل أنسجة النباتات دون إحداث أعراض مرضية، وقد تسهم في تعزيز مقاومة النبات لمسببات الأمراض والإجهادات غير الحيوية مثل الملوحة والمعادن الثقيلة. يُعد نبات القصب الشائع *Phragmitesaustralis (Cav.) Trin. ex Steud.*من النباتات المنتشرة في البيئات الساحلية والرطبة في ليبيا، إلا أن الفطريات الداخلية المرتبطة به لم تُدرس سابقًا، جُمعت عينات من الأوراق والريزومات والجذور من خمسة نباتات نامية في منطقة الشعاب بميناء طرابلس البحري، طرابلس، ليبيا، خلال يونيو 2024، وعُقمت سطحيًا وزُرعت على بيئة أجار البطاطا والدكستروز المدعمة بالكلورامفينيكول لعزل الفطريات القابلة للزراعة. تم التعرف مورفولوجيًا على ثلاثة عشر صنفًا فطريًا، أظهرت الأنسجة تحت الأرض تنوعًا أعلى، حيث عُزلت ثلاثة عشر صنفًا من الريزومات وعشرة من الجذور مقارنة بثمانية من الأوراق. تم تسجيل ستة أصناف.(*Acremonium* sp. ، *Alternaria sp.*، *Aspergillus niger*، *Aspergillus* sp.، *Cladosporium* sp.، ونمط مورفولوجي غير معرف من الخمائر) في جميع الأنسجة، مما يشير إلى كونها مكونات أساسية للمجتمع الفطري الداخلي. لم يُظهر اختبار كوشران Q فروقًا معنوية في حدوث الأصناف

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بين الأجزاء النباتية (P=0.066 df=2,،Q=5.43)، وكانت أعلى درجات التشابه وفق مؤشري سورنسن وجاكراد بين الريزومات والجذور .تمثل هذه الدراسة أول سجل للفطريات الداخلية القابلة للزراعة المرتبطة بـ *P. australis*في ليبيا، وتوفر قاعدة بيانات أولية للدراسات المستقبلية حول التعرف الجزيئي والوظائف البيئية لهذه الفطريات في الأراضي الرطبة بشمال أفريقيا.

الكلمات المفتاحية: الفطريات الداخلية؛ القصب الاسترالي، الفطريات الداخلية القابلة للزراعة، ليبيا، الأراضي الرطبة، النسيج النباتي.

1. Introduction

Endophytic fungi occupy the intercellular and intracellular spaces of plant tissue, and although the relationship is largely symptomless, it is rarely neutral: hosts often gain protection against pathogens and herbivores, along with greater tolerance of abiotic pressures such as salinity and heavy-metal-laden soil, while the fungus secures shelter and nutrition in return (Alam et al., 2021; Baron and Rigobelo, 2022). Based on colonization pattern, host range and mode of transmission, fungal endophytes have been grouped into a small number of functional classes, with most culturable isolates from non-grass hosts falling into the broad group of horizontally transmitted endophytes that dominates temperate and wetland floras worldwide (Rodriguez et al., 2009).

Studying this community requires care to avoid mistaking surface contaminants for true endophytes. After plant material is collected, tissue segments are first disinfected to strip away epiphytic microbiota (Li et al., 2016), and only then plated on nutrient media for culture-based isolation. Culture-dependent surveys remain the standard tool for building regional endophyte inventories, even though they are known to miss part of the community that culture-independent, sequence-based methods can detect.

Phragmites australis is among the world's most widespread wetland grasses and forms dense stands along Libya's coastal lagoons, brackish marshes and freshwater seeps, stabilizing shorelines and filtering water as it spreads. Its fungal endophytes have been studied elsewhere and tend to show strong tissue-specific patterns, sometimes contributing to host tolerance of salt and metal stress (Clay et al., 2016; Khalmuratova et al., 2020). Globally, root- and rhizosphere-associated fungal communities of coastal wetland plants remain comparatively understudied relative to their bacterial counterparts, and recent systematic reviews highlight substantial geographic and taxonomic gaps in this literature (Lumibao et al., 2024). In Libya specifically, fungal surveys have so far been confined to the marine environment, most recently documenting Ascomycota-dominated assemblages associated with the seagrass *Posidonia oceanic* along the Tripoli coastline (Benzayed et al., 2026), while the mycobiota of terrestrial and semi-aquatic wetland plants has not previously been examined. This study addresses that gap by describing, for the first time, the endophytic fungal community associated with *P. australis* in Libya, comparing taxon richness across leaf, rhizome and root tissue.

2. Materials and Methods

2.1 Collection and sampling of plant materials

Plant material was collected on 1 June 2024 from the Al-Shaab area of Tripoli Sea port, Tripoli, Libya. Because endophyte diversity is best captured when more than five individual hosts are sampled per site (Fan et al., 2020), leaves, rhizome segments, and root segments were taken from five reed plants spaced roughly 50 m apart. Samples were bagged in sterile plastic, transported to the laboratory, and processed within 24 hours of collection.

2.2 Surface disinfection of plant materials

Each sample was first rinsed under running tap water to remove debris and the bulk of surface bacteria, yeasts and fungi. Leaf, rhizome and root material was then cut into small segments, immersed in 70% ethanol for 1–3 minutes, followed by immersion in sodium hypochlorite solution for 3 minutes, and rinsed twice with sterile distilled water. The segments were blotted dry between sterile filter paper before being plated on potato dextrose agar (PDA).

2.3 Isolation and culture of endophytic fungi

Four surface-sterilized segments were plated per dish, with three replicate plates per tissue type (leaves, rhizomes, roots) for each host plant, giving a total of 45 plates and 180 tissue segments in total across the five plants (3 tissue types × 3 plates × 5 plants; 4 segments × 45 plates). PDA is the standard medium for recovering endophytic fungi but also supports bacterial growth, so it was fortified with the broad-spectrum antibiotic chloramphenicol, following Hamzah et al. (2018), to keep contamination down. Plates were held at room temperature for 2–4 weeks and checked macroscopically and microscopically as colonies emerged. Representative culture plates showing surface-sterilized tissue segments and the emergence of fungal colonies are presented in Figure 1.

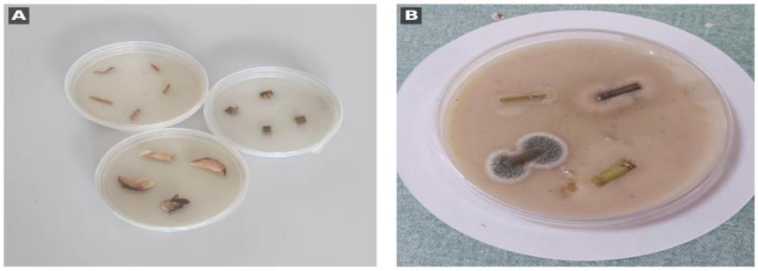


Figure 1. Isolation of endophytic fungi from surface-sterilized tissue segments of Phragmites australis on chloramphenicol-amended PDA. (A) Freshly plated leaf, rhizome and root segments at the start of incubation. (B) A representative plate after incubation, showing fungal colonies growing out from the tissue segments

2.4 Fungal identification

Isolates were identified to genus level using colony morphology and reproductive structures, referring to the identification keys of Barnett and Hunter (1998) and, where needed, Leslie and Summerell (2005).

3. Results

3.1 Taxonomic composition and tissue distribution

Across the three plant compartments, thirteen fungal taxa representing eight genera were recovered from the surface-sterilized segments (Table 1). Richness was greatest below ground, with thirteen taxa recovered from rhizome segments and ten from root segments, compared with eight from leaves. *Acremonium* sp., *Alternaria* sp., *Aspergillus niger*, *Aspergillus* sp. and *Cladosporium* sp., together with a yeast morphotype, appeared in all three tissues and stand out as the assemblage's shared, dominant taxa. The *Fusarium* complex (*F. oxysporum*, *F. solani* and *Fusarium* sp.), together with two unidentified isolates, was restricted to rhizome and/or root tissue. This below-ground restriction of *Fusarium* taxa parallels rhizosphere and root-endosphere fungal surveys of *P. australis* from other pollution-affected wetlands (Kalu et al., 2021).

Table 1. Distribution of fungal taxa isolated from leaf, rhizome, and root tissues of *Phragmites australis* collected from the Al-Shaab area , Tripoli Sea port, Tripoli, Liby.

Fungal taxon	Leaves	Rhizomes	Roots	Occurrence
Acremonium sp.	+	+	+	3/3
Alternaria sp.	+	+	+	3/3
Aspergillus niger	+	+	+	3/3
Aspergillus sp.	+	+	+	3/3
Chaetomium sp.	+	+	−	2/3
Cladosporium sp.	+	+	+	3/3
Fusarium oxysporum	−	+	+	2/3
Fusarium solani	−	+	+	2/3
Fusarium sp.	−	+	+	2/3
Penicillium sp.	+	+	−	2/3
Yeasts	+	+	+	3/3
Unidentified fungus 1	−	+	−	1/3
Unidentified fungus 2	−	+	+	2/3
Total taxa recorded per compartment	**8**	**13**	**10**	**13**

Note: "+" indicates the presence and "−" indicates the absence of each taxon in the corresponding tissue. The Occurrence column reports the number of tissue types (out of three) in which each taxon was recovered. The total number of unique fungal taxa recovered across all tissues was 13.

3.2 Statistical analysis of tissue-wise distribution

Because the same thirteen taxa were scored as present or absent across three related compartments from the same host plants, so we used Cochran's Q test (Zar.2010), the appropriate non-parametric test for k related binary samples, to check for differences in overall occurrence among tissues. No significant difference emerged ($Q = 5.43$, $df = 2$, $p = 0.066$), although the result approached the conventional significance threshold: tissue type did not significantly predict whether a taxon was recovered, despite the numerically higher richness below ground, at least at this sample size.

Pairwise McNemar's exact tests were broadly consistent with these findings, though the leaves-versus-rhizomes comparison approached statistical significance (leaves vs. rhizomes: $p = 0.063$; rhizomes vs. roots: $p = 0.250$; leaves vs. roots: $p = 0.688$). Community similarity between tissue pairs, measured using the Jaccard (Jaccard, 1912) and Sørensen–Dice indices(Sorensen,1948) (Table 2), was highest between rhizomes and roots (Jaccard = 0.77, Sørensen = 0.87), which is not surprising given their shared subterranean setting, and lowest between leaves and roots (Jaccard = 0.50, Sørensen = 0.67).

Table 2. Pairwise community similarity between plant tissues (Jaccard and Sørensen–Dice indices) and McNemar exact test p-values.

Tissue pair	Shared taxa	Jaccard index	Sørensen–Dice index	McNemar p-value
Leaves – Rhizomes	8	0.62	0.76	0.063
Rhizomes – Roots	10	0.77	0.87	0.250
Leaves – Roots	6	0.50	0.67	0.688

3.3 Shared and unique taxa (Venn analysis)

Figure 2 summarizes how taxa were distributed across the three compartments. Six taxa (46.2% of the total) were common to all three compartments; four were shared exclusively between rhizomes and roots; two were shared exclusively between leaves and rhizomes; none was shared exclusively between leaves and roots. Compartment-specific taxa were rare overall: only one rhizome-only taxon (Unidentified fungus 1) was recorded, with none unique to leaves or roots.

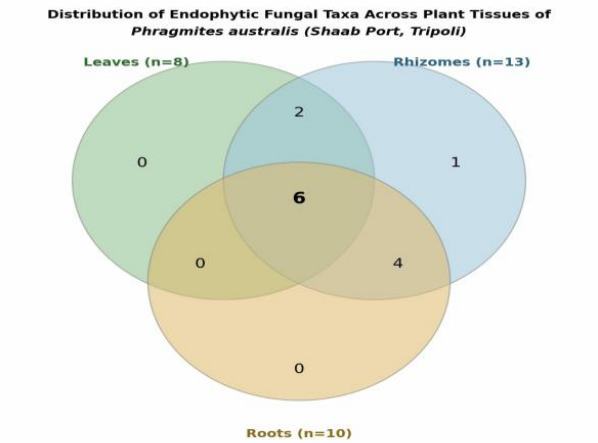


Figure 2. Venn diagram showing the distribution of endophytic fungal taxa shared among and unique to the leaf, rhizome and root tissues of Phragmites australis.

4. Discussion

Thirteen fungal taxa across eight genera were identified from *Phragmites australis* collected from the Al-Shaab area of Tripoli Sea port, Tripoli, Libya, in 2024. The six taxa present in every compartment (*Acremonium* sp., *Alternaria* sp., *Aspergillus niger*, *Aspergillus* sp. and *Cladosporium* sp., plus an unidentified yeast morphotype) were dominated by Ascomycota genera, the phylum that dominates most surveys of graminoid and wetland-plant endophytes over Basidiomycota and other groups; the phylum-level placement of the yeast isolate itself was not resolved by morphology alone. A similarly Ascomycota-dominated profile, rich in *Alternaria* sp. and *Aspergillus niger*, was recently reported from marine fungal surveys of the seagrass *Posidonia oceanica* along the Libyan coastline (Benzayed et al., 2026), suggesting these genera are broadly dominant, stress-tolerant colonizers across Libya's coastal and wetland plant hosts regardless of habitat salinity. The three *Fusarium* taxa, by contrast, stayed below ground tissues,as illustrated in (Figure 3), matching reports elsewhere that treat *Fusarium* as characteristically root- and rhizome-associated in reeds and other graminoids. Confirming these morphology-based identifications with ITS sequencing would be a useful next step, particularly for the two isolates that could only be labeled “unidentified fungus.”

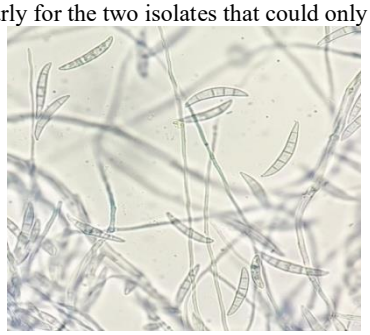


Figure 3. Fusarium sp. isolated from Phragmites australis tissue, showing the macroconidia (large, elongate, multi-septate) and microconidia (small, one- to few-celled) characteristic of the genus (magnification 40×).

Rhizome and root tissue carried more taxa than leaves, a pattern also seen by (Clay et al. (2016) in non-native *P. australis* populations of the Great Lakes, where rhizome tissue held the greatest endophyte diversity. Closer contact with the soil fungal reservoir, along with the comparatively stable microclimate of subterranean tissue versus light-exposed, physiologically variable leaves, could explain this bias. The numerical gap in richness was not statistically significant on Cochran’s Q test ($Q = 5.43$, $df = 2$, $p = 0.066$), though that result deserves a cautious reading given the modest sample of five host plants in this first regional survey. The similarity indices tell a more convincing story: rhizomes and roots shared 0.87 on the Sørensen index, well above the 0.67–0.76 linking leaves to either below-ground tissue, pointing to a real compositional split between above- and below-ground assemblages. The Venn analysis backs this up: four taxa were shared exclusively by rhizomes and roots, while none was shared exclusively by leaves and roots.

Alternaria (Figure 4) and *Cladosporium* stood out among the generalist core taxa here, matching findings from other wetland and halophytic hosts.(Khalmuratova et al.,2020), surveying five salt-marsh species including *P. australis*, likewise found *Alternaria* among the most widely distributed endophytic genera, alongside *Cladosporium* and *Fusarium*. Genera recovered this broadly are usually read as core, host-adapted members of the assemblage. The one taxon recorded in a single tissue only in this study — Unidentified fungus 1, confined to rhizomes — more plausibly represents a transient or opportunistic colonizer (Li et al., 2016; Fan et al., 2020).



Figure 4. Alternaria sp. conidia isolated from Phragmites australis tissue, showing the muriform, multi-septate, catenulate (chain-forming) morphology characteristic of the genus (magnification 40×).

Endophytic colonization by genera such as *Alternaria* and *Aspergillus* has repeatedly been tied to greater host tolerance of salinity and heavy metals, and to bioactive secondary metabolites with antimicrobial or plant-growth-promoting effects (Alam et al., 2021; Baron and Rigobelo, 2022). Given that *P. australis* in Libya often grows in brackish, metal-influenced coastal ground, the community described here could serve as a useful reservoir for future bioprospecting and stress-tolerance research. This survey is culture-dependent, so it only captures fungi able to grow on PDA under the incubation conditions used, and almost certainly underestimates true endophyte diversity relative to culture-independent, high-throughput sequencing (Fan et al., 2020). Culture-based recovery still has real value, though: it produces viable isolates for physiological, biochemical and bioprospecting work that sequence-only approaches cannot offer.

5. Conclusion

This study provides the first record of the culturable endophytic fungal community associated with *Phragmites australis* in Libya. Thirteen taxa were recovered, richness was greatest in rhizome and root tissue and lowest in leaves, and *Acremonium* sp., *Alternaria* sp., *Aspergillus niger*, *Aspergillus* sp. and *Cladosporium* sp., plus an unidentified yeast morphotype, emerged as shared, tissue-generalist dominants. Cochran's Q and McNemar tests found no statistically significant differences in richness among tissues at this sample size, while the Jaccard and Sørensen indices and the Venn analysis together point to a genuine compositional split between above-ground (leaf) and below-ground (rhizome and root) assemblages. These results give Libyan wetlands a baseline inventory of reed-associated mycobiota, with clear next steps ahead: molecular confirmation of isolate identities, larger and geographically broader geographic sampling beyond the Al-Shaab area of Tripoli Sea port, Tripoli, Libya, and functional assays testing what these endophytes contribute to host stress tolerance.

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