Print ISSN: 0375-9237
Online ISSN: 2357-0350

EGYPTIAN JOURNAL OF BOTANY (EJBO)

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PUBLISHED BY
THE EGYPTIAN
BOTANICAL SOCIETY

Diversity of culturable mycoendophytes in Egyptian Red Sea mangrove *Avicennia marina*

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Mangroves are distinct ecosystems that harbor rich biodiversity. Fungal endophytes play an important role in the biogeochemical processes within these ecosystems, making them an essential ecological element. Eleven grey mangrove stands on Egypt's Red Sea coast were examined to assess the diversity of culturable endophytic fungal populations. 450 endophytic fungal isolates were obtained from pneumatophores (60.9%) and leaves (39.1%) of *Avicennia marina*. Significant differences were observed in both colonization and isolation rates ($P < 0.001$). In every research site, the rates of colonization in pneumatophores were greater than in leaves. Shannon-Wiener diversity indices varied significantly among the studied sites, ranging from 1.50 to 2.19 ($P < 0.001$). The highest level of diversity was observed in Marsa Alam, Qalaan, and Safaga, while the lowest was reported for the Sharm Bahry mangrove. Out of the 450 isolates, 354 (79%) were identified to at least the genus level, whereas the rest were unidentified morphotypes, comprising 31 black yeasts and 65 mycelia sterilia. The identified isolates were classified into two phyla: Ascomycota was the most abundant, accounting for 98.3% of the isolates, while Basidiomycota accounted for only 1.7%. Fourteen genera belonging to four taxonomic classes, namely Sordariomycetes, Eurotiomycetes, Dothideomycetes, and Agaricomycetes, were recognized, with *Chaetomium, Aspergillus,* and *Penicillium* being the predominant. Based on available literature, this is the first comprehensive report documenting the biodiversity of mycoendophytes associated with *A. marina* in Egypt. It provides in-depth information on their occurrence and distribution within mangrove ecosystems at eleven locations along the Egyptian Red Sea coastline.

Keywords: Endophytic fungi, Biodiversity, Gray Mangrove, Egyptian Red Sea coast

ARTICLE HISTORY
Submitted: July 03, 2024
Accepted: September 09, 2024

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DOI: 10.21608/ejbo.2024.301366.2907

EDITED BY: K. Ghanem

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INTRODUCTION

Mangrove plants are vital to the ecosystem as they develop in tropical and subtropical areas where the land meets the sea. These areas usually have rocky or muddy coastal soils. They cover over a quarter of the world's tropical coastline and act as a natural barrier against strong waves and storms (Spalding et al., 1997; Ayyam et al., 2019). There are about 70 genuine mangrove species, belonging to 17 families. With an estimated total size of 137,760 square kilometers, only 15 nations have almost 75% of the world's mangroves (Alongi, 2002; Giri et al., 2011). A wide range of bioactive phytochemicals that have several pharmacological and biotechnological uses are produced by mangrove plants (Sadeer et al., 2023; Bibi et al., 2020). Moreover, some mangrove species are also commonly used in traditional medicines to treat conditions such as hypertension, diabetes, diarrhea, and indigestion (Zhu et al., 2009; Sadeer et al., 2019).

High-salt coastal habitats with regular high and low tides as well as muddy, anaerobic conditions are ideal for mangrove growth. Their supportive root system, known as rhizophores (e.g., *Rhizophora mucronata*, Lam.), and aerial roots, or pneumatophores (e.g., *Bruguiera gymnorhiza* (L.) Lam., and *Avicennia marina* (Forssk.) Vierh.) allow them to adapt to these harsh conditions (Sadeer et al., 2019). Seabirds and other tropical marine species depend on mangrove forests as their nesting grounds. A wide variety of microorganisms are also hosted by them. Marine endophytes, which make up a significant portion of this ecosystem, have an excellent home in the mangrove habitats. Mangroves' high salinity levels, high organic matter content, and high redox potential provide the ideal environment for certain endophytic microbe species, which are essential to the biogeochemical processes taking place in these marine environments (Nagelkerken et al., 2008; Ghizelini et al., 2012; Debbab et al., 2013; Eldeen and Effendy, 2013; Lin et al., 2019; Allard et al., 2020).

Mangrove forests are a biodiversity "hotspot" for marine endophytic fungi, which are physiologically well-adapted to thrive in highly saline environments and can withstand fluctuations in pH and temperature (Bhimba et al., 2012; Bramhachari et al., 2019). Coevolving with mangrove species, fungal endophytes are known to improve nutrient intake and increase tolerance to environmental stresses. Additionally, they act as a barrier of defense against infections. A variety of bioactive metabolites,

including alkaloids, flavonoids, phenolic acids, steroids, benzopyranones, quinones, and terpenoids, are produced because of activating many metabolic pathways. These characteristics make them excellent resources for prospecting novel bioactive substances (Eldeen and Effendy, 2013; Simões et al., 2015; Palanichamy et al., 2018; Bibi et al., 2020; Tchinda et al., 2021).

In Egypt, mangrove stands occupy approximately 525 hectares and are in various areas along the Egyptian Red Sea coasts. These mangroves are mainly mono-specific, consisting only of *A. marina* (grey mangroves; Avicenniaceae), except for a few locations near the Egyptian-Sudanese border area, where *R. mucronata* (loop-root mangrove; Rhizophoraceae) also exists alongside *A. marina* (Zahran, 1993; Cabahug, 2002). *A. marina* forms flourish between Haleib, Hurghada, and the Red Sea shore in Sinai for many kilometers along the coast. This mangrove is often found in scattered groupings and is restricted to tiny coastal regions. Furthermore, it has colonized small Red Sea islands such as Wadi el Gemal, Safaga, Abu Minqar, and El Qusair (Zahran and Willis, 2009).

Because of their harsh coastal environment, Egyptian mangroves are an ideal habitat for endophytic fungi. This offers an excellent chance to investigate the diversity of mycoendophytes and the useful metabolites they generate. Unfortunately, due to minimal research, little is known about the variety of endophytic fungi in Egyptian mangrove habitats (Shebany, 2012; Basheer et al., 2018; Khalil et al., 2021). Therefore, this study aimed to evaluate the diversity of the endophytic fungal communities inhabiting the predominant mangrove species in Egypt, *A. marina,* across eleven mangrove stands along the Red Sea coastline. This work also affords preliminary data on mangrove fungal endophytes for a subsequent study investigating their bioactive natural products (Unpublished Data).

MATERIAL AND METHODS

Study Sites and Samples Collection

Samples were collected from eleven *Avicennia marina* mangrove stands (nature reserves). These mangroves are found in separate locations scattered along the Egyptian Red Sea Coastline, namely Nabq mangrove, Ras-Muhammed mangrove, Abu Shaara mangroves, Abu Minqar mangrove, Safaga mangrove (17^{th} km south of Safaga city), Al Quseir mangrove (40^{th} km south of Al Quseir city), Sharm Bahry mangrove, Sharm Qebly mangrove, Marsa Alam mangrove (near the opening of Wadi El Gemal natural reserve), Qulaan mangrove, Hamata mangrove. Each collection site was carefully documented on a map by recording its GPS coordinates (Figure 1). Coordinates of all locations were also shown in Supplementary Table S1. All samples were collected during September 2019 except for samples from Marsa Alam, Qalaan, and Hamata, which were collected in October 2021. Ten trees of *A. marina* (about 1.5 m tall) were randomly selected from each study site. Ten healthy mature leaves (from the lowest branch) and one pneumatophore (aerial roots) were sampled from each tree. After being transferred to the lab in portable cool chambers, the plant samples were put in sterile sampling polythene bags from Whirl-Pak. There, they underwent prompt processing to isolate endophytic fungi.

Isolation and Identification of Entophytic Fungal Isolates

After collecting the *A. marina* leaves and pneumatophores, they were first carefully cleaned with running tap water to remove any attached debris, following the isolation process described by Kjer et al. (2010) with minor adjustments. Then, all samples were surface sterilized by submerging them in 70% (v⁄v) ethanol for 1 minute, immersing them in sterile water for 1 minute, and dipping them in 4% (v⁄v) sodium hypochlorite for 3 minutes. Finally, they were rinsed several times in sterile water and dried on sterilized filter papers. After samples had dried, the selected plant leaves were cut into 0.5 cm^2 segments while dried aerial roots were excised into 1 cm long fragments. The surface sterilized sample segments were plated on Petri dishes containing 2% (w⁄v) malt-extract agar (MEA; Difco, Sparks, MD, USA) supplemented with chloramphenicol (0.2 g/L). All plates were incubated at 25°C for 30 days and were checked daily for fungal growth. After incubation, hyphal tips from the cultivated plant fragments were subcultured onto fresh MEA plates. According to (Schulz et al., 1993), sterilized leaf and aerial root segments were imprinted onto MEA plates and incubated at 25° for 7 days to ensure the efficacy of surface sterilization, the absence of any fungal growth indicated that the surface sterilization protocol was effective.

For long-term preservation of purified cultures, isolates were maintained on MEA slants and 20% (v/v) glycerol in distilled water at 4 °C and − 20 °C, respectively, till further use (Wang et al., 2019). Following the guidelines of Leslie and Summerell (2006), the single-spore technique was used to ensure

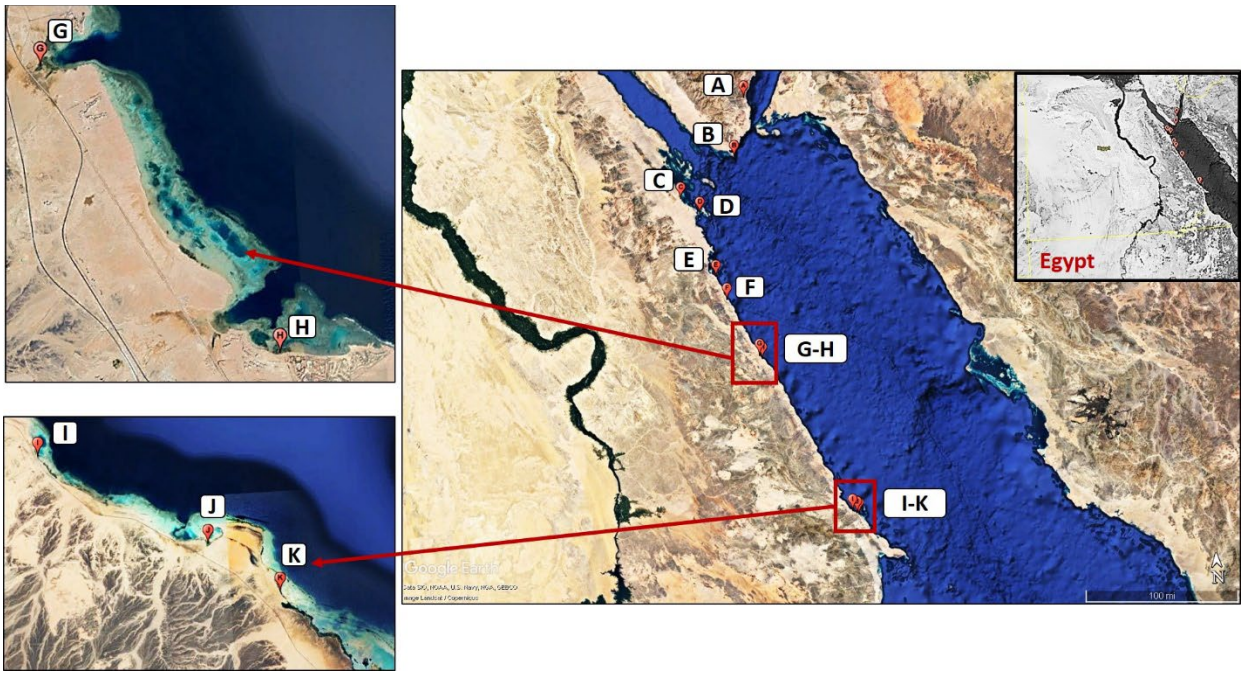

Figure 1. Map of Egyptian Red Sea coastline showing the locations of selected study sites where mangroves were collected (Constructed by Google Earth Pro; accessed date. 12/2023). (A): Nabq mangrove, (B): Ras-Muhammed mangrove, (C): Abu Shaara mangroves, (D): Abu Minqar mangrove, (E): Safaga mangrove, (F): Al Quseir mangrove, (G): Sharm Bahry mangrove, (H): Sharm Qebly mangrove, (I): Marsa Alam mangrove, (J): Qulaan mangrove, (K): Hamata mangrove.

the purity of isolates, immediately before identifying them. Obtained monocultures of mycoendophytes were grouped based on their morphological features and growth rate. Isolates that failed to sporulate on MEA, potato dextrose agar (PDA; Difco Sparks, MD, USA), and corn meal agar (CMA; Difco Sparks, MD, USA) were subcultured on low-nutrient media to enhance sporulation and were examined periodically. These media include water agar media 2% (w⁄v), half- and 1/4-strength PDA, and Synthetic nutrient-poor agar (SNA; Difco Sparks, MD, USA). To encourage sporulation, sterile *A. marina* leaf pieces embedded in water agar were used as well.

When isolates did not sporulate under these conditions, they were recognized as "mycelia sterilia" (Guo et al., 2000; Su et al., 2012). The phenotypic identification of sporulating isolates based on their macroscopic and microscopic characteristics was aided by the universal identification manuals of Ellis (1971); Klich (2002); Leslie and Summerell (2006); Pitt and Hocking (2009); Seifert, et al., (2011); and Visagie et al., (2014). Five isolates were identified at Assuit-University Mycological Center (Assiut University, Egypt) depending on the keys provided by Moubasher (1993); Barnett and Hunter (1998); and Domsch et al. (2007). Tedersoo et al. (2018) and the MycoBank database (https://doi.org/www.mycobank.org) were used to verify the classification and nomenclature of the identified fungi.

Data Analysis

The occurrence of fungal endophytes was determined using colonization and isolation rates, which were computed using the following equations: Colonization rate = (*Ncol*/*Nt*)×100, where *Ncol* is the number of segments colonized by an individual taxon, and *Nt* is the total number of cultured segments. Isolation rate = (*n*/*Nt*)×100, where *n* is the number of isolates of an individual taxon, and *Nt* is the total number of cultured segments (Li et al., 2020). To determine the abundance of a certain endophytic taxon isolated from mangrove tissues, the relative frequency (RF) was employed. It was computed by dividing the number of isolates of a particular taxon from the assemblage by the total number of isolates of all taxa (Zhou et al., 2018). The Shannon-Wiener diversity index (*H*'), which expresses the number of individuals (taxa/species) living in a community and their relative abundance, was used to estimate the mycoendophytic diversity of each mangrove stand. This indicator assesses the number of diverse species/taxa (richness), considering their relative abundance (evenness) in the sample (Heip et al., 1998; Nolan and Callahan, 2006). This Shannon-

Wiener diversity index is calculated using ELMStats 2x2 G-statistic software version 12 according to the formula*: H*' =−∑[(pi)×ln(pi)]*,* where *Pi* is the proportion of individuals *I* taxon/species in a whole community *Pi*=n/N, ***ln*** is the natural log, *Σ* is the sum of the calculations, and N is the number of taxa (Okuda et al., 2024).

The Shannon divers, colonization, and isolation rates were measured in triplicate, and the results were shown as mean ± standard deviation (SD). These data were analyzed using a statistical package (SAS, 2002) to test the significance ($p < 0.05$) of the studied fixed effect; Location (study sites) on the examined results using the following model: $Y_{jk} = \mu + L_j + e_{jk,}$ where: Y_{jk} = the experimental observations (Shannon divers, colonization rate, and isolation rate) measured by j^{th} locations, μ = the overall mean, L_j = the fixed effect of j^{th} locations where j = 1 to 11, and the random error (ejk) which is normally and independently distributed (NID), with a variance of σe^2 and a mean of 0.

RESULTS

Occurrence of Endophytic Fungi

450 culturable endophytic fungal isolates were retrieved from pneumatophores (roots) and leaves segments of *Avicennia marina* collected from eleven study sites; 274 of them (i.e. 60.9%) were recovered from roots, while 176 of them (i.e. 39.1%) were recovered from leaves. To assess the fungal endophyte occurrence, colonization, and isolation rates were computed (Table. 1). In the entire plant, the colonization rate of endophytic fungi ranged from 22.66 ± 0.47% to 70.79 ± 0.51% and the isolation rate ranged from 28.40 ± 0.33 % to 88.26 ± 0.20% among the eleven study sites. Safaga mangroves had the highest colonization and isolation rates, whereas Al Quseir mangroves had the lowest. There was a statistically significant difference (P<0.001) in the total colonization rates of endophytic fungi among the eleven locations. Similarly, the statistical analysis demonstrated that study locations had a substantial impact on the isolation rate of endophytic fungi (P< 0.001). Furthermore, in every research site, the rate of endophytic fungal colonization in pneumatophores was much greater than that in leaves.

Biodiversity and Composition of Endophytic Fungal Community

Based on their morphological features, 354 (79%) of the 450 endophytic fungal isolates that were found were identified at least to the genus level. The remaining isolates made up 21% of the total and included 6.8% black yeasts (isolates with mucoid to pasty colonies with dark olive-brown to black shades and producing yeast-like bud cells) and 14.4% mycelia sterilia (cultures growing as dark sterile mycelia and failing to sporulate under the previously described study conditions). These findings are shown in Supplementary Table S2.

The Shannon-Wiener diversity index was used to compare isolates from each of the eleven research locations to describe the diversity of the endophyte community (Table 1 and Figure 2). The Shannon-Wiener diversity index varied between the culturable mycoendophytes of the eleven sites, which ranged from 1.50 ± 0.02 to 2.19 ± 0.10, indicating significant variation ($P < 0.001$) in endophytic fungal biodiversity from the different study sites. The highest diversity indices were recorded in Marsa Alam, Qalaan, and Safaga, which accounted for 2.19 ± 0.10, 2.14 ± 0.07, and 2.08 ± 0.01, respectively. Whereas the lowest index was reported for the Sharm Bahry mangrove.

As per the findings presented in Figures 3 and Supplementary Table S.2, the 354 isolates that were identified morphologically belong to three taxonomic classes: Sordariomycetes, Eurotiomycetes, and Dothideomycetes. Their respective relative isolation frequencies (RF) are 25.1%, 12.7%, and 39.6%. These isolates represent thirteen Ascomycota genera. In addition, only one genus (RF = 1.33%) belonged to the class Agaricomycetes (phylum Basidiomycota). *Chaetomium* (RF = 33.8%), *Aspergillus* (RF = 17.3%), and *Penicillium* (RF = 7.6 %) were the most abundant mycoendophytic genera. The least frequent genera were *Nigrospora*, *Philophora*, and *Aureobasidium*, with only one isolate each representing 0.2% of the total.

The distribution of the isolated fungal endophytes is displayed in Figure 4, along with a comparison of their relative abundance throughout the eleven research sites. Of all eleven sites, *Chaetomium* sp. was the most frequently isolated culturable endophytic genus from roots (RF 40-70%), while poorly detected in the leaf parts (RF < 10%). In contrast, black yeasts were highly represented in leaves, especially in Nabq and Ras-Mohammed sites (RF > 60%), whereas poorly reported in roots. At the Sharm-Bahry site, the frequency of both Mycelia sterilia and *Aspergillus terreus* (RF > 50% and RF > 20%, respectively) was the second highest, after *Chaetomium* sp. The remaining endophytic genera were *Penicillium, Cladosporium*, *Stachybotrys*, *Alternaria*, *Fusarium*, *Curvularia*, *Ulocladium*, *Epicoccum*, *Rhizoctonia*, *Nigrospora*,

Table 1. Frequency of occurrence and diversity index of culturable fungal endophytes isolated from leaves and pneumatophores (roots) of *Avicennia marina* collected from the eleven study sites.

Study site	No. of +ve Segments[⍰]					No. of Fungal isolates					Occurrence and diversity in a given location		
	Root		Leaf		Total count*	Root		Leaf		Total count*			
	Segment count*	CR (%)*	Segment count*	CR (%)*		Isolate count*	IR (%)*	Isolate count	IR (%)*		Colonization rate (%)**	Isolation rate (%)**	Shannon diversity index**
Nabq	15	50.0	5	16.6	20	15	50.0	9	30.0	24	32.60±0.53^{j}	40.55±0.64^{f}	1.84±0.01^{cb}
Ras Muhammed	16	53.3	10	33.3	26	16	53.3	16	53.3	32	43.40±0.33^{g}	53.62 ± 0.54^{e}	1.73±0.01^{c}
Abu- Shaara	22	73.3	10	33.3	32	22	73.3	14	46.6	36	52.23±0.84^{f}	60.71 ± 0.42^{d}	1.94±0.01^{b}
Abu-Minqar	23	76.6	11	36.6	34	23	76.6	13	43.3	36	56.06±0.33^{e}	61.18 ± 0.61^{d}	1.82±0.05^{cb}
Safaga	28	93.3	15	50.0	43	28	93.3	20	66.6	48	70.79±0.51^{a}	88.26 ± 0.20^{a}	2.08±0.01^{a}
Al Quseir	9	30.0	5	16.6	14	9	30.0	8	26.6	17	22.66±0.47^{k}	28.40 ± 0.33^{g}	1.82±0.04^{cb}
Sharm Bahry	17	56.6	7	23.3	24	17	56.6	8	26.6	25	40.32±0.45^{i}	41.50 ± 0.34^{f}	1.50±0.02^{d}
Sharm Qebly	23	76.6	5	30.0	28	23	76.6	9	30.0	32	45.84±0.48^{h}	53.59 ± 0.50^{e}	1.73±0.04^{c}
Marsa Alam	27	90.0	11	36.6	40	27	90.0	16	53.3	43	66.22±0.25^{b}	74.59 ± 0.36^{b}	2.19±0.10^{a}
Hamata	24	80.0	10	33.3	36	24	80.0	15	50.0	39	60.36±0.47^{d}	65.68 ± 0.59^{c}	1.90±0.03^{b}
Qulaan	26	86.6	12	63.3	38	26	86.66%	19	63.33%	45	63.65± 0.57^{c}	75.48 ± 0.26^{b}	2.14± 0.07^{a}
P-value											<0.001	<0.001	<0.001

[⍰]Number of segments colonized by endophytic fungi, the total number of cultured segments was 60 segments per study site (30 from root segments and 30 from leaf), * data were expressed as mean, ** data were expressed as least square means ± SE, values without shared superscripts (a, b, c, cb, d, e, f, g, h, i, j, and k) are significantly different at $P < 0.05$, CR: Colonization rate (%), IR: isolation rate (%). The total count of isolates obtained from pneumatophores (roots) and leaves collected from the eleven study sites were 274 and 176, respectively.

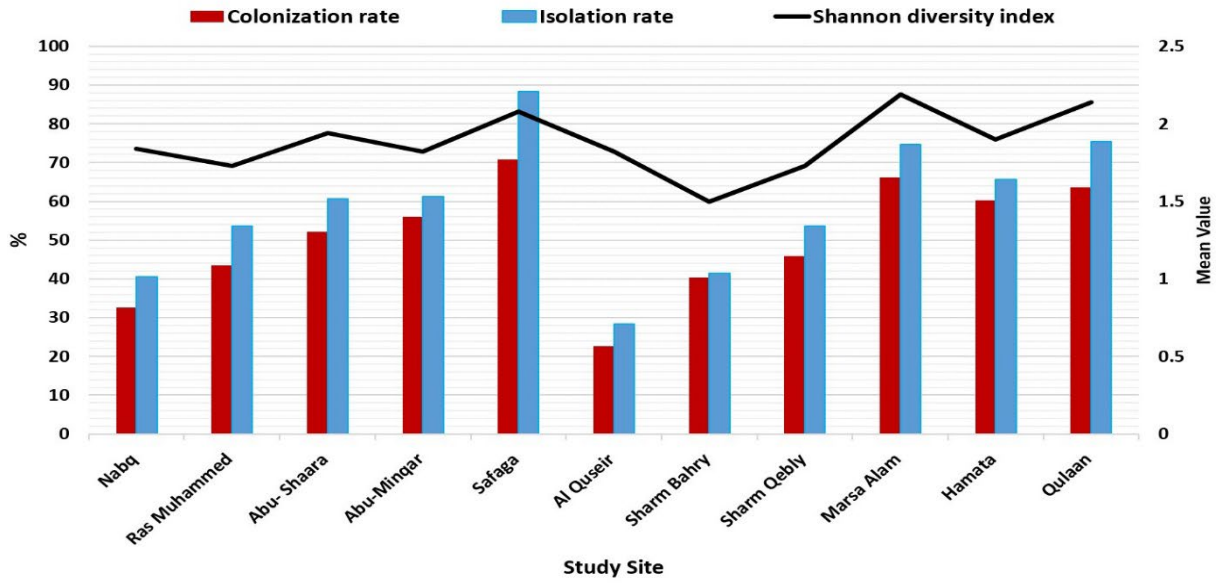

Figure 2. Shannon diversity index and rate of isolation and colonization of fungal endophytes isolated from *A. marina* along the eleven study sites.

Phialophora, and *Aureobasidium,* with *Penicillium* sp. being the most represented, especially in the leaves collected from Al- Qusayr site (RF > 50%). The rest had low relative frequencies ranging from less than 10% to 20%.

DISCUSSION

Many endophytic bacteria and fungi inhabit the mangrove ecosystem, creating a rich hub of biodiversity for these endophytes. Mangrove trees' leaves, stems, roots, and bark are all colonized by endophytes. The distinctive ecological characteristics, variety, and abundance of unique secondary metabolites produced by mangrove-associated endophytic fungi have garnered significant interest. These mycoendophytes are highly bioactive, which enables them to produce numerous bioactive chemicals with a variety of biotechnological and medicinal uses (Cadamuro et al., 2021). Given the ecological relevance of mangroves and the function that their associated fungal endophytes perform, this study examined the biodiversity of the endophytic fungal community in the leaves and pneumatophores of the grey mangrove *Avicenna marina* collected from eleven stands along the Egyptian Red Sea coast.

450 mycoendophytic isolates were obtained from pneumatophore (root) and leaf segments of collected

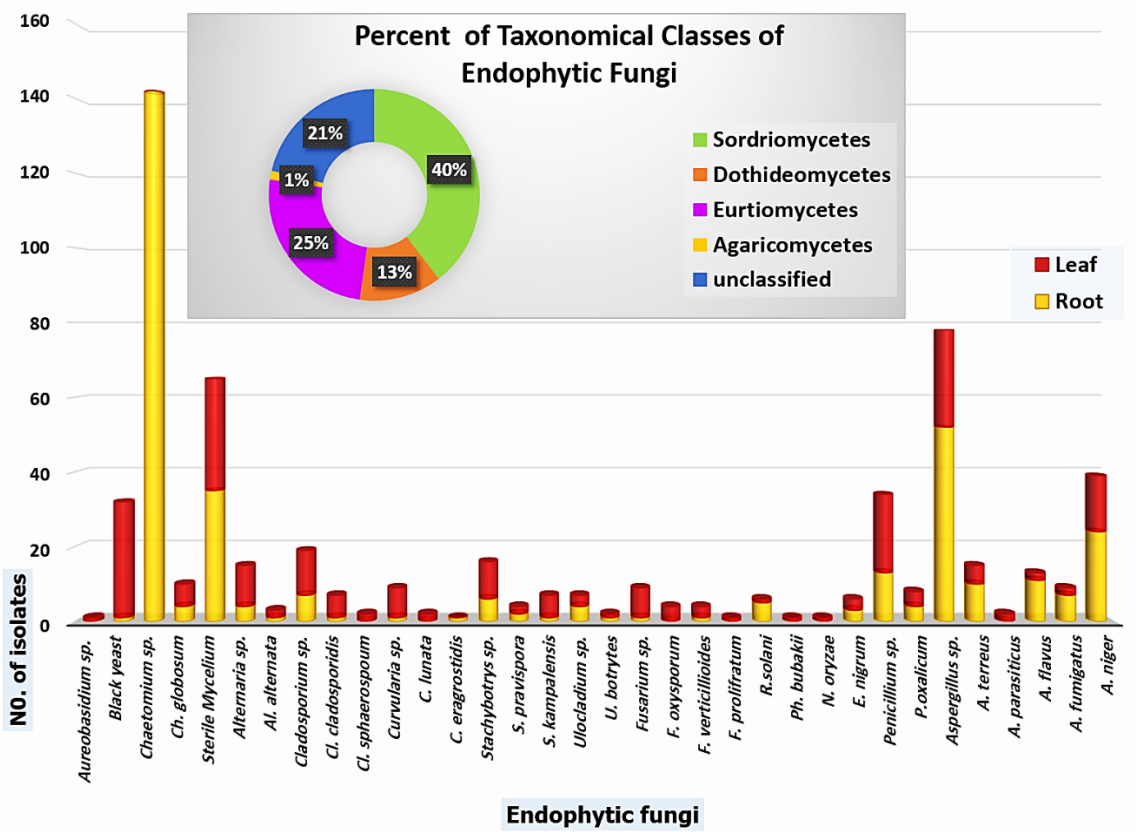

Figure 3. Taxonomic distribution of different fungal endophytes isolated from leaves and pneumatophores (roots)of *A. marina* collected from the eleven sites during the study period.

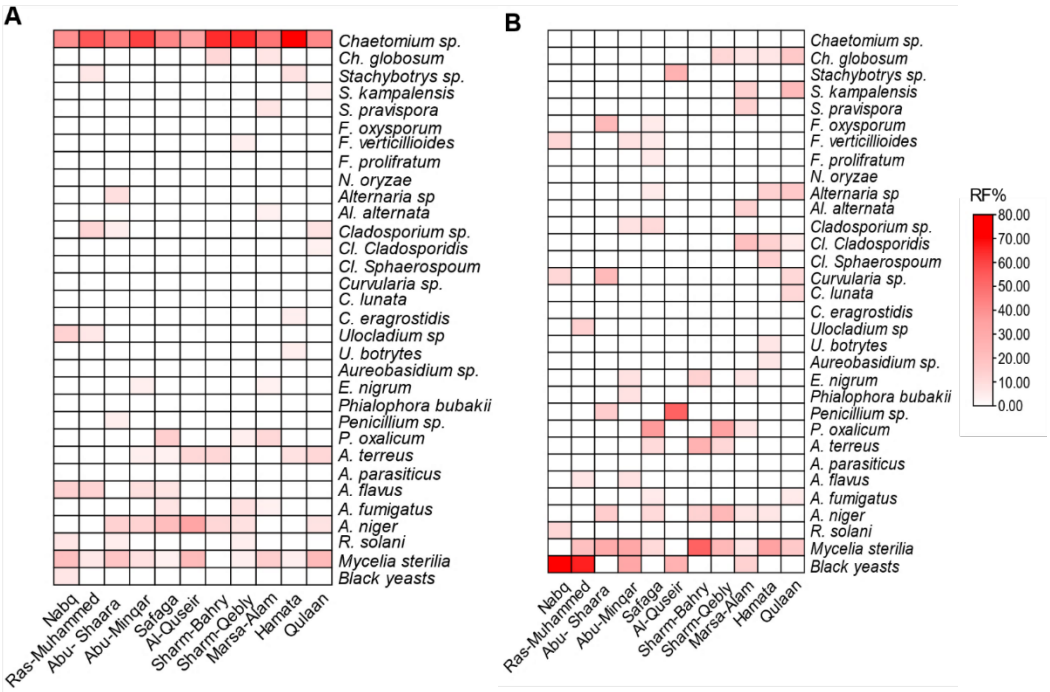

Figure 4. Heatmap showing the relative abundance of the endophytic fungal community in *A. marina* root (panel A) and leaf (panel B) along the eleven studied sites. The columns represent the clustering between the studied sites and the endophytic fungal species according to their composition similarity in the columns. Each species corresponds to a colored line indicating its relative frequency (RF%). Heatmap analyses and visualization were carried out by TBtools v2.09 (Chenc et al. 2023).

A. marina from the eleven studied sites, 60.9% were recovered from collected mangrove pneumatophores and 39.1% from the leaves, with significantly higher colonization and isolation rates in pneumatophores than in leaves at all study sites. In consistency with this finding, Xing et al. (2011) isolated endophytic fungi from five different mangrove species on the south coast of China. The recorded colonization rate ranged from 12.5 to 41.7% in roots, and from 12.5 to 25.1% in leaves. A similar tissue preference was reported by Vinodkumar et al. (2023) who studied the endophytic fungi associated with pneumatophores, twigs, and leaves of *A. marina* collected from Godrej mangrove forest in Mumbai- Maharashtra, western India. The authors reported that the maximum colonization rate of fungal endophytes was recorded from pneumatophores (85%) followed by leaves (77%). This difference in colonization between above- and below-ground parts might be attributed to differences in biotic and abiotic aspects. Humidity, for example, is much lower in the air than in the soil, which might result in a lower colonization rate in leaves than in roots, as endophyte colonization is positively correlated with humidity. Likewise, the comparatively damp and rich-in-nutrient soil substrate improves the capacity of fungal propagules to infiltrate plant roots (Herrera et al. 2010; Massimo et al. 2015).

The eleven research locations' variations in the occurrence of the isolated fungal endophytes throughout the plant were assessed using estimated colonization and isolation rates, which varied from around 22 to 71 for colonization and 28 to 88 for isolation rate. Statistical analysis revealed that the isolation rate of endophytic fungi was significantly different across the eleven collection sites. Likewise, the studied sites significantly influenced endophytic fungal colonization rates ($P< 0.001$). Colonization and isolation rates were highest for mangroves in South Safaga and lowest for mangroves in Al-Quseir. The high levels of pollution recorded in this region of the Red Sea shoreline might be the cause of the heightened rates of colonization and isolation in South Safaga (Abd-ElHadi, 2008). This result is in line with the findings of a study conducted by Kiti et al. (2021) in Gazi Bay, Tudor, and Mida Creeks on the coast of Kenya, who recorded a significant variation in the colonization rates of endophytic fungi associated with mangroves, ranging from 38.9 to 98%. The authors discovered that Tudor Creek had the highest fungal community whereas Gazi Bay had the lowest. They explained this by pointing out that Tudor Creek is situated in an area that is highly contaminated with significant levels of pollution coming from both industrial and residential sources. According to Thatoi et al. (2013), fungal endophytes in plant tissues aid in the host's adaptation to biotic and abiotic stressors. As a result, fungal endophytes are frequently found in higher frequencies in the tissues of plants that grow in polluted environments.

The Shannon diversity index varied among the endophytic fungal communities at the eleven study sites, ranging from 1.50±0.02 to 2.19±0.10. Based on statistical analysis, the endophytic fungal biodiversity varied significantly ($P<0.001$) among all locations, suggesting a relatively high level of diversity among isolates obtained from different stands. A comparable range of diversity index values (1.2±0.29 to 4±0.3) was reported in a previous study on fungal endophytes associated with halophytic plants conducted by Li et al. (2020). In the Gurbantonggut desert, which borders a subtropical Chinese Sea coast, the authors examined the diversity of endophyte communities in ten halophytic species, which are adapted to multiple stresses, including low water availability, high salinity, and nutrient deficiency—conditions that are like those in which Egyptian mangroves grow. As a result, these ecosystems provide distinct environments for a wide variety of endophytes impacted by these harsh settings.

354 isolates, i.e. 79% of the total, were identified, at least to the genus level, as sporulating mold fungi based on their morphological features. Sixty-five of the isolated (14.4%) were dark, sterile mycelia, or "mycelia sterilia". This morphotype lacks reproductive structures such as spores or fruiting bodies, which limits the ability to classify them (Naik, 2009). Mycelia sterilia isolates recovered from roots (35) were more than those from (30). In line with these findings, previous studies revealed that they can extensively colonize internal tissues, especially roots, of woody plants including *A. marina*, forming a symbiotic relationship with the plant (Shebany, 2012; Bonito et al., 2014).

Numerous advantages of Mycelia Sterilia for plants have been documented. In nutrient-poor environments, where *A. marina* frequently thrives, they improve nutrient absorption, which can be very beneficial. Furthermore, they aid overall growth, help plants resist environmental challenges such as salinity and drought, and contribute to defense against pathogens through their ability to produce bioactive metabolites with antimicrobial properties (Hyde and

Soytong, 2008). A prominent finding in earlier research on fungal endophytes is the occurrence of Mycelia sterilia, which has been observed at varying frequencies among endophytes associated with mangroves. About 37% of endophytes isolated from three mangrove species in northeastern Brazil were mycelia sterilia (Costa et al., 2012). Similarly, on the East Coast of Demerara, Guyana, approximately 23% of the obtained mangrove-associated-endophytes were unclassified mycelia sterilia (Craig and Ansari, 2021).

The 354 isolates that have been identified fall into two main Dikaryotic phyla: Ascomycota, which makes up 98.3% of the group, and Basidiomycota, which makes up 1.7%. Several earlier studies confirmed that Ascomycota predominated in the mangrove-associated endophytic fungal populations in India's Godrej Mangrove Forest (Vinodkumar et al., 2023), in Santa Catarina Island, Brazil (da Silveira Bastos et al., 2024), and Tanzania (Myovela et al., 2024). Ascomycota members have been shown to perform important ecological roles in maritime environments, including the mangrove ecosystem. Ascomycetes are a group of saprophytic and symbiotic fungi that are crucial to the environment because they help with nutrient recycling and usage. In marine habitats, these fungi are frequently found on various substrates that are high in chitin, cellulose, or lignin. Because of their capacity to break down substrates, these complex compounds can enter the food chain (Liu et al., 2015; Velez et al., 2015; Muwawa et al., 2024).

Among the Ascomycetes, genera belonging to three classes: Sordariomycetes, Eurotiomycetes, and Dothideomycetes were dominant with relative isolation frequencies of 39.6%, 25.1%, and 12.7%, respectively, of the total isolates retrieved throughout the study. Studies on the diversity of endophytes associated with coastal mangroves in Brazil by de Souza Sebastianes et al. (2013), and da Silveira Bastos et al. (2024), and that conducted by Myovela et al. (2024) in Tanzania, observed similar trends in their findings regarding the predominance of these classes of Ascomycota among endophytic fungal community. Furthermore, substantial proportions of these often-reported classes have been discovered in sea sediments, indicating their ubiquitous presence in marine environments (Zhang et al., 2015; Li et al., 2016; da Silva et al., 2024).

The class Agaricomycetes (phylum: Basidiomycota) was the least recorded in this study. This result aligns with da Silveira Bastos et al. (2024), who reported a similarly low proportion of this class in their work regarding mangrove endophytes in Brazil. The low frequency of basidiomycetes found in this study and numerous other investigations about fungal endophytes, including mangrove-associated endophytes, could be attributed to the employment of traditional culture-based techniques, which present difficulties because most ascomycetes grow more quickly than basidiomycetes, and many of them lack useful morphological traits. This might lead to a decrease in the number of basidiomycetes that are reported or classified as mycelia sterilia or unclassified (Wang et al., 2005; Arnold et al., 2007; Martin et al., 2015).

Chaetomium, Aspergillus, and *Penicillium* were the dominant identified genera. *Chaetomium* sp. which belongs to the class Sordariomycetes, was the most prevalent with a count of 152 and a total relative frequency of colonization of 33.9%. The genus *Chaetomium* has been repeatedly reported as an endophyte associated with several plant species including *A. marina* and is a source of bioactive secondary metabolites with many pharmaceutical applications. (Shebany, 2012; Kaur et al., 2020; Khalil et al., 2020; Rao et al., 2023). Isolates of this genus were found to predominantly colonize pneumatophores (roots) with a higher frequency of occurrence, reaching 52.5% of all root isolates, compared to the leaves (only 3.4% out of all leaf isolates). The genus *Chaetomium* has frequently been described as an endophyte associated with roots, but the exact causes of this preference for root colonization are unknown. Potential influences include tissue phytochemistry, surface characteristics, and the existence or absence of competing microflora (Khan et al., 2012; Haruma et al., 2018). Such root-specific association suggests a potentially specialized symbiosis of *Chaetomium sp.* with the root tissues, which may participate in nutrient cycling, promoting plant growth, or even providing protection against biotic and abiotic stressors. (Al Husnain et al., 2023).

The second most frequent genus was *Aspergillus* (RF = 17.3%), followed by *Penicillium* (RF = 7.6%). Endophytic species of *Aspergillus* and *Penicillium* have been commonly isolated from mangroves and revealed as a significant source of several bioactive metabolites. (Basheer et al., 2018; Bibi et al., 2021; Trivedi and Thumar, 2023; Mwawa et al., 2024). According to earlier research, the number of these two endophytic species in plant tissues may be linked to improved development under adverse environmental circumstances, pollution, and other

stressors as well as increased resistance to diseases (Ismail et al., 2018; Attia et al., 2022; Akram et al., 2023; Gowtham et al., 2024). Moreover, other genera including *Cladosporium*, *Stachybotrys*, *Alternaria*, *Fusarium*, *Curvularia*, *Ulocladium*, *Epicoccum*, and *Rhizoctonia* were recovered with lower relative frequencies, ranging between 4.2% and 1.3% of the total recorded isolates. Members of these genera are also common among fungal endophyte communities in different mangrove species (Li et al., 2016; Hamzah et al., 2018; Trivedi and Thumar, 2023).

Although there is a shortage of information on endophytic fungi connected to mangroves, a comparable endophytic variety of fungal species was previously documented in Egypt, which is consistent with the current results. Shebany (2012) isolated each of *Aspergillus sp., Chaetomium sp., Cladosporium sp. Fusarium sp.* and *Ulocladium sp.* from *A. marina* in the Red Sea coast of Egypt. While *Aspergillus aculeatus, A. niger, A. terreus,* and *Eurotium amstelodami*, were the most dominant endophytes recovered from South Safaga and Abu Hamrah mangrove stands (Basheer et al., 2018). Khalil et al. (2021) successfully isolated *Chaetomium sp., C. globosum, C. madrasense, Aspergillus hiratsukae, Alternaria tenuissima,* and *Curvularia lunata* from leaves of *A. marina* growing in a semi-arid environment in Egypt.

Environmental parameters including salinity, pH, soil texture, nutrient levels, and pollution may cause variations in colonization rate and biodiversity in research locations. Previous research has shown that, depending on environmental conditions, plant-associated microbial communities can be impacted by geographic variances in study locations (Sanka Loganathachetti et al., 2017; Muwawa et al., 2024). In addition, the high occurrence and diversity of fungi in pneumatophores compared to leaves may be due to direct penetration of pneumatophores by soil fungi, as soil, especially the rhizosphere, is a rich core of microbial biodiversity.

Finally, the overall findings of the current study add to the growing body of information and documentation regarding the fungal resources associated with Egypt's mangrove nature reserves. Given the impacts of global climate change on biological resources, the present study facilitates monitoring of the mangrove ecosystem along the coast of the Red Sea. Nevertheless, there is still a knowledge gap concerning the relationship between mycoendophytes associated with mangrove plants and their host. Further research is still required to thoroughly understand this relationship, especially their ability to produce bioactive metabolites as unique as those produced by the host.

CONCLUSION

The present work aimed to thoroughly investigate the culturable endophytic fungal communities associated with *Avicennia marina* in Egypt. The study covered eleven gray mangrove stands along the Red Sea coastline, to provide in-depth insights concerning these communities' occurrence, biodiversity, and composition. Across all research locations, there was a notable diversity in the colonization rate of fungal endophytes, with a greater rate in pneumatophores than in leaves. Ascomycetous fungi were dominant, with *Chaetomium, Aspergillus,* and *Penicillium* being the most abundant genera. Most of the recovered genera occurred in both plant parts. However, some showed certain tissue preferences. It is worth noting that this work also provides preliminary data on mangrove mycoendophytes for a subsequent prospective study investigating their bioactive natural products (Unpublished data).

REFERENCES

Abd-ElHadi, M. Z. (2008). Evaluation of pollution rate by petroleum oil and its effect on mangrove communities along the red seacoast, Egypt. Journal of Soil Sciences and Agricultural Engineering, 33(1), 793-803.

Akram, S., Ahmed, A., He, P., He, P., Liu, Y., Wu, Y., ... & He, Y. (2023). Uniting the role of endophytic fungi against plant pathogens and their interaction. *Journal of Fungi*, *9*(1), 72.

Al Husnain, L., Alajlan, L., AlKahtani, M. D., & Ameen, F. (2023). *Avicennia marina* endophytic fungi show antagonism against tomato pathogenic fungi. Journal of the Saudi Society of Agricultural Sciences, 22(4), 214-222.

Allard, S. M., Costa, M. T., Bulseco, A. N., Helfer, V., Wilkins, L. G. E., Hassenrück, C., Zengler, K., Zimmer, M., Erazo, N., Mazza Rodrigues, J. L., Duke, N., Melo, V. M. M., Vanwonterghem, I., Junca, H., Makonde, H. M., Jiménez, D. J., Tavares, T. C. L., Fusi, M., Daffonchio, D., Duarte, C. M., Peixoto, R. S., Rosado, A. S., Gilbert, J. A., & Bowman, J. (2020). Introducing the Mangrove Microbiome Initiative: Identifying Microbial Research Priorities and Approaches to Better Understand, Protect, and Rehabilitate Mangrove Ecosystems. mSystems, 5. https://doi.org/10.1128/msystems.00658-20

Alongi, D. M. (2002). Present state and future of the world's mangrove forests. Environmental Conservation, 29(3), 331–349. doi:10.1017/S0376892902000231

Arnold, A. E., Henk, D. A., Eells, R. L., Lutzoni, F., & Vilgalys, R. (2007). Diversity and phylogenetic affinities of foliar fungal endophytes in loblolly pine inferred by culturing and environmental PCR. *Mycologia*, *99*(2), 185-206.

Attia, M. S., Salem, M. S., & Abdelaziz, A. M. (2022). Endophytic fungi *Aspergillus* spp. reduce fusarial wilt disease severity, enhance growth, metabolism and stimulate the plant defense system in pepper plants. *Biomass Conversion and Biorefinery*, 1-11

Ayyam, V., Palanivel, S., Chandrakasan, S., Ayyam, V., Palanivel, S., & Chandrakasan, S. (2019). Coastal ecosystems and services. *Coastal ecosystems of the Tropics-Adaptive management*, 21-47.

Barnett, H. L., & Hunter, B. B. (1998). Illustrated Genera of Imperfect Fungi. APS Press.

Basheer, M. A., Mekawey, A. A., El-Kafrawy, S. B., & Abouzeid, M. A. (2018). Antimicrobial activities of endophytic fungi of Red Sea aquatic plant *Avicennia marina*. *Egyptian Journal of Microbiology*, *53*(1), 231-240.

Bhimba, B. V., Franco, D. A. D., Mathew, J. M., Jose, G. M., Joel, E. L., & Thangaraj, M. (2012). Anticancer and antimicrobial activity of mangrove derived fungi *Hypocrea lixii* VB1. *Chinese journal of natural medicines*, *10*(1), 77-80.

Bibi, F., Faheem, M., Azhar, E. I., Yasir, M., Alvi, S. A., & Kamal, M. A. (2021). Diversity and bioactive potential of endophytic fungi associated with the mangrove plant *Rhizophora mucronata*. Frontiers in Microbiology, 12, 732731.

Bibi, S. N., Gokhan, Z., Rajesh, J., & Mahomoodally, M. F. (2020). Fungal endophytes associated with mangroves–Chemistry and biopharmaceutical potential. South African Journal of Botany, 134, 187-212

Bonito, G., Reynolds, H., Robeson, M. S., Nelson, J., Hodkinson, B. P., Tuskan, G., ... & Vilgalys, R. (2014). Plant host and soil origin influence fungal and bacterial assemblages in the roots of woody plants. Molecular ecology, 23(13), 3356-3370.

Bramhachari, P.V., Anju, S., Sheela, G.M., Komaraiah, T.R., Venkataiah, P., & Prathyusha, A.M.V.N. (2019). Correction to: Secondary Metabolites from Marine Endophytic Fungi: Emphasis on Recent Advances in Natural Product Research. In: Singh, B. (eds) *Advances in Endophytic Fungal Research. Fungal Biology. Present Status and Future Challenges,* Springer, Cham. https://doi.org/10.1007/978-3-030-03589-1_16

Cabahug, D. M. (2002). Rehabilitation, conservation and sustainable utilization of mangroves in Egypt. Community-based mangrove rehabilitation and ecotourism development and management in the Red Sea Coast, Egypt. FAO Final report.

Cadamuro, R.D., da Silveira Bastos, I.M.A., Silva, I.T., da Cruz, A.C.C., Robl, D., Sandjo, L.P., Alves Jr, S., Lorenzo, J.M., Rodríguez-Lázaro, D., Treichel, H. and Steindel, M., & Fongaro, G. (2021). Bioactive compounds from mangrove endophytic fungus and their uses for microorganism control. Journal of fungi, (Basel, Switzerland), 7(6), 455. https://doi.org/10.3390/jof7060455

Chen C., Wu Y., Li J., Wang X., Zeng Z., Xu J., Liu Y., Feng J., Chen H., He Y., and Xia R. (2023). TBtools-II: A "one for all, all for one" bioinformatics platform for biological big-data mining. Mol. Plant. 16, 1733–1742.

Costa, I. P., Maia, L. C., & Cavalcanti, M. A. (2012). Diversity of leaf endophytic fungi in mangrove plants of northeast Brazil. *Brazilian Journal of Microbiology*, *43*, 1165-1173.

Craig, K., & Ansari, A. (2021). A preliminary investigation of endophytic fungal diversity at Hope, East Coast Demerara, Guyana. *GSC Biological and Pharmaceutical Sciences*, *17*(2), 044-048.

da Silva, M. K., Barreto, D. L. C., Vieira, R., Neto, A. A., de Oliveira, F. S., Convey, P., ... & Rosa, L. H. (2024). Diversity and enzymatic, biosurfactant and phytotoxic activities of culturable Ascomycota fungi are present in marine sediments obtained near the South Shetland Islands, maritime Antarctica. Extremophiles, 28(2), 20.

da Silveira Bastos, I. M. A., Cadamuro, R. D., de Freitas, A. C. O., da Silva, I. T., Stoco, P. H., Sandjo, L. P., ... & Steindel, M. (2024). Diversity of fungal endophytes from mangrove plants of Santa Catarina Island, Brazil. Brazilian Journal of Microbiology, 1-11.

de Souza Sebastianes, F. L., Romão-Dumaresq, A. S., Lacava, P. T., Harakava, R., Azevedo, J. L., De Melo, I. S., & Pizzirani-Kleiner, A. A. (2013). Species diversity of culturable endophytic fungi from Brazilian mangrove forests. Current genetics, 59, 153-166.

Debbab, A., Aly, A. H., & Proksch, P. (2013). Mangrove-derived fungal endophytes–a chemical and biological perception. *Fungal diversity*, *61*(1), 1-27.

Domsch, K. H., Gams, W., & Anderson, T. (2007). Compendium of soil fungi (2nd ed.). IHW-Verlg Eching-Germany.

Eldeen, I. M., & Effendy, M. A. (2013). Antimicrobial agents from mangrove plants and their endophytes. *Microbial pathogens and strategies for combating them: science, technology and education*, *2*, 872-882.

Ellis MB (1971) Dematiaceous hyphomycetes. Kew, UK: Commonwealth Mycological Institute, 608 pp. https://doi.org/10.1079/9780851986180.0000

Ghizelini, A. M., Mendonça-Hagler, L. C. S., & Macrae, A. (2012). Microbial diversity in Brazilian mangrove sediments: a mini review. *Brazilian Journal of Microbiology*, *43*, 1242-1254.

Giri, C., Ochieng, E., Tieszen, L. L., Zhu, Z., Singh, A., Loveland, T., Masek, J., & Duke, N. (2011). Status and distribution of mangrove forests of the world using earth observation satellite data (version 1.4, updated by UNEP-WCMC). Global Ecology and Biogeography, 20(1), 154-159.

Gowtham, H. G., Hema, P., Murali, M., Shilpa, N., Nataraj, K., Basavaraj, G. L., ... & Amruthesh, K. N. (2024). Fungal Endophytes as Mitigators against Biotic and Abiotic Stresses in Crop Plants. *Journal of Fungi*, *10*(2), 116.

Guo, L. D., Hyde, K. D., & Liew, E. C. Y. (2000). Identification of endophytic fungi from *Livistona chinensis* based on

morphology and rDNA sequences. *The New Phytologist*, *147*(3), 617-630.

Hamzah, T. N. T., Lee, S. Y., Hidayat, A., Terhem, R., Faridah-Hanum, I., & Mohamed, R. (2018). Diversity and characterization of endophytic fungi isolated from the tropical mangrove species, *Rhizophora mucronata*, and identification of potential antagonists against the soil-borne fungus, *Fusarium solani*. Frontiers in microbiology, 9, 1707

Haruma, T., Yamaji, K., Masuya, H., & Hanyu, K. (2018). Root endophytic *Chaetomium cupreum* promotes plant growth and detoxifies aluminum in Miscanthus sinensis A ndersson growing at the acidic mine site. Plant species biology, 33(2), 109-122.

Heip, C. H., Herman, P. M., & Soetaert, K. (1998). Indices of diversity and evenness. *Oceanis*, *24*(4), 61-88.

Herrera, J., Khidir, H. H., Eudy, D. M., Porras-Alfaro, A., Natvig, D. O., & Sinsabaugh, R. L. (2010). Shifting fungal endophyte communities colonize Bouteloua gracilis: effect of host tissue and geographical distribution. *Mycologia*, *102*(5), 1012-1026.

Hyde, K. D., & Soytong, K. (2008). The fungal endophyte dilemma. *Fungal divers*, *33*(163), e173.

Ismail, Hamayun, M., Hussain, A., Iqbal, A., Khan, S. A., & Lee, I. J. (2018). Endophytic fungus *Aspergillus japonicus* mediates host plant growth under normal and heat stress conditions. *BioMed Research International*, *2018*(1), 7696831.

Kaur, N., Arora, D. S., Kalia, N., & Kaur, M. (2020). Bioactive potential of endophytic fungus *Chaetomium globosum* and GC–MS analysis of its responsible components. Scientific Reports, 10(1), 18792.

Khalil, A. M. A., Abdelaziz, A. M., Khaleil, M. M., & Hashem, A. H. (2021). Fungal endophytes from leaves of *Avicennia marina* growing in semi-arid environment as a promising source for bioactive compounds. Letters in Applied Microbiology, 72(3), 263-274

Khalil, M. S., Abdel-Lateef, A. A., El-Beih, A. A., & Ismail, N. S. (2020). Diversity and bioprospection of endophytic fungi associated with the mangrove plant *Avicennia marina* in the Red Sea coast of Egypt. Journal of Fungi, 6(2), 91.

Khan, A. L., Shinwari, Z. K., Kim, Y. H., Waqas, M., Hamayun, M., Kamran, M., & Lee, I. J. (2012). Role of endophyte *Chaetomium globosum* LK4 in growth of *Capsicum annuum* by production of gibberellins and indole acetic acid. Pak. J. Bot, 44(5), 1601-1607

Kiti, H. M., Munga, C. N., Odalo, J. O., Guyo, P. M., & Kibiti, C. M. (2021). Diversity of mangrove fungal endophytes from selected mangrove species of coastal Kenya. *Western Indian Ocean Journal of Marine Science*, *20*(1), 125-136.

Kjer, J., Debbab, A., Aly, A. H., & Proksch, P. (2010). Methods for isolation of marine-derived endophytic fungi and their bioactive secondary products. *Nature Protocols*, *5*(3), 479-490.

Klich, M. A. (2002). Identification of common Aspergillus species. Centraalbureau voor schimmelcultures.

Leslie, J.F. and Summerell, B.A. (2006) The Fusarium Laboratory Manual. Blackwell Publishing, Hoboken,1-2. https://doi.org/10.1002/9780470278376

Li, J. L., Sun, X., Chen, L., & Guo, L. D. (2016). Community structure of endophytic fungi of four mangrove species in Southern China. Mycology, 7(4), 180-190.

Li, J. L., Sun, X., Zheng, Y., Lü, P. P., Wang, Y. L., & Guo, L. D. (2020). Diversity and community of culturable endophytic fungi from stems and roots of desert halophytes in northwest China. *MycoKeys*, *62*, 75.

Li, W., Wang, M. M., Wang, X. G., Cheng, X. L., Guo, J. J., Bian, X. M., & Cai, L. (2016). Fungal communities in sediments of subtropical Chinese seas as estimated by DNA metabarcoding. Scientific reports, 6(1), 26528.

Lin, X., Hetharua, B., Lin, L., Xu, H., Zheng, T., He, Z., & Tian, Y. (2019). Mangrove sediment microbiome: adaptive microbial assemblages and their routed biogeochemical processes in Yunxiao mangrove national nature reserve, China. *Microbial ecology*, *78*, 57-69.

Liu, S., Ren, B., Chen, J., & Dang, K. (2015). Diversity and ecological functions of fungal communities in the marine sediments of Xiamen Western Harbor. Journal of Oceanography, 71(6), 689-702.

Martin, R., Gazis, R., Skaltsas, D., Chaverri, P., & Hibbett, D. (2015). Unexpected diversity of basidiomycetous endophytes in sapwood and leaves of Hevea. *Mycologia*, *107*(2), 284-297.

Massimo, N. C., Nandi Devan, M. M., Arendt, K. R., Wilch, M. H., Riddle, J. M., Furr, S. H., ... & Arnold, A. E. (2015). Fungal endophytes in aboveground tissues of desert plants: infrequent in culture, but highly diverse and distinctive symbionts. *Microbial ecology*, *70*, 61-76.

Moubasher, A. H. (1993). Soil fungi in Qatar and other Arab countries. The Centre for Scientific and Applied Research, University of Qatar.

Muwawa, E. M., Makonde, H. M., Obieze, C. C., de Oliveira, I. G., Jefwa, J. M., Kahindi, J. H., & Khasa, D. P. (2024). Diversity and assembly patterns of mangrove rhizosphere mycobiome along the Coast of Gazi Bay and Mida Creek in Kenya. Plos one, 19(4), e0298237.

Myovela, H., Hussein, J., & Tibuhwa, D. (2024). The hidden diversity of mangrove endophytic fungi from Tanzania: insights from a preliminary study. Biologia, 79(2), 669-683.

Nagelkerken, I. S. J. M., Blaber, S. J. M., Bouillon, S., Green, P., Haywood, M., Kirton, L. G., ... & Somerfield, P. J. (2008). The habitat function of mangroves for terrestrial and marine fauna: a review. *Aquatic botany*, *89*(2), 155-185.

Naik, B. S. (2009). Taxonomic placement for mycelia sterilia in endophytic fungal research: a molecular approach. Current Science, 97(9), 1276-1277.

Nolan, K. A., & Callahan, J. E. (2006, July). Beachcomber biology: The Shannon-Weiner species diversity index. In Proc. workshop able (Vol. 27, pp. 334-338).

Okuda, N., Karube, Z. I., Sakai, Y., Takeyama, T., Tayasu, I., Yoshimizu, C., & Nagata, T. (2024). Trophic energy flows

increase in more diverse communities of coastal food webs: Testing the vertical diversity hypothesis. Authorea Preprints.

Palanichamy, P., Krishnamoorthy, G., Kannan, S., & Marudhamuthu, M. (2018). Bioactive potential of secondary metabolites derived from medicinal plant endophytes. *Egyptian Journal of Basic and Applied Sciences*, *5*(4), 303-312.

Pitt, J. I., & Hocking, A. D. (2009). *Fungi and food spoilage* (Vol. 519, p. 388). New York: Springer.

Rao, Q. R., Rao, J. B., & Zhao, M. (2023). Chemical diversity and biological activities of specialized metabolites from the genus Chaetomium: 2013–2022. Phytochemistry, 210, 113653.

Sadeer, N. B., Mahomoodally, M. F., Zengin, G., Jeewon, RAlongi., Nazurally, N., Rengasamy, K. R. R., et al. (2019). Ethnopharmacology, phytochemistry, and global distribution of mangroves—A comprehensive review. Marine Drugs, 17, 231.

Sadeer, N. B., Zengin, G., & Mahomoodally, M. F. (2023). Biotechnological applications of mangrove plants and their isolated compounds in medicine-a mechanistic overview. *Critical Reviews in Biotechnology*, *43*(3), 393-414.

Sanka Loganathachetti, D., Poosakkannu, A., & Muthuraman, S. (2017). Fungal community assemblage of different soil compartments in mangrove ecosystem. Scientific reports, 7(1), 8560

Schulz, B., Wanke, U., Draeger, S., & Aust, H. J. (1993). Endophytes from herbaceous plants and shrubs: effectiveness of surface sterilization methods. *Mycological research*, *97*(12), 1447-1450.

Seifert, K. A., & Gams, W. (2011). The genera of Hyphomycetes–2011 update. *Persoonia-Molecular Phylogeny and Evolution of Fungi*, *27*(1), 119-129.

Shebany, Y. M. (2012). Antimicrobial activity of endophytic fungi isolated from *Avicennia marina* plant, Red Sea, Egypt. Egypt J Microbiol, 47(1), 141-152.

Simões, M. F., Antunes, A., Ottoni, C. A., Amini, M. S., Alam, I., Alzubaidy, H., ... & Bajic, V. B. (2015). Soil and rhizosphere associated fungi in gray mangroves (*Avicennia marina*) from the Red Sea—a metagenomic approach. *Genomics, Proteomics and Bioinformatics*, *13*(5), 310-320.

Spalding, M., Blasco, E., & Field, C. (1997). World Mangrove Alias. *The International Society for Mangrove Ecosystems: Okinawa, Japan*.

Su, Y. Y., Qi, Y. L., & Cai, L. (2012). Induction of sporulation in plant pathogenic fungi. *Mycology*, *3*(3), 195-200.

Tchinda, V. T., Toghueo, R. M. K., Mbazoa, C. D., & Boyom, F. F. (2021). Isolation, identification, and diversity of culturable endophytic fungi from the mangrove plant *Sonneratia alba*. Journal of Fungi, 7(3), 200

Tedersoo, L., Sánchez-Ramírez, S., Koljalg, U., Bahram, M., Döring, M., Schigel, D., ... & Abarenkov, K. (2018). High-level classification of the Fungi and a tool for evolutionary ecological analyses. *Fungal diversity*, *90*, 135-159.

Thatoi, H., Behera, B. C., & Mishra, R. R. (2013). Ecological role and biotechnological potential of mangrove fungi: a review. *Mycology*, *4*(1), 54-71

Trivedi, N. S., & Thumar, J. T. (2023). Characterization and antibacterial activity of endophytic fungi isolated from *Avicennia marina*. Vegetos, 36(1), 163-172.

Velez, P., Gonzalez, M. C., Capello-Garcia, S., Rosique-Gil, E., & Hanlin, R. T. (2015). Diversity of marine ascomycetes from the disturbed sandy beaches of Tabasco, Mexico. Journal of the Marine Biological Association of the United Kingdom, 95(5), 897-903.

Vinodkumar, K., Monica, S., Pinky, K., Tulsi, R., & Lal-Sahab, Y. (2023). Endophytic fungi from mangrove plant *Avicennia marina* and their enzyme cellulase activity. *The International Journal of Science, Mathematics and Technology Learning, 30*(2), 117-130.

Visagie, C. M., Houbraken, J., Frisvad, J. C., Hong, S. B., Klaassen, C. H. W., Perrone, G., Seifert, K. A., Varga, J., Yaguchi, T. and Samson, R. A. (2014). Identification and nomenclature of the genus Penicillium. Studies in Mycology 78, 343-371.

Wang, L., Ren, L., Li, C., Gao, C., Liu, X., Wang, M., & Luo, Y. (2019). Effects of endophytic fungi diversity in different coniferous species on the colonization of *Sirex noctilio* (Hymenoptera: Siricidae). *Scientific reports*, *9*(1), 5077.

Wang, Y. U., Guo, L. D., & Hyde, K. D. (2005). Taxonomic placement of sterile morphotypes of endophytic fungi from Pinus tabulaeformis (Pinaceae) in northeast China based on rDNA sequences. *Fungal Diversity*.

Xing, X. K., Chen, J., Xu, M. J., Lin, W. H., & Guo, S. X. (2011). Fungal endophytes associated with Sonneratia (Sonneratiaceae) mangrove plants on the south coast of China. *Forest Pathology*, *41*(4), 334-340.

Zahran, M. A. (1993). Mangroves and shoreline development in Egypt. *Journal of Environmental Sciences*, *5*, 119-140.

Zahran, M.A., Willis, A.J. (2009). The Eastern Desert. In: The Vegetation of Egypt. Plant and Vegetation, vol 2. Springer, Dordrecht, 101-211. https://doi.org/10.1007/978-1-4020-8756-1_4.

Zhang, T., Fei Wang, N., Qin Zhang, Y., Yu Liu, H., & Yan Yu, L. (2015). Diversity and distribution of fungal communities in the marine sediments of Kongsfjorden, Svalbard (High Arctic). Scientific reports, 5(1), 14524.

Zhou, J., Diao, X., Wang, T., Chen, G., Lin, Q., Yang, X., & Xu, J. (2018). Phylogenetic diversity and antioxidant activities of culturable fungal endophytes associated with the mangrove species *Rhizophora stylosa* and *R. mucronata* in the South China Sea. PloS one, 13(6), e0197359.

Zhu, J., Zhang, J., Wang, Y., Zhang, S., & Guo, X. (2009). Antimicrobial activity of mangrove plant *Sonneratia paracaseolaris* Burm. F. Asian Journal of Traditional Medicines, 4(2), 70-72.

Supplementary Files

Table S1. GPS data of the selected collection sites

1	Nabq Nature Reserve	28.278056, 28°16′41″N	34.38, 34°22′48″E
2	Ras Muhammed Nature Reserve	27.722222, 27°43′20″N	34.253889, 34°15′14″E
3	Abu- Shaara Mangrove	27.356662, 27°21'23.9"N	33.685201, 33° 41' 6.7"E
4	Abu-Minqar Island	27.211873, 27°12'42"N	33.874588, 33° 52'28.5"E
5	Safaga Mangrove (17 Km South of Safaga City)	26.616389, 26°36′ 59″N	34.011389, 34° 00′ 41″E
6	Al Quseir Mangrove (40 km south of the Al Quseir city)	26.398320, 26°23'53.9"N	34.112018, 34° 6' 43"E
7	Sharm Bahry Mangrove	25.867240, 25°52'2"N	34.413879, 34° 24'49.9"E
8	Sharm Qebly Mangrove	25.834979, 25°50'5.9"N	34.440355, 34° 26'25"E
9	Marsa Alam Mangrove (at the mouth of Wadi El Gemal)	24.381170, 24°22'52"N	35.262199, 35° 15'43.9"E
10	Hamata Mangrove	24.345219, 24°20'42.7"N	35.322981, 35° 19' 22.7"E
11	Qulaan Mangrove	24.357696, 24°21'27.7"N	35.305037, 35° 18'18"E

Table S2. Distribution of different fungal endophytes isolated from leaves and pneumatophores (roots) of A. marina collected from the eleven sites during the study period

Class	Genus	Identified species	Total *		Root*		Leaf*	
			Count	RF (%)	Count	RF (%)	Count	RF (%)
Sordariomycetes			178	39.55	155	55.95	23	13.06
	Chaetomium		152	33.77	146	52.55	6	3.41
		Ch.. globosum	10	2.22	4	1.45	6	3.41
		Chaetomium sp.	142	31.55	142	51.82	0	0.00
	Stachybotrys		16	3.55	8	2.18	8	5.68
		S. kampalensis	7	1.55	5	1.80	2	1.13
		S. pravispora	4	0.88	2	0.73	2	1.13
		Stachybotrys sp.	5	1.11	1	1.09	4	1.13
	Fusarium		9	2.00	1	0.36	8	4.54
		F. oxysporum	4	0.88	0	0.00	4	2.27
		F. verticillioides	4	0.88	1	0.36	3	1.70
		F. prolifratum	1	0.22	0	0.00	1	0.56
	Nigrospora	*N. oryzae*	1	0.22	0	0.00	1	0.56
Dothideomycetes			57	12.66	19	6.86	38	21.59
	Alternaria		15	3.33	4	1.45	11	6.25
		Al. alternata	3	0.66	1	0.36	2	1.13
		Alternaria sp.	12	2.66	3	1.09	9	5.11
	Cladosporium		19	4.22	7	2.55	12	6.81
		Cl. cladosporidis	7	1.55	1	0.36	6	3.41
		Cl. sphaerospoum	2	0.44	0	0.00	2	1.13
		Cladosporium sp.	10	2.22	6	2.19	4	2.27
	Curvularia		9	2.00	1	0.36	8	4.54
		C. lunata	2	0.44	0	0.00	2	1.13
		C. eragrostidis	1	0.22	1	0.36	0	0.00
		Curvularia sp.	6	1.33	0	0.00	6	3.41
	Ulocladium		7	1.55	4	1.45	3	1.70
		U. botrytes	2	0.44	1	0.36	1	0.56
		Ulocladium sp.	5	1.11	3	1.09	2	1.13
	Aureobasidium	*Aureobasidium sp.*	1	0.22	0	0.00	1	0.56
	Epicoccum	*E. nigrum*	6	1.33	3	1.09	3	1.70
Eurotiomycetes			113	25.11	59	21.53	54	30.68
	Phialophora	*Ph. bubakii*	1	0.22	0	0.00	1	0.56
	Penicillium		34	7.55	12	4.37	22	12.5
		P. oxalicum	8	1.78	4	1.46	4	2.27
		Penicillium sp.	26	5.7	8	2.88	18	1023
	Aspergillus		78	17.33	47	17.15	31	17.61
		A. terreus	15	3.33	9	3.28	6	.3.40
		A. parasiticus	2	0.44	0	0.00	2	1.13
		A. flavus	13	2.88	10	3.60	3	1.70
		A. fumigatus	9	2.00	7	2.55	2	1.13
		A. niger	39	8.66	21	7.66	18	10.22
Agaricomycetes (Basidiomycota)	*Rhizocotonia*	*R. solani*	6	1.33	5	1.82	1	0.56
Unclassified			96	21.33	36	13.13	60	34.09
	Dark Sterile Mycelium (mycelia sterilia)		65	14.44	35	12.77	30	17.04
	Black yeast		31	6.88	1	0.36	30	17.04
Total			450	100.00	274	60.88	176	39.11

*Total isolates were 450, count of isolates obtained from pneumatophores (roots) and leaves were 274 and 176, respectively, RF: Relative frequency of isolation.