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Bioprospecting Endophytic Fungi from salt adapted plants

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Abstract

Abiotic stress is one of the major constraints which restrain plant growth and productivity by disrupting physiological processes and stifling defense mechanisms. Hence, the present work focused on the isolation and identification of salt tolerant endophytes. The obtained results clarified that 158 fungal species were recovered from different halophytic plants including, (*Olea europaea*, *Ficus carcia*, *Psidium guajava*). Among them, 120 isolates belonged to three genera: *Aspergillus*, *Penicillium*, and *Cladosporium*. Additionally, 26 isolates were identified as belonging to the genus *Trichoderma*, 4 isolates to *Curvularia*, 3 isolates to *Rhizopus*, and 5 isolates to *Fusarium*. Furthermore, the most common fungi were recovered and identified morphologically. The identified genera were *Aspergillus terreus* AUMC 16944, *Trichoderma longibrachiatum* AUMC 16945, *A. niger van Tieghem* AUMC 16942 and *Penicillium purpurogenum stoll* AUMC 16943. Thus, these isolates are recommended as bio-priming with salt tolerant endophytes to mitigate the salt stress consequences and develop a potential salt resistance in crop plants.

Keywords: Endophytic fungi, salinity stress, morphological characterization.

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1- Introduction

Endophytes are a diverse group of microorganisms including bacteria and fungi that inhabit the internal tissues of plants without causing any visible harm to their hosts. These symbionts have garnered significant attention due to their capacity to enhance host plant fitness and improve tolerance to environmental stresses. By establishing a mutualistic association, endophytes contribute to the plant's defense system and stress resilience, offering a sustainable and environmentally friendly strategy for improving crop adaptation under adverse conditions (Pallavi et al., 2022). Endophytic fungi, in particular, have demonstrated the ability to activate systemic resistance in their host plants. This resistance not only enhances tolerance to abiotic stresses such as drought and salinity but also provides protection against biotic challenges including pathogens and pests (Ampangi-Ramaiah et al., 2020; Manasa et al., 2020). Given their beneficial attributes, endophytic fungi are increasingly recognized as promising bio-inoculants that can be used to promote plant growth in stress-prone environments (Abo Nouh et al., 2021).

In addition to their plant growth-promoting effects, endophytic fungi serve as natural sources of a wide array of bioactive compounds such as flavonoids, terpenoids, phenolics, saponins, alkaloids, carbohydrates, tannins, and nematicidal agents (Liu et al., 2016; Bogner et al., 2017). Members of the genus *Aspergillus*, a common group of filamentous fungi found in soil and plant tissues, are well-known for their adaptability and metabolic diversity, which include growth-promoting capabilities (Yoo et al., 2018). Soil salinity is a major constraint to global agriculture, particularly in arid and semi-arid regions where it threatens the productivity of millions of hectares of farmland. Current estimates indicate that approximately 3.2 million hectares are already affected by salinity (Benmahioul et al., 2009), and this figure is projected to rise, with over 20% of irrigated agricultural land currently considered unsuitable for cultivation due to salinity-related stress (Shrivastava and Kumar, 2015). By 2050, nearly half of all cultivable land may be impacted by salt stress (Munns and Tester, 2008). Salinity disrupts key physiological processes in plants by inducing osmotic and ionic stress, interfering with nutrient uptake, altering hormonal balance, and inhibiting photosynthesis and protein synthesis, which ultimately suppresses plant growth (Abeer et al., 2015).

In light of these challenges, researchers are seeking innovative and eco-conscious strategies to improve plant stress tolerance.

Fungal endophytes have emerged as a particularly promising solution, even under extreme environmental conditions such as drought, salinity, and high temperatures (Rodriguez et al., 2009). Studies have shown that endophytes adapted to such habitats can transfer their stress tolerance traits to non-host plant species, enhancing resilience and performance in stressful environments (Rodriguez et al., 2008; Sangamesh et al., 2018; Ripa et al., 2019).

Salt-tolerant plants, or halophytes, represent an important source of endophytic fungi with adaptive traits suited for saline conditions. These plants have evolved physiological mechanisms to cope with high salinity, and their associated endophytes are likely to possess similar stress tolerance characteristics (Navarro-Torre et al., 2023). Isolation of endophytes from halophytic hosts provides an efficient strategy for identifying fungal strains capable of producing osmoprotectants, antioxidant enzymes, and plant growth-promoting compounds—traits that are highly valuable for the development of biofertilizers and sustainable crop management systems in salt-affected soils (Airin et al., 2023; Das et al., 2025).

Notably, species such as *Aspergillus terreus* are known for their low nutritional requirements and ability to colonize a variety of organic and inorganic substrates, including those contaminated by trace organic pollutants. These traits make them suitable for biotechnological applications in agriculture, including biocontrol and plant growth promotion (Waqas et al., 2015; Halo et al., 2018). Salinity has already affected approximately 1.13 billion hectares of land globally (Wicke et al., 2011), and it continues to reduce the growth and yield of crops at all developmental stages, particularly in arid and semi-arid regions (Nawaz et al., 2010).

Given their ability to colonize diverse plant species and enhance stress resilience, fungal endophytes are increasingly being explored as biofertilizers to improve crop performance under saline conditions (Rodriguez et al., 2008). Soil salinization—primarily caused by sodium chloride accumulation—disrupts plant metabolism, limits water uptake, and reduces yield. However, salt-tolerant microorganisms have shown promise in restoring plant function and productivity under such conditions (Gupta et al., 2020).

This study aims to address existing gaps in knowledge by isolating and characterizing endophytic fungi from halophytic plants, particularly *Olea europaea* (olive) and *Ficus carica* (fig), which are naturally adapted to saline environments. We hypothesize that these endophytes possess intrinsic salt tolerance traits that can be harnessed to improve the stress resilience of economically important, salt-sensitive crops. Identifying and validating such strains could provide a sustainable and effective strategy for mitigating the adverse effects of salinity on agriculture.

It is estimated that more than 300,000 plant species exist globally, the majority of which harbor microbial endophytes (Smith et al., 2008). These symbiotic organisms are integral to plant health, and their absence renders plants more susceptible to environmental stress and pathogenic attack (Timmusk et al., 2011). Harnessing the potential of endophytes represents a promising direction for future agricultural innovation.

2- Materials and methods

2.1. Collection of the host plant materials

Plant materials that used for the isolation of endophytic fungi were taken aseptically in sterile containers and transported under refrigeration to microbiology laboratory in Faculty of Science, Benha University for subsequent analyses. The plant samples were collected from north of Sinai, Marsa matrouh, Ismailia and Qalubyia governorate.

2.2. Isolation and maintenance of endophytic fungi

Plant Leaves and stems were washed in running tap water to remove dust. The plants have been segmented into small parts with an area of 5 mm^2. Their surface was sterilized by 70% (v/v) ethanol for 1 min followed by treatment with sodium hypochlorite 2.5% (w/v) for 2 min followed by treatment with 70% (v/v) ethanol for 30s and washed with sterile distilled water three times to remove any epiphytic microbial flora. The sterilized plant samples were planted onto potato dextrose agar (PDA) (Neergard, 1977) supplemented with Ampicillin (1 µg/ml). After incubation for 15 days at 28 ± 2°C, the developed hyphal tips of the fungal colonies were purified by subculturing on the corresponding medium and incubated for 7-15 days at standard conditions. The purified isolates were preserved at 4°C and used during the course of study.

2.3. Identification of fungal endophytes

All fungal isolates were identified at Faculty of Science Botany Dep. according to Nelson *et al*. (1983). By using light microscope provided with a camera and slides had been continuously observed under various powers of microscope i.e., 10 and 40X. based on cultural and morphological characteristics on specific media and available of literature as compared with the description given by (Raper and Fennell 1965; Maren and Johan 1988) for the genus *Aspergillus*, Nelson *et al*. (1983) for *Fusarium*, the isolates of *penicillium* spp. were determined according to Ramirez (1982) and (Barnett and Hunter, 1977; Pitt, 1979) for the genera of imperfect fungi, All developing fungi were cultured on PDA slants then stored in a refrigerator.

The strains were grown on Czapek-Dox agar, PDA, and malt extract agar media for morphological identification (Moubasher 1993; Barnett and Hunter1994; John and Brett 2006; Leslie and Summerell 2008). Fungal identification carried out based on morphology approach. Morphological identifications were made through observing both of macroscopic and microscopic phenotypic characters. Macroscopic characterization includes observation on colour, colony shape, surface, texture, exudates drop, and reverse colour. For microscopic observation, fungal mycelia were mounted in one drop of 1% lactophenol blue solution. Microscopic characterizations were conducted under light microscope by observing hyphae, hyphae pigmentation, septate, clamp connection, spore, and another reproductive structures.

3- Results

3.1. Isolation and identification of endophytic fungi

Collected plant parts, leaves and stems of different plants (**Figure 1**) were used for fungal isolation. A total of 158 purified endophytic fungal isolates were recovered from different plants.

It could be observed that the number of fungal endophytes from *Citrus limon* (24 fungal isolates), *Portulica oleracea* (20 fungal isolates) and *Olea europaea* media (19 fungal isolates) was more than that isolated from *Diospyros kaki* (10 fungal isolates) as shown in **Table 1**. In addition, the obtained data revealed that the recovered fungi were greater in the stems than leaves of various plants. The obtained fungi were identified to the species level based on their morphological and physiological characteristics. Among them, 120 isolates belonged to three genera: *Aspergillus*, *Penicillium*, and *Cladosporium*. Additionally, 26 isolates were identified as belonging to the genus *Trichoderma*, 4 isolates to *Curvularia*, 3 isolates to *Rhizopus*, and 5 isolates to *Fusarium*. According to Raper and Fennell (1965), the species of Aspergilli were belonging to five groups as follows; *A. niger* group (*A. niger*), *A. flavus* group (*A. flavus, A. oryzae*), *A. fumigatus* group (*A. fumigatus*) and *A. terreus* group (*A. terreus*). The genus

Penicillium was represented by two species, *Penicillium italicum*, *Penicillium chrysogenum* according to Pitt (1979). The genus *Cladosporium* was represented by one species, *Cladosporium oxysporum* according to Domsch (1993). According to Booth (1971), the genus of *Fusarium* has four species, *Fusarium moniliforme*, *Fusarium oxysporum*, *Fusarium solani*, *Fusarium proliferatum*.

It could be observed; *Aspergillus* species were the most frequent among the collected endophytic fungal isolates. The commonness of genus *Aspergillus* among the selected endophytic fungal species was noted by 46%. Among these selected fungal endophytes, four isolates of *A. niger*, three isolates were belonging to *A. flavus* group (*A. flavus* (2 isolates), *A. oryzae* (1 isolate) and three isolated of *A. terrus,* and one isolate of *A. parasiticus* and two of *A. fumigatus*. Three isolates of the genus *Fusarium* (*F. oxysporum, F. solani,* and *Fusarium* sp.), three isolates of *Penicillium* (*P. italicum, P. chrysogenum,* and *Penicillium purpurogenum*.), three isolates of *Alternaria* (*Alternaria* sp.) (**Table 2**). Based on the obtained results four promising isolates namely, *A. niger* , *Penicillium sp, Aspergillus terreus, Trichoderma longibrachiatum* were recovered and deposited on AUMC (**Table 3**).

Figure 1: Morphological view of the collected plants. *Ficus carcia* (A), *Olea europaea* (B), *Psidium guajava* (C), *Portulica oleracea* (D), *Coleus amboinicus* (E), *Mangifera indica* (F), *Citrus sinensis* (G), *Malva parviflora* (H), *Mentha spicata* (I), *Citrus limon* (J), *Ocimum basilicum* (K), *and Ficus sycomorus* (L).

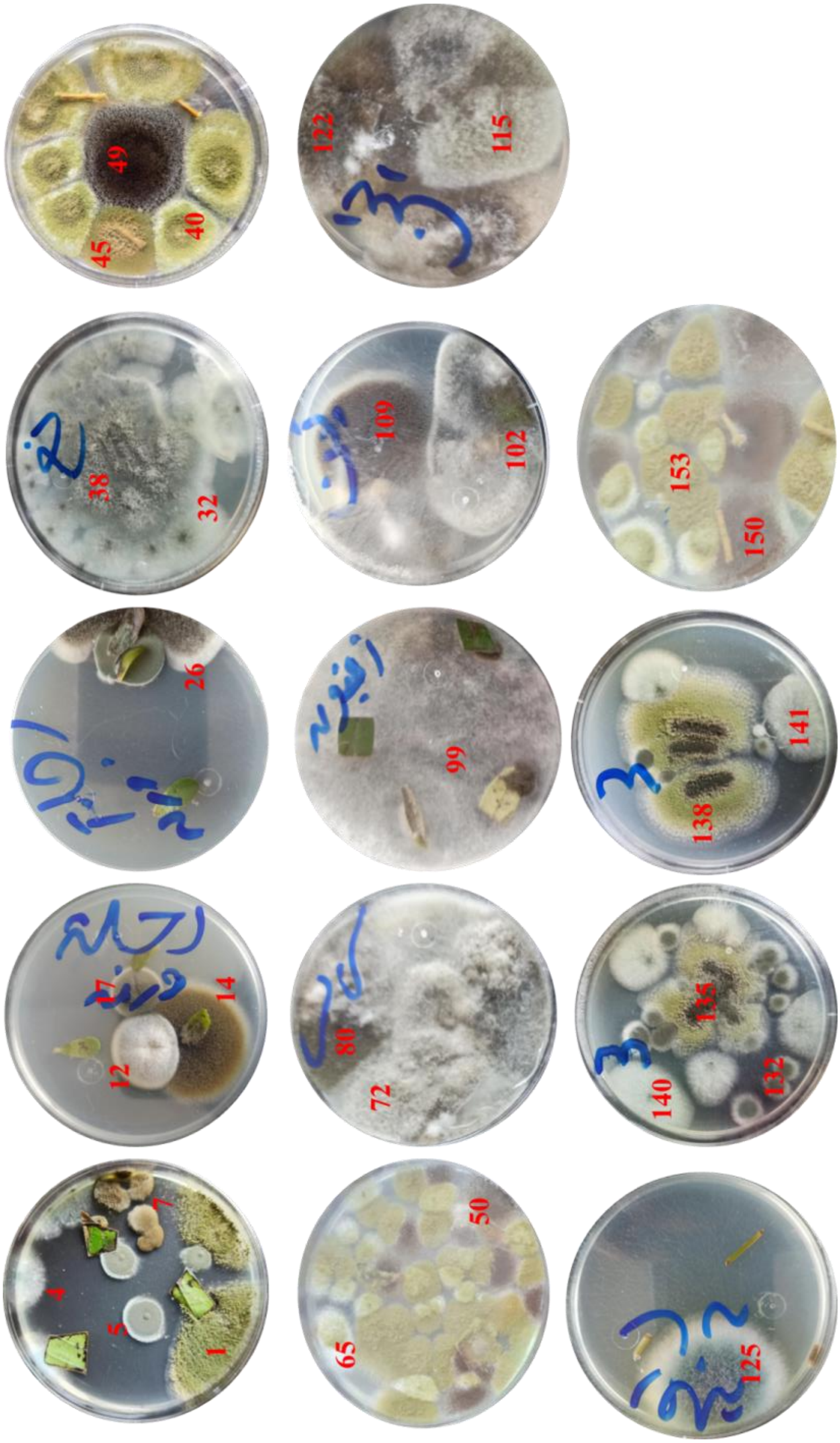

Figure 2: Isolation of endophytic fungi from different plants. Appearance of fungal growth from different plant parts including leaves and stems.

Table 1: Overall frequency of the recovered fungal endophytes from different plants on PDA medium.

Plant part / Plant	Leaf	Stem	Total No. of fungal endophytes
Olea europapaea	7	12	19
Ficus carcia	6	7	13
Psidium guajava	7	8	15
Portulica oleracea	8	12	20
Ficus sycomorus	7	9	15
Citrus limon	9	15	24
Diospyros kaki	2	4	6
Coleus amboinicus	5	7	12
Mangifera indica	4	5	9
Ocimum basilicum	4	8	12
Mentha spicata	6	7	13

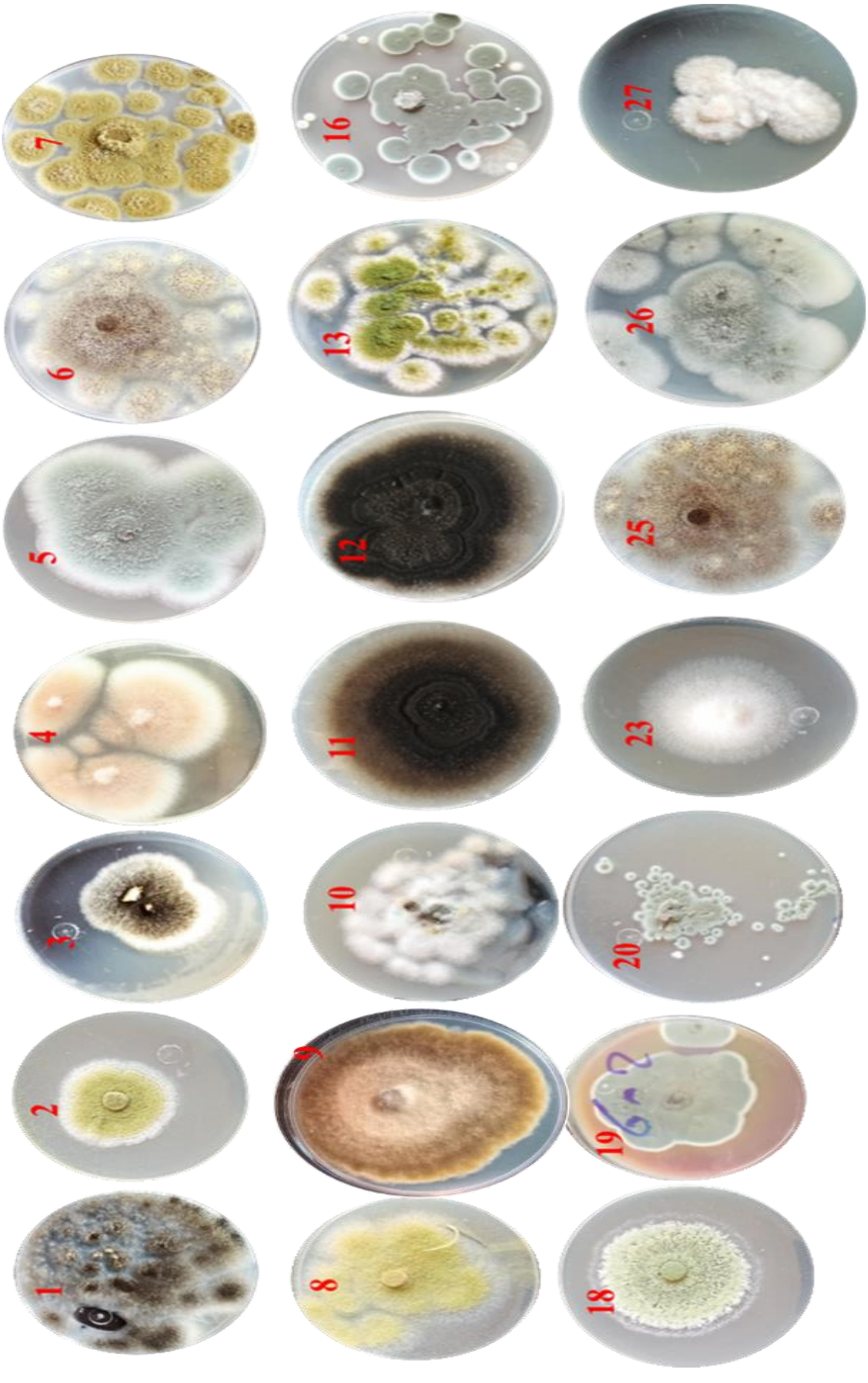

Figure 3: Purified fungal endophytes isolated from plant parts including leaves and stems.

Table 2: Morphological identification of the selected fungal endophytes from different plant parts.

Isolate No.	Plant Name	Isolate Source	Fungal Isolate
1	*Olea europaea*	Leaf	*Aspergillus niger*
2			*A. flavus*
3		stem	*A. niger*
4			*A. terrus*
5	*Ficus carcia*	Leaf	*Penicillium sp.*
6	*Psidium guajava*	Leaf	*A. niger*
7		Stem	*A. parasiticus*
8	*Portulica oleracea*	Leaf	*A. flavus*
9	*Ficus sycomorus*	Leaf	*Alternaria* sp.
10		Stem	*Fusarium* sp.
11			*Curvularia* sp.
12	*Citrus limon*	Leaf	*Cladosporium* sp.
13			*A. oryzae*
14	*Citrus sinensis*	Leaf	*Alternaria* sp.
15			*Alternaria* sp.
16			*A. fumigatus*
17	*Diospyros kaki*	Leaf	*Tricoderma* sp.
18	*Coleus amboinicus*	Leaf	*Tricoderma* sp.
19	*Mangifera indica*	Leaf	*Penicillium chrysogenum*
20			*A. fumigatus*
21	*Mentha spicata*	Leaf	*Curvularia* sp.
22			*A. niger*
23		Stem	*Fusarium exosporium*
24			*A. terrus*
25			*A. terrus*
26			*P. italicum*
27	*Allium cepa*	Stem	*F. solani*
28	*Malva parviflora*	Leaf	*Tricoderma* sp.

Table 3: Promising endophytic fungal isolates.

No.	Isolate
1	*A. niger van Tieghem* AUMC 16942
2	*Penicillium purpurogenum stoll* AUMC 16943
3	*Aspergillus terreus* AUMC 16944
4	*Trichoderma longibrachiatum* AUMC 16945

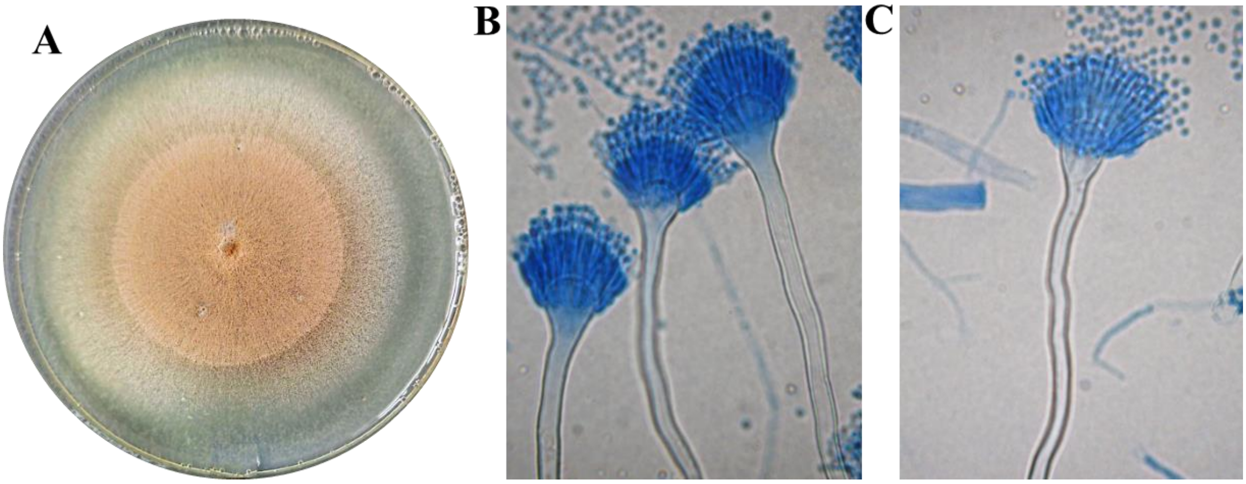

Figure 4: Culture characteristics of potent fungal endophytes. A; Morphological view of *Aspergillus terreus* AUMC 16944 growing on the surface of PDA medium, B & C, The microscopical view of the conidial heads at 1000× magnification (Light Microscope).

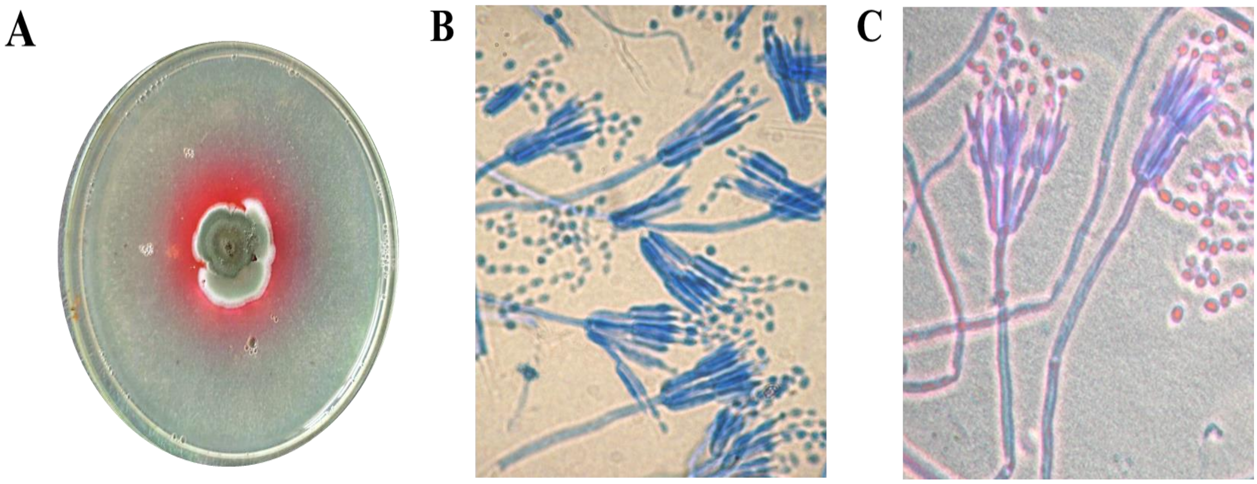

Figure 5: Culture characteristics of potent fungal endophytes. A; Morphological view of *Penicillium purpurogenum stoll* AUMC 16943 growing on the surface of PDA medium, B & C, the microscopical view of the conidial heads at 1000× magnification (Light Microscope).

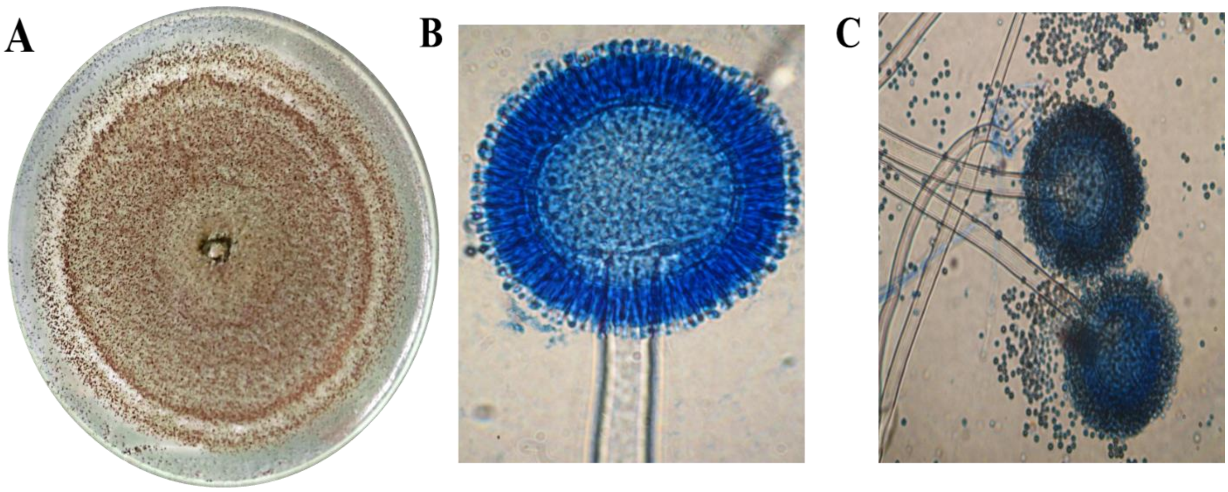

Figure 6: Culture characteristics of potent fungal endophytes. A; Morphological view of *Aspergillus niger van Tieghem* AUMC 16942 growing on the surface of PDA medium, B & C, the microscopical view of the conidial heads at 1000× magnification (Light Microscope).

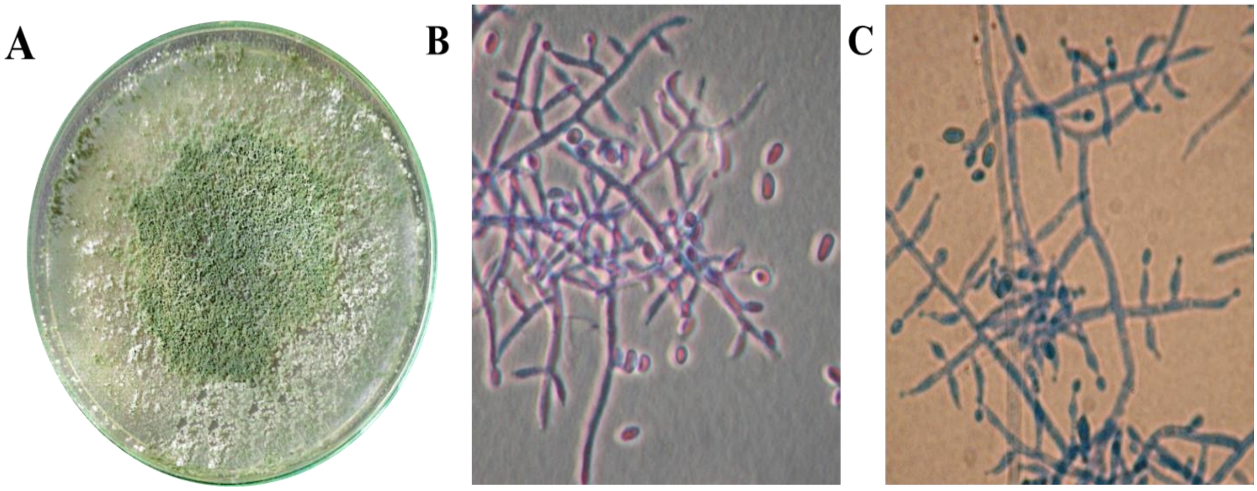

Figure 7: Culture characteristics of potent fungal endophytes. A; Morphological view of *Trichoderma longibrachiatum AUMC 16945* growing on the surface of PDA medium, B & C, the microscopical view of the conidial heads at 1000× magnification (Light Microscope).

4. Discussion

Endophytic fungi inhabit the internal parts of plants and promote their growth. Study aimed to isolate endophytic fungi from the different organs of the various plants in relation to different PGP activities. The data revealed that endophytic fungi are more predominant in the stem than in the leaves. Therefor the colonization rate and species richness of endophytic fungi varied among halophyte species in the current study. In this field Xing et al. (2011) recovered 39 distinct endophytic species in five mangrove species and found the colonization rate of endophytic fungi ranging from 8.0 to 54.0% in stems, and from 12.5 to 25.1% in leaves. Sun et al. (2012b) identified 21 endophyte species from eight desert halophytes and found the colonization rates ranging from 35 to 100% in stems and leaves. Furthermore, we found that the colonization rate and species rich ness of endophytic fungi was generally higher in stem than in leaves. The difference in endophyte assemblage in various tissues indicated that some microbial endophytes have an affinity for different tissue types. Few endophytic fungi were isolated from the leaves, which may be because the relative surface area of the leaves of C. multiflorus was small or the nutrients contained were less susceptible to strain infection than other parts, or the adaptability of endophytic fungi in the leaves was weak in artificial PDA medium, which was similar to the results by Shuhang et al.

(2020). The difference in endophyte assemblage in various tissues indicated that some microbial endophytes have an affinity for different tissue types. This attraction could be due to their ability to use or survive on a particular substrate (different tissue texture and chemistry) (Huang et al., 2008). There are differences in endophytic fungi in different tissue distribution, which may be due to the different structures or chemical components of different organization, so the endophytic fungal communities that invade and grow in each organization are different. As halophyte plants can survive in high salt conditions, the study and evaluation of their symbiotic fungi would be very beneficial for crop production in salty areas. In the present study158 isolates encompassing genera such as *Aspergillus*, *Penicillium*, *Fusarium*, and *Trichoderma*, reflecting the protocol's suitability for saline habitats.The prominence of *Aspergillus*, especially *A. niger* and *A. terreus*, aligns with known salinity tolerance and environmental resilience of these taxa (Gupta et al.,2021). Hosts including *Citrus limon*, *Olea europaea*, and *Portulaca oleracea* yielded particularly abundant isolates, suggesting these plants harbor enriched endophytic communities in saline soils (Sun et al.,2021). Additionally, repeated isolation of *Aspergillus*, *Penicillium*, and *Trichoderma* aligns with reports of their metabolic versatility and stress-related secondary metabolite production, supporting their candidacy as bio-inoculants for saline agriculture (Omomowo et al.,2023).

5- Conclusion

In this study fungal endophytes were recovered from salt adapted plants such as *Olea europaea*, *Ficus carica*, and *Psidium guajava* and identified morphologically. The most common fungal species were deposited at Assuit University. The fungal colonization in halophyte plants represents valuable resources for enhancing crop resilience to salinity stress. Future research should focus on field validation and elucidation of the underlying mechanisms by which these endophytes confer salt tolerance to their host plants.

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