



## Diversity of endophytic fungi from extreme habitats and their promising biochemical markers

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### ABSTRACT

Climate change exacerbates abiotic stresses, including drought and salinity, significantly affecting agriculture. Overreliance on chemical fertilizers compounds environmental pollution, necessitating sustainable alternatives. Endophytic fungi adapted to extreme habitats present a viable solution by promoting plant growth and enhancing stress tolerance. This study explored the potential of endophytic fungi from harsh environments as eco-friendly substitutes for chemical fertilizers. A total of 174 fungal isolates, representing 13 genera, were recovered from nine wild plant species along the coasts of Port Said Governorate, Egypt. *Alternaria* dominated the isolates (52.3%), while *Talaromyces* was the least represented (0.6%). Isolates were tested for drought and salinity tolerance using polyethylene glycol (PEG-6000) and NaCl. Ten highly tolerant isolates were further evaluated for plant growth-promoting traits, including enzymatic activities, nutrient mobilization, antioxidant properties, and phytohormone production. Notable findings included *Aspergillus terreus* (P2R2M1) and *Chaetomium globosporum* (P5S1M1), which exhibited multiple enzymatic activities, and *Trichoderma viride* (P14R3M1), which showed exceptional phosphate solubilization (256 µg/mL). *Cladosporium tenuissimum* (P4R3M1) demonstrated strong siderophore production (89%), while *Trichoderma viride* (P12R1M1) exhibited the highest antioxidant capacity (77%) and phenolic content (44 µg GAE/mg DW). *Acremonium hyalinulum* (P14R1M1) produced the most indole acetic acid (16 µg/mL), and P12R1M1 synthesized the highest gibberellic acid (671 µg/mL). Additionally, this study identified new records of endophytic fungi in Egypt, such as *Chaetomium globosporum* and *Pseudoseptoria* sp. These findings underscore the potential of endophytic fungi as sustainable agricultural inputs and highlight their role in reducing reliance on chemical fertilizers.

**Keywords:** Abiotic stress, Climate change, Endophytic fungi, Harsh habitats, Plant growth.

## 1. INTRODUCTION

Climate change poses a considerable risk to agriculture, and the progression of global climate change is projected to accelerate and intensify in the coming decades [1]. These rapid shifts exacerbates abiotic stresses such as salinity, extreme heat, drought, cold and soil pH variations, which collectively impact crop yield and quality [2]. In response, addressing these challenges has become a primary focus for researchers in agricultural and environmental sciences.

While synthetic fertilizers are widely used to enhance crop productivity and mitigate abiotic stresses, they have adverse effects on soil quality and contribute to long-term pollution [3]. Consequently, identifying sustainable alternatives is essential. Endophytic fungi, a class of beneficial microorganisms that inhabit plant tissues without causing disease, are promising candidates for this role. They are known to confer adaptive advantages to their host plants under stress conditions.

Fungal endophytes contribute to plant growth and stress tolerance through various mechanisms. They secrete hydrolytic enzymes that degrade complex organic matter into plant-available nutrients, produce siderophores to enhance iron uptake, and promote phosphate solubilization to increase phosphorus availability [4, 5]. Additionally, they synthesize plant growth hormones like gibberellins, cytokinins and auxins, which improve shoot and root development [6].

Endophytic fungi confer stress tolerance through mechanisms such as reducing water loss, improving water use efficiency, enhancing root development, increasing antioxidant activity, and adjusting osmotic pressure [7, 5]. These traits make them valuable tools for sustainable agriculture, especially in arid and semi-arid regions.

Recent studies have shown that habitat adapted fungal endophytes can impart significant abiotic stress tolerance to non-native host plants [2, 6, 8]. Therefore, understanding their diversity, community structure, and ecological roles is crucial for their application in agricultural biotechnology. However, habitat-adapted fungal endophytes from extreme habitats in Egypt are rarely studied. Hence, this study aimed to isolate and characterize culturable endophytic fungi from the coastal ecosystems of Port Said Governorate, Egypt, along with assessing their growth under water deficit and salt stress conditions. Furthermore, the plant growth-promoting traits of the most promising isolates, including enzymatic activity, phosphate solubilization, siderophore production, phenolic compound production, antioxidant activity, and phytohormones production, were properly investigated *in vitro*.

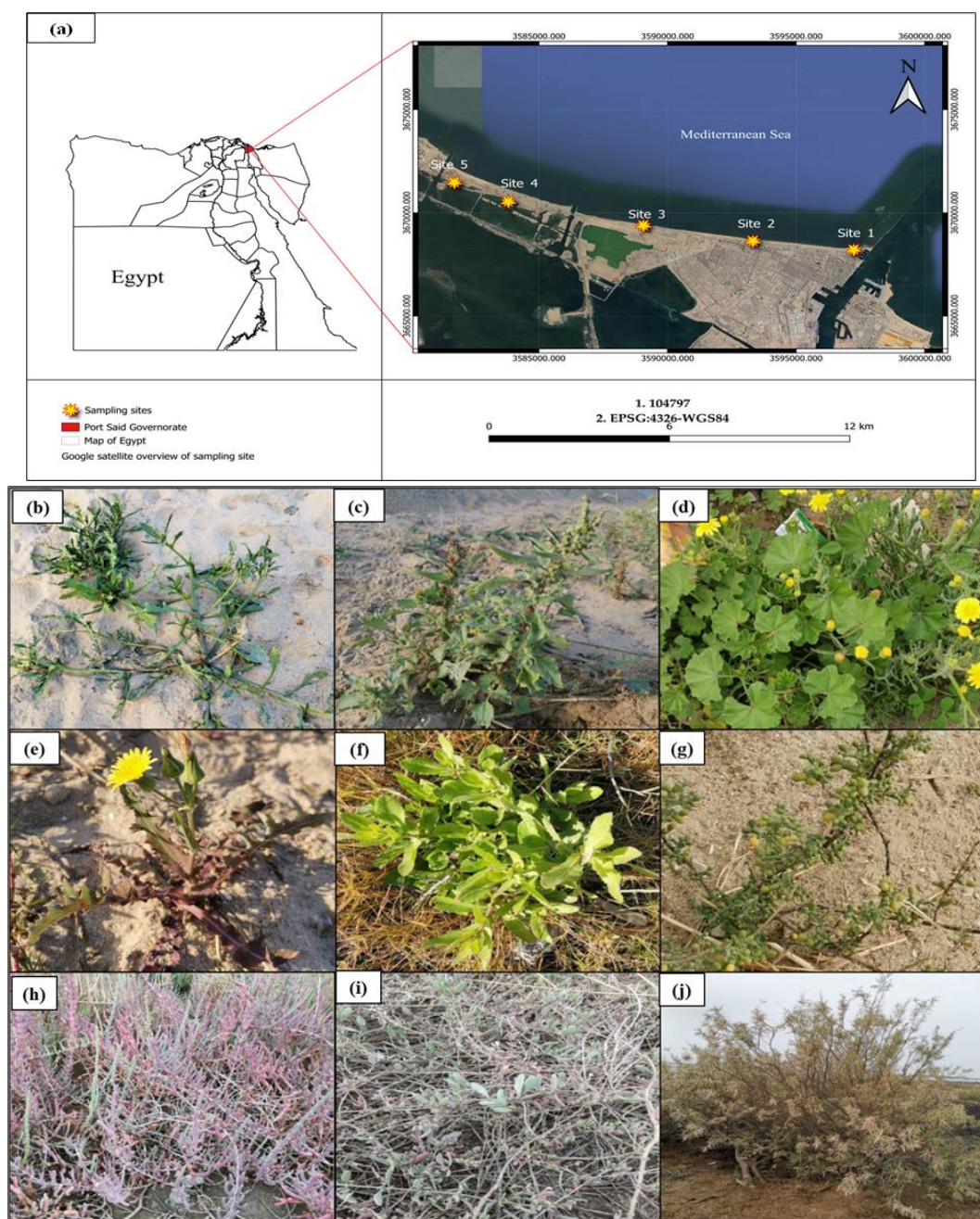
## 2. MATERIALS AND METHODS

**2.1 Sampling and isolation of endophytic fungi:** Nine healthy plant samples with associated rhizosphere soil were collected from five coastal sites in Port Said, Egypt, during autumn 2021 (Fig. 1). Soil physicochemical properties including electrical conductivity (EC), sodium chloride (NaCl) concentration (%), total dissolved solids (TDS), and pH were analyzed using a Mi306 conductivity meter and an ST3100-F-OHAUS pH meter, respectively. The soil moisture content was determined using standard gravimetric methods [9]. Plant samples were sterilized and processed for isolation of fungal endophytes following the procedures described in [10]. Plant segments (roots, stems, and leaves) were

separately plated on potato dextrose agar (PDA) supplemented with 50 mg/L chloramphenicol (4 segments per plate, 5 replicates per plant part) and incubated at  $28^{\circ}\text{C} \pm 2^{\circ}\text{C}$ . Plant segments were closely monitored for fungal growth, and the emergent fungal hyphal tips were promptly transferred to fresh PDA for purification and subsequent preservation at  $4^{\circ}\text{C}$ .

**2.2 Selection of drought and salt tolerant isolates:** To initiate a preliminary selection process, recovered endophytic fungal isolates were subjected to *in vitro* drought and salinity stress assessments. Isolates were cultivated in potato dextrose (PD) broth supplemented with increasing concentrations of polyethylene glycol (PEG-6000; 0%, 10%, 20%, and 30%) or on PDA media containing various NaCl levels (0M, 1M, 2M, and 3 M). After 10 days of incubation at  $28 \pm 2^{\circ}\text{C}$ , fungal dry biomass was assessed and results expressed as grams of dry weight per mL broth medium [11]. The most resilient isolates to drought and salinity stress were selected for subsequent analyses.

**2.3 Morphological identification of endophytic fungi:** All recovered endophytic fungal isolates were subjected to morphological identification at the genus level using standard microscopic techniques. Drought- and salt-tolerant isolates were further characterized at the species level based on macroscopic and microscopic features, following the criteria outlined in references [12, 13, 14, 15, 16, 17]. Colony morphology, encompassing size, color, margin, texture, and pigmentation, was recorded. Microscopic examination of hyphae, conidiophores, phialides, and conidia was performed using a Leica DM750 microscope equipped with a Leica MC170 HD camera.



**Fig (1): Study area and the nine wild plants sampled for isolation of endophytic fungi:** (a) A google satellite map of sampling sites of extreme habitat coastal at Port Said Governorate. (b) *Cakile maritima* Scop.2., sampled from site (1). (c, d & e) *Chenopodium murale* L., *Malva parviflora* & *Launaea capitata* (Spreng.) Dandy, sampled from site (2). (f & g) *Pluchea dioscoridis* (L.) DC & *Zygophyllum album* L f., sampled from site (3). (h & i) *Arthrocnemum macrostachyum* (Moric.) K.Koch & *Atriplex portulacoides* L., sampled from site (4). (j) *Tamarix nilotica* (Ehrenb.) Bunge, sampled from site (5).

**2.4 Estimation of fungal diversity indices:** Fungal diversity was evaluated using statistical indices as outlined by [7, 18]. Colonization frequency (CF%) was calculated as the number of segments colonized by endophytic fungi divided by the total number of incubated segments. The isolation rate (IR%) was calculated as the ratio of the number of isolates obtained from tissue segments to the total number of incubated tissue segments. Absolute frequency (f) represented the total count of isolated endophytes, while relative frequency (fr) indicated the proportion of each fungal genus within the collection of isolates. Richness (R) was used to quantify the total number of fungal genera recovered from each plant.

The Shannon diversity index ( $H'$ ) was calculated using the formula  $H' = -\sum(p_i * \ln p_i)$ , where  $p_i$  represents the proportion of individuals belonging to species  $i$ . Evenness ( $E$ ) was derived from the ratio  $H'/\ln S$ , with  $S$  representing species richness. The Simpson dominance index ( $C$ ) was calculated using the formula  $C = -\sum(n_i/N)^2$ , where  $n_i$  is the total number of isolates of a particular genus, and  $N$  is the total number of isolates across all genera. Simpson's diversity index ( $1-D$ ) was computed as  $1 - \sum(p_i^2)$ , where  $p_i$  is the proportion of individuals belonging to species  $i$ . The Berger-Parker Dominance Index ( $p_{\max}$ ) was calculated as the number of individuals in the most dominant taxon relative to the total number of individuals.

**2.5 Screening of fungal endophytes extracellular enzymatic activity:** Endophytic fungal enzyme production was qualitatively assessed using an agar plate-based method. Mycelial plugs from each isolate were inoculated onto minimal medium agar plates containing specific substrates for the detection of carboxymethyl cellulase (CMC), amylase, pectinase, protease, and chitinase [8]. The medium composition was as follows: [6 g  $\text{NaNO}_3$ , 1.5 g  $\text{KH}_2\text{PO}_4$ , 5g KCl, 0.5 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.01 g  $\text{FeSO}_4$ , 0.01 g  $\text{ZnSO}_4$ ; pH 7.0]. For ligninase activity, PDA medium supplemented with Azure B dye was employed [19]. Cultures were incubated at  $28 \pm 2^\circ\text{C}$  for seven days. Congo red staining followed by NaCl washing was used to visualize CMCase activity [20], while iodine reagent was used for amylase, pectinase, and chitinase detection [21]. The enzymatic index (EI) was calculated as follows:  $\text{EI} = \text{Diameter of the halo zone (in mm)} / \text{Diameter of the fungal colony (in mm)}$ .

**2.6 Assessment of Inorganic phosphate solubilization and siderophores production:** A quantitative bioassay using Pikovskaya's broth supplemented with 0.5% tricalcium phosphate was performed to assess the phosphate solubilization capacity of fungal isolates, and the available phosphorus in culture supernatants was determined using the molybdenum blue method adapted from [22]. Absorbance measurements at 820 nm were correlated to phosphorus concentration using a standard curve prepared with potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ), with results expressed as micrograms of phosphorus per milliliter of culture supernatant. Siderophore production by fungal isolates was assessed using the Chrome Azurol S (CAS) liquid assay [23]. Siderophore concentration was determined by measuring the absorbance at 630 nm and calculating percent siderophore units based on the following formula: % Siderophore units =  $[(A_r - A_s)/A_r] \times 100$ , where  $A_r$  and  $A_s$  represent the absorbance of the control (uninoculated broth with CAS solution) and the sample at 630 nm, respectively.

**2.7 Determination of total phenolic content and antioxidant capacity:** For the assessment of total phenolic content (TPC) and antioxidant capacity (TAC), fungal endophytes were cultured in Czapek-Dox (CD) broth and incubated at  $28 \pm 2^\circ\text{C}$  for 21 days. Following liquid-liquid extraction with ethyl acetate and subsequent drying, the crude extract was re-dissolved in 80% ethanol for analysis. TPC was measured using the Folin & Ciocalteu phenol reagent method, and the results were expressed as microgram gallic acid equivalents per milligram of dry weight of the ethanolic extract ( $\mu\text{g GAE/mg DW}$  of EA extract) [24]. Total antioxidant capacity was evaluated using the potassium permanganate reducing antioxidant capacity (PRAC) assay, as described by [25]. PRAC activity was calculated using the formula:  $\text{PRAC (\%)} = [(A_c - A_s)/A_c] \times 100$ , where  $A_c$  is the absorbance of the reagent control and  $A_s$  is the absorbance of the test sample.

**2.8 Phytohormone production by fungal endophytes:** Gibberellin-like substances were conducted by inoculating endophytic fungal isolates in CD broth and gibberellic acid ( $\text{GA}_3$ ) was quantified using colorimetric methods as described by [6]. Absorbance was measured at 254 nm, with gibberellic acid ( $\text{GA}_3$ ) serving as the standard for quantification. For the assessment of auxin-like substances, each isolate

was inoculated into modified Czapek-Dox medium (CDM) supplemented with 2 mg/mL L-tryptophan [8]. Following incubation, the supernatants were acidified and extracted with ethyl acetate (2:1 v/v). The organic phase (ethyl acetate) was evaporated to dryness, and the concentrated residues were re-dissolved in methanol to measure IAA concentration [26]. Salkowski reagent was used for the colorimetric determination of IAA. Absorbance was recorded at 530 nm using a double-beam UV-visible spectrophotometer (ST-UV-1901PC) [6]. The IAA concentration was calculated from a standard curve created using pure indole-3-acetic acid. The results were expressed as micrograms of the respective compounds (IAA or GA<sub>3</sub>) per mL of filtrate.

**2.9 Statistical analysis:** The data were analyzed using one-way analysis of variance (ANOVA) performed with CoStat software (version 6.45) and are expressed as the mean  $\pm$  standard error from three replicates. Differences between means were considered statistically significant at  $p \leq 0.05$ , as determined by the least significant difference (LSD) post-hoc test.

### 3. RESULTS

**3.1 Variations in soil aspects and diversity of endophytic fungi:** Data presented in Table (1) revealed significant variations in soil properties among the rhizospheres of sampled plant hosts. *Tamarix nilotica* rhizosphere soil exhibited the highest salinity levels, with an electrical conductivity (EC) of 12.2 mS/cm, a sodium chloride (NaCl) percentage of 23.2%, and a total dissolved solids (TDS) concentration of 5.6 g/L. *Arthrocnemum macrostachyum* rhizosphere had the highest soil moisture content at 25.8%, while *Zygophyllum album* rhizosphere displayed the lowest soil moisture content at 1.5%. A total of 174 fungal isolates were obtained from 540 tissue fragments, belonging to thirteen genera. Table (2) demonstrates that Amaranthaceae was the most abundant plant family. Roots were found to harbor more endophytic fungi than other plant parts, with *Cakile maritima* and *Malva parviflora* exhibiting the highest colonization and isolation rates: 45% and 13%, respectively. Table (3) shows that *Alternaria* sp.



**Table1:** Isolation sites at Port Said Governorate and their physiochemical parameters.

Sampling site	Coordinate		Host plant	Soil physiochemical parameters					Soil type
	longitude	latitude		EC (mS/cm)	NaCl (%)	TDS (g/L)	pH	Moisture content (%)	
1	32°18'52"	31°16'21"	<i>Cakile maritima</i> Scop.2	0.50±0.01 <sup>d</sup>	1.07±0.07 <sup>e</sup>	0.25±0.00 <sup>d</sup>	7.22±0.11 <sup>d</sup>	2.33±0.33 <sup>d</sup>	Sandy soil
			<i>Chenopodium murale</i> L.	1.44±0.05 <sup>c</sup>	2.77±0.12 <sup>cd</sup>	0.72±0.03 <sup>c</sup>	7.27±0.03 <sup>cd</sup>	2.67±0.67 <sup>d</sup>	
2	32°17'20"	31°16'29"	<i>Malva parviflora</i>	0.84±0.01 <sup>cd</sup>	1.63±0.03 <sup>de</sup>	0.42±0.01 <sup>cd</sup>	7.63±0.09 <sup>ab</sup>	1.83±0.17 <sup>d</sup>	
			<i>Launaea capitata</i> (Spreng.) Dandy	0.65±0.01 <sup>d</sup>	1.30±0.00 <sup>e</sup>	0.33±0.00 <sup>d</sup>	7.43±0.03 <sup>bcd</sup>	1.83±0.17 <sup>d</sup>	
3	32°15'56"	31°16'40"	<i>Pluchea dioscoridis</i> (L.) DC	1.42±0.16 <sup>c</sup>	3.13±0.13 <sup>c</sup>	0.71±0.08 <sup>c</sup>	7.54±0.18 <sup>abc</sup>	1.63±0.09 <sup>d</sup>	
			<i>Zygophyllum album</i> L f.	0.69±0.02 <sup>d</sup>	1.43±0.03 <sup>e</sup>	0.34±0.01 <sup>d</sup>	7.53±0.05 <sup>abc</sup>	1.52±0.02 <sup>d</sup>	
4	32°11'30"	31°17'30"	<i>Arthrocnemum macrostachyum</i> (Moric.) K.Koch	6.16±0.05 <sup>b</sup>	11.77±0.15 <sup>b</sup>	3.07±0.02 <sup>b</sup>	7.75±0.13 <sup>a</sup>	25.75±0.69 <sup>a</sup>	
			<i>Atriplex portulacoides</i> L.	5.94±0.09 <sup>b</sup>	10.93±0.18 <sup>b</sup>	2.99±0.06 <sup>b</sup>	7.36±0.02 <sup>bcd</sup>	12.50±0.20 <sup>c</sup>	
5	32°10'30"	31°17'51"	<i>Tamarix nilotica</i> (Ehrenb.) Bunge	12.21±0.61 <sup>a</sup>	23.20±1.25 <sup>a</sup>	5.99±0.31 <sup>a</sup>	7.73±0.07 <sup>a</sup>	19.09±0.60 <sup>b</sup>	
LSD				0.63	1.27	0.33	0.28	1.21	

\*Values are mean of three replicates ± SE. Values with different letter (s), in each column, are significantly different at  $P \leq 0.05$  using least significant difference (LSD) test.

was the most abundant fungal genus, accounting for 52.3% of the recovered isolates. *Talaromyces* sp. was the least abundant, colonizing one stem segment of only one host. Table (4) highlights the diversity of endophytic fungi (EF) isolated from the nine wild plant hosts. *Cakile maritima* 2 was the most hospitable, with 27 recovered fungal isolates, while *Chenopodium murale* and *Launaea capitata* were the least hospitable with only 12 recovered isolates. *Malva parviflora* demonstrated exceptional genus richness, with 6 distinct fungal genera recovered with evenness of 0.21, indicating a diverse fungal community. Regarding diversity indices, *Atriplex portulacoides* L. exhibited the highest overall fungal diversity as assessed by the Simpson's diversity index (0.99), suggesting a more evenly distributed community. Conversely, *Zygophyllum album* displayed a higher dominance index (Berger-Parker index = 90.5%), indicating a community dominated by a single fungal genus, *Alternaria* sp.

**Table 2:** Colonization rate (CR%) and isolation rate (IR) of fungal endophytes from different plant hosts.

Host plant	Family	Colonization rate (CR) %			Isolation rate (IR)		
		Root	Stem	Shoot	Root	Stem	Shoot
<i>Arthrocnemum macrostachyum</i> (Moric.) K.Koch	Amaranthaceae	35	65	15	0.1	0.15	0.05
<i>Atriplex portulacoides</i> L.	Amaranthaceae	40	40	50	0.05	0.05	0.1
<i>Cakile maritima</i> Scop.1	Brassicaceae	50	45	40	0.15	0.15	0.05
<i>Chenopodium murale</i> L.	Amaranthaceae	20	5	35	0.1	0.05	0.1
<i>Launaea capitata</i> (Spreng.) Dandy	Asteraceae	10	0	50	0.05	0	0.1
<i>Malva parviflora</i>	Mallows	40	30	10	0.2	0.15	0.05
<i>Pluchea dioscoridis</i> (L.) DC	Asteraceae	25	20	65	0.1	0.1	0.1
<i>Tamarix nilotica</i> (Ehrenb.) Bunge	Tamaricaceae	35	35	5	0.15	0.1	0.05
<i>Zygophyllum album</i> L f.	Zygophyllaceae	25	25	55	0.1	0.05	0.1

**Table 3:** Absolute (f) and relative frequency (fr) of fungal endophytes from different plant hosts.

Endophytic fungi	P1			P2			P3			P4			P5			P6			P7			P8			P9			(f)	fr (%)
	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L		
<i>Acromonium sp.</i>	2			2			2 1												4						1			12	6.9
<i>Alternaria sp.</i>	2 8			1 6			2 2 2			2			4 11			4 5 10			5 3			8 7			2 6 1			91	52.3
<i>Aspergillus sp.</i>	7																											7	4.0
<i>Chaetomium sp.</i>																			6									6	3.4
<i>Cladosporium sp.</i>	1 5			2																								8	4.6
<i>Drechslera sp.</i>										9																		9	5.2
<i>Fusarium sp.</i>	2						1						2			1			1 4									11	6.3
<i>Pseudoseptoria sp.</i>							2																					2	1.1
<i>Stemphyllum sp.</i>				1									2			1												4	2.3
<i>Talaromyces sp.</i>							1																					1	0.6
<i>Trichoderma sp.</i>													3									8			4			15	8.6
<i>Ulocladium sp.</i>										1												3						4	2.3
Sterile mycelium							3																		1			4	2.3
Total	10	9	8	4	2	6	9	5	2	2	0	10	5	4	13	5	5	11	7	13	3	8	8	10	7	7	1	174	100
Species richness	3	3	1	2	2	1	4	3	1	1	0	2	2	1	2	2	1	2	2	3	1	1	1	2	3	1	1		

\***L**, Leaf; **S**, Stem; **R**, Root. **P1–P9** correspond to *Cakile maritima* Scop., *Chenopodium murale* L., *Malva parviflora*, *Launaea capitata* (Spreng.) Dandy, *Pluchea dioscoridis* (L.) DC., *Zygophyllum album* L. f., *Arthrocnemum macrostachyum* (Moric.) K. Koch, *Atriplex portulacoides* L., and *Tamarix nilotica* (Ehrenb.) Bunge, respectively.

**Table 4:** Diversity indices of endophytic fungi isolated from different plant hosts.

Index	Host plant								
	P1	P2	P3	P4	P5	P6	P7	P8	P9
Total No. of isolates	27	12	16	12	22	21	23	26	15
Richness (R)	5	4	6	3	4	3	4	3	4
Shannon diversity index (H')	0.31	0.37	0.37	0.35	0.31	0.28	0.3	0.25	0.35
Evenness (E)	0.19	0.26	0.21	0.32	0.22	0.25	0.22	0.23	0.25
Simpson dominance index (C)	0.03	0.11	0.14	0.06	0.03	0.02	0.03	0.01	0.07
Simpson's diversity index (1-D)	0.97	0.89	0.86	0.94	0.97	0.98	0.97	0.99	0.93
Berger Parker Dominance Index (pimax)	37.04	58.33	37.50	75.00	68.18	90.48	0.35	57.69	60.00

\***P1–P9** correspond to *Cakile maritima* Scop., *Chenopodium murale* L., *Malva parviflora*, *Launaea capitata* (Spreng.) Dandy, *Pluchea dioscoridis* (L.) DC., *Zygophyllum album* L. f., *Arthrocnemum macrostachyum* (Moric.) K. Koch, *Atriplex portulacoides* L., and *Tamarix nilotica* (Ehrenb.) Bunge, respectively.

**3.2 Drought and salinity tolerance of fungal endophytes:** Table (5) presents the drought and salinity tolerance of recovered endophytic fungal isolates. Among the thirteen fungal genera, *Pseudoseptoria*, *Chaetomium* sp., *Talaromyces*, and *Trichoderma* species demonstrated high drought tolerance, exhibiting a mean increased mycelial dry biomass by 34%, 1%, 1%, and 1.3%, respectively at 30% PEG-6000. Conversely, *Drechslera* and *Stemphyllum* isolates exhibited low drought tolerance, with significant reductions in mean mycelial dry biomass by 100% and 71%, respectively, at 30% PEG-6000. Regarding salinity tolerance, as depicted in Table 5, isolates of genus *Drechslera* displayed the highest tolerance to








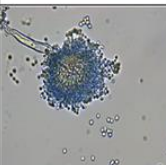
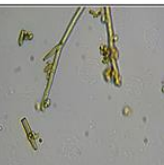
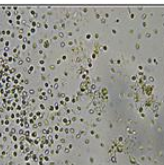


salinity followed by *Cladosporium* spp. and *Stemphylium* spp., with respective reductions in mycelial dry biomass of 62%, 76%, and 77% at 3M NaCl. In contrast, isolates of genus *Pseudoseptoria*, *Talaromyces*, and sterile mycelium exhibited the least salinity tolerance, with complete inhibition of mycelial growth at 3M NaCl. Ten isolates were selected based on their high drought and salinity tolerance for further investigation of plant growth enhancing characteristics.

**Table 5:** Effect of drought and salinity on mycelial dry biomass reduction (%) of culturable endophytic fungal isolates.






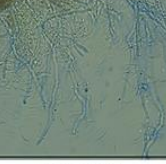
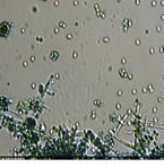



Isolate	Mycelial dry biomass reduction (%)					
	PEG (10%)	PEG (20%)	PEG (30%)	NaCl (1M)	NaCl (2M)	NaCl (3M)
<i>Acremonium</i> sp.	15.3±4.9 <sup>bc</sup>	24.1±7.7 <sup>bcd</sup>	33±9.3 <sup>cde</sup>	31.6±3.5 <sup>ab</sup>	44.9±5.2 <sup>f</sup>	78.0±2.8 <sup>bcd</sup>
<i>Alternaria</i> sp.	15.8±2.1 <sup>bc</sup>	36.3±3.8 <sup>bc</sup>	55±4.6 <sup>bcd</sup>	28.0±2.2 <sup>ab</sup>	59.3±2.0 <sup>cd</sup>	93.3±1.5 <sup>a</sup>
<i>Aspergillus</i> sp.	0.9±6.0 <sup>bcd</sup>	24±3.4 <sup>bcd</sup>	12±2.5 <sup>defg</sup>	37.4±2.1 <sup>ab</sup>	80.0±3.9 <sup>abc</sup>	83.0±2.6 <sup>abcd</sup>
<i>Chaetomium</i> sp.	-1.9±0.8 <sup>cd</sup>	-2±0.8 <sup>def</sup>	-1±0.8 <sup>efg</sup>	24.5±4.8 <sup>ab</sup>	46.7±2.1 <sup>def</sup>	95.0±1.4 <sup>ab</sup>
<i>Cladosporium</i> sp.	-6.3±6.3 <sup>d</sup>	9.2±2.8 <sup>de</sup>	19±4.3 <sup>def</sup>	27.2±3.6 <sup>ab</sup>	46.7±4.6 <sup>def</sup>	76.1±6.1 <sup>cd</sup>
<i>Drechslera</i> sp.	26.2±0.7 <sup>ab</sup>	73±0.4 <sup>a</sup>	100±0.0 <sup>a</sup>	48.6±2.3 <sup>a</sup>	52.7±0.8 <sup>cdef</sup>	61.9±1.5 <sup>d</sup>
<i>Fusarium</i> sp.	21.5±3.6 <sup>b</sup>	47.4±8.2 <sup>ab</sup>	60±6.9 <sup>abc</sup>	20.6±3.7 <sup>b</sup>	58.8±3.7 <sup>cde</sup>	88.7±3.9 <sup>ab</sup>
<i>Pseudoseptoria</i> sp.	-32.9±1.2 <sup>e</sup>	-35.4±1.3 <sup>f</sup>	-34±1.7 <sup>g</sup>	37.1±0.6 <sup>ab</sup>	86.9±0.3 <sup>ab</sup>	100.0±0.0 <sup>a</sup>
<i>Stemphylium</i> sp.	36.4±11.0 <sup>a</sup>	49±10.7 <sup>ab</sup>	71±12.1 <sup>ab</sup>	25.2±6.4 <sup>ab</sup>	45.4±6.5 <sup>ef</sup>	77.0±7.2 <sup>bcd</sup>
<i>Talaromyces</i> sp.	-13.4±8.2 <sup>de</sup>	-18.3±1.7 <sup>ef</sup>	-1±1.0 <sup>efg</sup>	31.6±2.3 <sup>ab</sup>	87.9±1.8 <sup>ab</sup>	100.0±0.0 <sup>a</sup>
<i>Trichoderma</i> sp.	-1.3±0.7 <sup>cd</sup>	-1.3±0.7 <sup>def</sup>	-1.3±0.7 <sup>efg</sup>	19.5±5.3 <sup>b</sup>	67.9±10.2 <sup>bcd</sup>	85.7±7.2 <sup>abc</sup>
<i>Ulocladium</i> sp.	8.7±2.4 <sup>bcd</sup>	14.4±3.2 <sup>cde</sup>	58±19.0 <sup>abcd</sup>	24.9±4.0 <sup>ab</sup>	53.4±5.4 <sup>cdef</sup>	83.0±7.7 <sup>abc</sup>
Sterile mycelium	10.5±4.2 <sup>bcd</sup>	29.8±7.1 <sup>bcd</sup>	62±17.5 <sup>abc</sup>	44.3±19.8 <sup>a</sup>	100.0±0.0 <sup>a</sup>	100.0±0.0 <sup>a</sup>
LSD	28.00	43.64	53.10	29	27.7	22.8

\*Values are mean of three replicates ± SE. Values with different letter (s), in each column, are significantly different at  $P \leq 0.05$  using least significant difference (LSD) test.

**3.3 Morphological characteristics of the most promising isolates:** The isolates were selected based on their high drought and salinity tolerance potential. Morphological identification at the species level was performed through a comprehensive analysis of macroscopic and microscopic features, as illustrated in (Fig. 2). The identified isolates included *Aspergillus terreus* (P2R2M1), *Cladosporium tenuissimum* (P4R3M1), *Talaromyces stipitatus* (P5R2M1), *Chaetomium globosporum* (P5S1M1), *Trichoderma viride* (P7R3M1, P12R1M1, and P14R3M1), *Pseudoseptoria* sp. (P10R4M1), *Alternaria tenuissima* (P12S4M1), and *Acremonium hyalinulum* (P14R1M1). Notably, two novel records for Egypt as endophytic fungi were identified: P5S1M1 (*Chaetomium globosporum*) and P10R4M1 (*Pseudoseptoria* sp.).

Fungal colony					
Microscopic view					
Code	P2R2M1	P4R3M1	P5R2M1	P5S1M1	P7R3M1
Fungal endophyte	<i>Aspergillus terreus</i>	<i>Cladosporium tenuissimum</i>	<i>Talaromyces stipitatus</i>	<i>Chaetomium globosporum</i>	<i>Trichoderma viride</i>
Host plant /Isolation part	<i>Cakile maritima</i> Scop. / Root	<i>Chenopodium murale</i> L. / Root	<i>Malva parviflora</i> / Root	<i>Malva parviflora</i> / Stem	<i>Pluchea dioscoridis</i> (L.) DC / Root

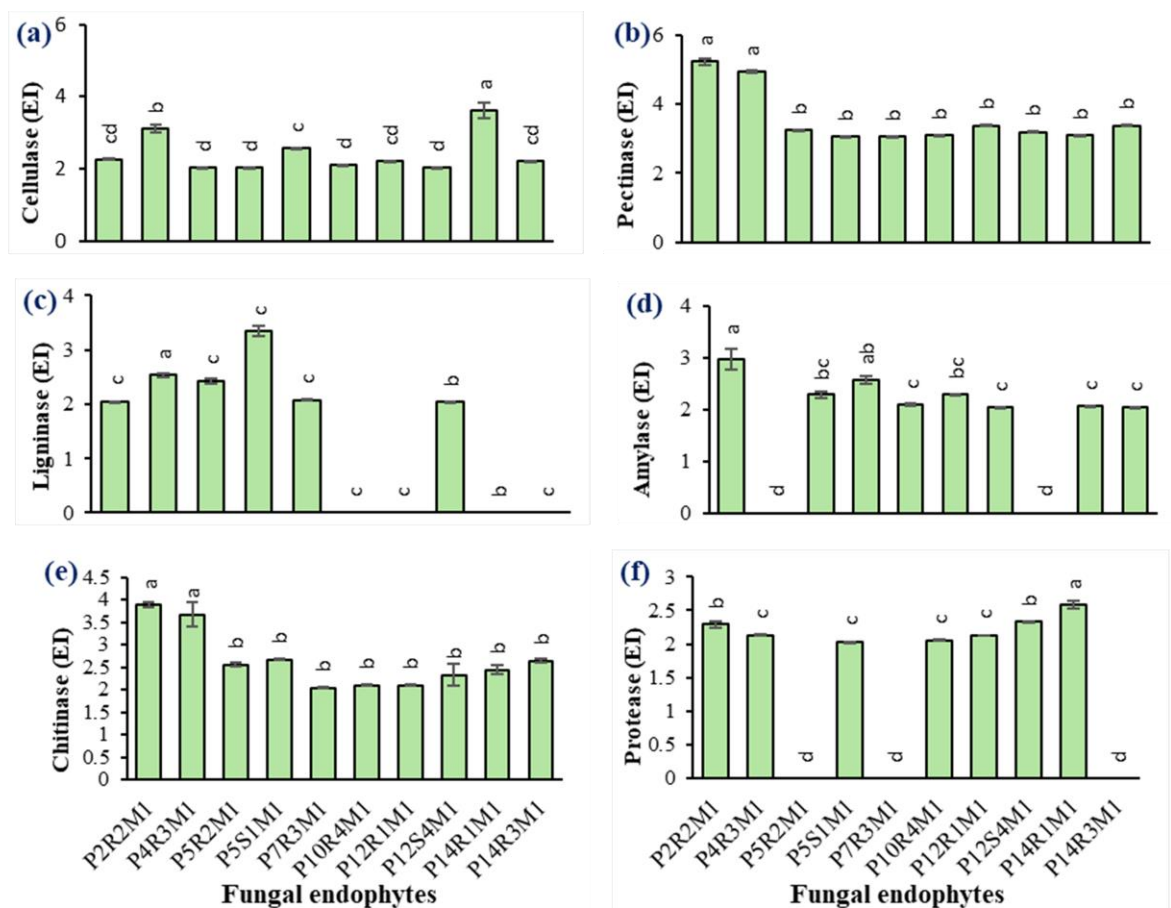
  

Fungal colony					
Microscopic view					
Code	P10R4M1	P12R1M1	P12S4M1	P14R1M1	P14R3M1
Fungal endophyte	<i>Pseudoseptoria</i> sp.	<i>Trichoderma viride</i>	<i>Alternaria tenuissima</i>	<i>Acremonium hyalinulum</i>	<i>Trichoderma viride</i>
Host plant/Isolation part	<i>Arthrocnemum macrostachyum</i> (Moric.) K.Koch / Root	<i>Atriplex portulacoides</i> L. / Root	<i>Atriplex portulacoides</i> L. / Stem	<i>Tamarix nilotica</i> / Root	<i>Tamarix nilotica</i> / Root

**Fig. (2): Morphological features of the ten selected endophytic fungal isolates exhibiting drought and salinity tolerance:** The macroscopic characteristics display fungal colonies on Malt Extract Agar observed after 7-14 days of inoculation. The microscopic view, captured at 400x magnification using lactophenol cotton blue stain, shows the fungal characteristics after 7 days of incubation.

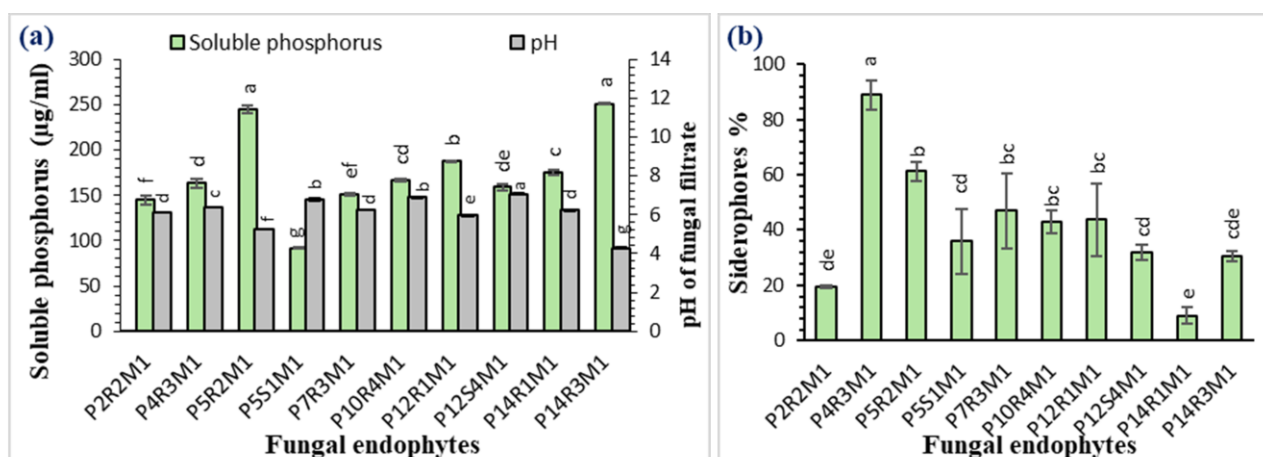
### 3.4 Divergences in enzymatic activity and plant growth enhancing characteristics:

**3.4.1 Extracellular enzymatic activity:** All isolates exhibited extracellular enzymatic activity for cellulase, pectinase, and chitinase, although significant variations were observed among isolates (Fig. 3). The highest cellulase activity was produced by P14R1M1, with an enzymatic index (EI) of 4.00, while the lowest was observed in P5S1M1, with an EI of 2.03 (Fig. 3, (a)). All isolates demonstrated high pectinase activity, with P2R2M1 exhibiting the highest level of 5.2 EI (Fig. 3, (b)). Ligninase activity varied among isolates, with P5S1M1 showing the highest activity of 3.3 EI, and P10R4M1, P12R1M1, P14R1M1 and P14R3M1 isolates displaying no activity (Fig. 3, (c)). For amylase, P2R2M1 displayed the highest activity with an EI of 3.1, whereas P4R3M1 and P12S4M1 showed no activity (Fig. 3, (d)). All isolates exhibited high chitinase activity, with enzymatic index value ranging from 4.0 in P2R2M1 to 2.04 in P7R3M1 (Fig. 3, (e)). Protease activity was highest in P14R1M1, with an EI of 3.0, while P5R2M1, P7R3M1, and P14R3M1 showed no activity (Fig. 3, (f)).



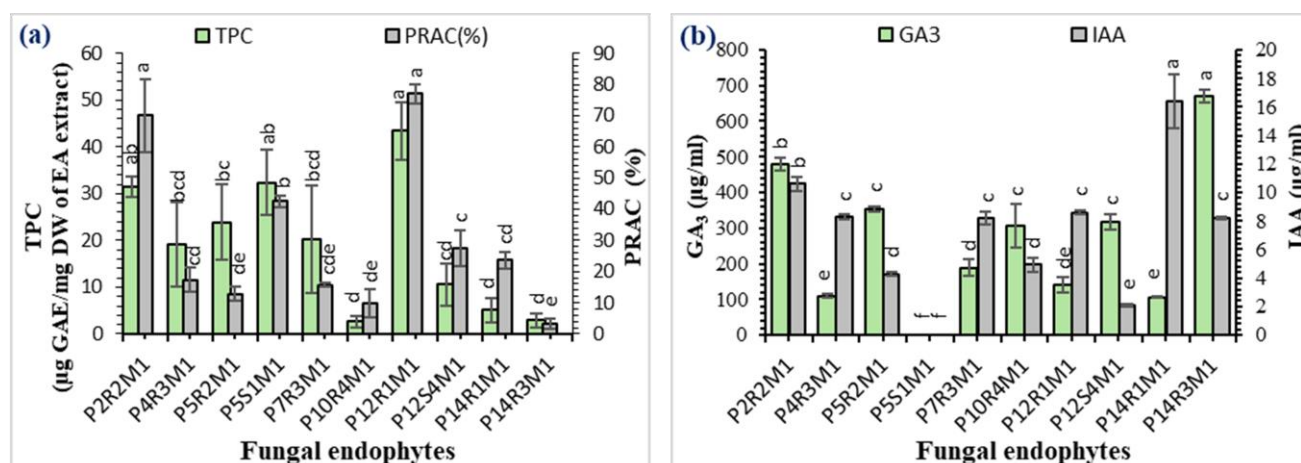
**Fig. (3): Enzymatic index (EI) of the ten drought and salinity-tolerant endophytic fungal isolates: (a) Cellulase activity. (b) Pectinase activity. (c) Ligninase activity. (d) Amylase activity. (e) Chitinase activity. (f) Protease activity.** Values are mean of three replicates  $\pm$  SE. Values with different letter (s) are significantly different at  $P \leq 0.05$ .

**3.4.2 Phosphate solubilization and siderophores production:** The ten endophytic fungal isolates, previously identified for their tolerance to drought and salinity, were evaluated for their ability to solubilize tricalcium phosphate (TCP) and produce siderophores. As shown in (Fig. 4, (a)), all isolates exhibited phosphate solubilization activity after a 7-day incubation period. P14R3M1 and P5R2M1 demonstrated the highest phosphate solubilization capacity, releasing 256 and 245  $\mu\text{g/mL}$  of phosphate, respectively. In contrast, P5S1M1 displayed the lowest activity, solubilizing only 92  $\mu\text{g/mL}$ . A correlation was observed between phosphate solubilization and pH reduction; isolates P14R3M1 (pH 4.3) and P5R2M1 (pH 5.2) exhibited significantly lower pH values compared to P5S1M1 (pH 7). Siderophore production was assessed using the Chrome Azurol S (CAS) assay as shown in (Fig. 4, (b)). P4R3M1 produced the highest siderophore content (89%), followed by P5R2M1 (61%). Conversely, P14R1M1 and P2R2M1 exhibited the lowest siderophore levels (9% and 19%, respectively), with no significant difference between them at  $P \leq 0.05$ .



**Fig.(4): Phosphate solubilization and siderophores production potential of the ten drought and salinity-tolerant endophytic fungal isolates: (a) soluble phosphorus concentration and pH levels in culture filtrates. (b) percentage of siderophore production in culture filtrates. Values are mean of three replicates  $\pm$  SE. Values with different letter (s) are significantly different at  $P \leq 0.05$ .**

**3.4.3 Antioxidant activity and phytochemicals production:** All the assessed endophytic fungal isolates demonstrated significant antioxidant activity as assessed by permanganate-reducing antioxidant capacity (PRAC %) and total phenolic content (TPC) (Fig.5, (a)). P12R1M1 exhibited the highest antioxidant potential, with a PRAC value of 77%, followed by P1R2M1 (70%). In contrast, P14R3M1 displayed the lowest PRAC value of 3.2%. Consistent with the PRAC results, P12R1M1 and P4R2M1 also exhibited the highest total phenolic content (TPC), with values of 44 and 32  $\mu$ g gallic acid equivalents (GAE)/mg dry weight (DW) of ethanolic extract, respectively. P14R3M1 demonstrated the lowest TPC level at 3  $\mu$ g GAE/mg DW. As illustrated in (Fig. 5, (b)), a majority of the ten endophytic fungal isolates investigated produced gibberellic acid ( $GA_3$ ) in their culture filtrates. The highest  $GA_3$  concentration was observed in P14R3M1 at 671  $\mu$ g/mL, followed by P2R2M1 at 478  $\mu$ g/mL. Similarly, most isolates demonstrated the ability to produce indole-3-acetic acid (IAA) in a culture medium supplemented with L-tryptophan. P14R1M1 and P2R2M1 exhibited the highest IAA concentrations, both reaching 16 and 10  $\mu$ g/mL, respectively. However, P5S1M1 neither produced IAA nor  $GA_3$ .



**Fig. (5): Antioxidant activity and phytohormones production by the ten drought and salinity-tolerant endophytic fungal isolates: (a) Total phenolic content (TPC) and permanganate reducing antioxidant capacity (PRAC) %. (b) Gibberellic acid (GA<sub>3</sub>) and indole acetic acid (IAA) production. Values are mean of three replicates ± SE. Values with different letter (s) are significantly different at P ≤ 0.05.**

#### 4. DISCUSSION AND CONCLUSION

The increasing severity of environmental factors, such as drought and salinity, caused by climate change, poses a serious threat to agriculture and food security worldwide. To address these challenges, innovative solutions are urgently needed. Endophytic fungi, which live inside plants, have shown great potential as biofertilizers to improve plant resilience and crop yields. Specifically, fungi isolated from extreme environments can help plants tolerate drought and salt stress by enhancing water absorption, producing protective substances, regulating stress hormones, and efficiently acquiring nutrients [27].

The Amaranthaceae family emerged as the most common host plant found in our study, accounting for approximately 33% of all hosts. This is likely due to the diverse range of habitats in which Amaranthaceae plants can thrive, including hot, dry, and saline environments, as well as coastal, humid, temperate, and tropical regions. Notable adaptive traits of these plants include C<sub>4</sub> photosynthesis, continuous flowering, a deep taproot system, a wide-reaching lateral root system, the accumulation of compatible solutes, efficient water use, and the activation of genes and regulatory proteins involved in stress responses [28]. In this study, a total of 174 fungal isolates, representing eleven different genera, were recovered from nine wild plant species adapted to extreme habitats in Port Said Governorate. The most abundant fungal genus was *Alternaria*, accounting for 55% of the total isolates. These findings corroborate those of [29], who reported *Alternaria* as the dominant endophytic fungus in xerophytic plants from the Northwest China desert, comprising 20–65% of the fungal community.

Regarding drought and salinity tolerance, *Pseudoseptoria*, *Talaromyces*, and *Trichoderma* species demonstrated superior drought resistance, indicative of their potential xerophytic adaptations. Additionally, most isolates demonstrated high salinity tolerance, continuing to grow even at high concentrations of NaCl (3M NaCl). These findings align with those of [2] who reported that endophytic strains *Microdochium majus*, *Meyerozyma guilliermondi*, and *Aspergillus aculeatus* isolated from the xerophytic plant *Carthamus oxycantha* L. exhibited drought tolerance, with fungal biomass increasing by 14-20% when exposed to 8% PEG-8000. Furthermore, [3] documented the salt tolerance of three endophytic fungi from plants of Hoz-e Soltan Salt Lake in Iran, including *Neocamarosporium chichastianum*, *Neocamarosporium goegapense*, and *Periconia macrospinosa*, which were capable of growth at 3M NaCl.



Endophytic fungi possess a diverse array of hydrolytic enzymes, including amylase, cellulase, protease, and pectinase. This enzymatic activity breaks down complex biomolecules into simpler forms that can be utilized by the host plant, enhancing nutrient uptake, promoting cell wall expansion, and improving the plant's stress resilience, ultimately contributing to robust plant development [30]. In this study, all ten drought- and salinity-tolerant endophytic fungi demonstrated cellulase, pectinase, and chitinase activity, with some isolates showing limited or no ligninase, amylase, or protease activity. Remarkably, *A. terreus* (P2R2M1) and *C. globosporum* (P5S1M1) isolates exhibited the ability to produce all six assessed enzymes. Specifically, P2R2M1 and P4R3M1 isolates displayed high production capacity for most of the assessed enzymes. These findings align with previous reports highlighting the high enzymatic productivity of endophytic fungi, including *Aspergillus* and *Cladosporium* species [4, 18, 31].

Nutrient mobilization, another critical trait of endophytic fungi, is facilitated through mechanisms like iron chelation using siderophores and phosphorus solubilization via organic acid secretion. These processes increase nutrient accessibility, promoting plant growth and resilience, thereby offering potential benefits for sustainable agriculture [32]. All ten endophytic fungal isolates demonstrated significant nutrient mobilization capabilities, including phosphate solubilization and siderophore production. Notably, *T. viride* (P14R3M1) and *T. stipitatus* (P5R2M1) exhibited particularly high phosphate solubilization activity, accompanied by a decrease in culture filtrate pH, suggesting the presence of organic acids. *Cladosporium tenuissimum* (P4R3M1), on the other hand, produced the highest levels of siderophores. These findings align with previous researches on the nutrient mobilization abilities of endophytic fungi, including *Talaromyces*, *Trichoderma*, and *Cladosporium* species [23, 33, 34].

Endophytic fungi, which are well-adapted to their environments, can also mitigate the negative effects of both abiotic and biotic stressors, including oxidative stress caused by reactive oxygen species (ROS), on plants by producing phenolic and antioxidant metabolites. These metabolites directly scavenge ROS and stimulate the plant's own antioxidant production [37]. The selected endophytic fungal isolates exhibited notable antioxidant capacity and total phenolic content, with a strong correlation between high TPC and antioxidant capacity. This finding suggests that these fungi are highly tolerant to salt or drought stress and have the potential to alleviate plant abiotic stress. These results align with previous studies highlighting the antioxidant potential of endophytic fungi adapted to harsh environments [2, 24, 36]. Notably, *A. terreus* (P2R2M1) and *T. viride* (12R1M1) exhibited the highest antioxidant capacity and TPC, corroborating the findings of [37], who observed a predominance of phenolic compounds in *Aspergillus terreus* isolated from the desert plant *Hibiscus sabdariffa* (204.5 mg/mL in ethyl acetate extract) and a high total antioxidant capacity of 1.3 mg/ml.

Phytohormone production, including auxins and gibberellins, represents one of the most significant plant growth-promoting traits of endophytic fungi. These hormones play critical roles in plant development under various conditions [8]. In this study, all selected isolates produced IAA and GA<sub>3</sub>, except for *C. globosporum* (P5S1M1). The highest IAA production was observed in *A. hyalinulum* (P14R1M1), while *T. viride* (P14R3M1) exhibited the highest GA<sub>3</sub> production (671 µg/mL). These findings align with previous research on the phytohormone production capabilities of endophytic fungi, including *Acremonium* sp and *Trichoderma* sp. [33, 38]. In addition, several studies have demonstrated the role of phytohormones produced by habitat-adapted endophytic fungi in mitigating the negative effects of biotic and abiotic stresses on plants [34, 39, 40]. These findings collectively highlight the potential of the endophytic fungi isolated in this study from extreme habitats to serve as effective plant growth promoters.

In conclusion, this study demonstrated that endophytic fungi from the harsh coastal habitats of Port Said Governorate exhibit exceptional tolerance to drought and salinity. These fungi also displayed high extracellular enzymatic activity, efficient nutrient mobilization (such as phosphate solubilization and siderophore production), and significant antioxidant properties. Additionally, they produced essential



phytohormones, including indole-3-acetic acid (IAA) and gibberellic acid (GA<sub>3</sub>), underscoring their potential to promote plant growth and enhance tolerance to abiotic stress. Furthermore, this research documented new records of endophytic fungi in Egypt, specifically *Chaetomium globosporum* and *Pseudoseptoria* sp. Future research would focus on *in vitro* and greenhouse evaluations of the most promising isolates to improve salt and drought tolerance in some economically important plants.

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