



Diversity, Tolerance and Antioxidant Enzymes of Indigenous Fungi Isolated from Xerophytes Grown in Stressed Soil

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Abstract

Introduction: This study aimed at isolating and characterizing endophytic fungi from xerophytic plants collected from Egypt west deserts.

Materials and Methods: Soil samples and different plant species were collected from Egypt west deserts. The pH and electrical conductivity of soil samples were measured. Endophytic fungi were then isolated from collected plants and identified. Salt and pH tolerance of fungal species were assessed. Antioxidant enzymes of the most tolerant fungi were screened.

Results: The collected soil samples were highly saline and alkaline compared to Nile shore soil. Czapek Dox's agar media was the most suitable tested media for catching the highest count of endophytes from the xerophytic plants *Kochia indica*, *Zygophyllum coccineum*, *Amaranthus viridis* L, and *Dodonaea viscosa*. Moreover, *D. viscosa* was colonized with a high count of endophytic fungi. Low diversity of indigenous fungi was detected, where the 36 endophytic isolates constituted only four species. The tolerance assay toward different concentrations of salt and pH values proved that *A. terreus* and *A. flavus* were halotolerant and alkali-tolerant species, where they tolerate up to 3 M NaCl and pH values up to 11. Molecular identification of the most tolerant isolates resulted in *Aspergillus terreus* and *Aspergillus flavus* with accession numbers OQ271446 and OQ271456, respectively. It was found that the two antioxidant enzymes catalase and peroxidase displayed significant increases in their activity under high salinity and alkalinity compared to the control.

Conclusions: The isolated fungi were tolerant to salinity and alkalinity which significantly stimulate the antioxidant enzyme activities of these fungal species.

Keywords: Xerophytes, Endophytes, Stressed Soil, Antioxidant Enzymes

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Introduction

Plants are subjected to various environmental stresses stimulated by living organisms, from which they cannot escape because of their immobility. The main abiotic factors that injure plants include low or high temperatures, salt stress, drought, acidic environments, heavy metal stress, nutritional stress, and starvation.¹ Living organisms, such as viruses, bacteria, fungi, insects, parasites, and weeds, cause plant damage, known as biotic stress. Abiotic stresses such as salinity, high temperatures, and drought have a negative impact on crop production. One technique for coping with the detrimental impacts of unfavorable environmental conditions is the development of stress tolerance in plants.² Plants develop diverse physiological, biochemical, and molecular mechanisms to combat these stressors.³ Over the past few decades, changes in the Earth's climate have caused irregular and erratic precipitation in addition to an increase in temperature, the emergence of drought-prone regions, and the intensification of abiotic stress impacts on crops.⁴ Erosion, soil degradation, and salinity affect approximately 5.2 billion hectares of agricultural land.⁵ Soil salinization

occurs when water-soluble salts build-up in the soil to the point where they adversely affect agricultural production, environmental health, and the economy. Salinity affects the metabolism of soil organisms and reduces soil productivity during the early stages. However, in later stages, it obliterates all plants and other species living in the soil, converting fertile and productive land into barren and desertified areas.^{6,7} In general, saline soil has an exchangeable sodium content of 15%, and in the root zones saturation extract has an electrical conductivity (EC) exceeding 4 dS m⁻¹ (about 40 mM NaCl) at 25 °C. At this EC, the majority of the crop yields decreased.^{8,9}

Primary salinity, resulting from the long-term natural buildup of salts in the soil or surface water, is the primary cause of salinity in the soil. This natural process is induced mainly by the weathering of parent materials containing soluble salts due to the breakdown of rocks containing Cl⁻, Na⁺, Ca²⁺, and Mg²⁺, as well as occasionally SO₄²⁻ and CO₃²⁻. Furthermore, sea salt deposits carried by wind also affect the soil type. The second reason for soil salinity is

human-induced salinity, which results from anthropogenic activities that disturb the hydrological balance of the soil between applied water (via irrigation or rainfall) and water utilized by crops.^{9,10}

Soil pH significantly impacts soil biogeochemical activity in the natural environment. It affects various soil chemical, biological, and physical properties as well as processes that affect plant development and biomass yield. The soil pH is a "master soil variable".¹¹ Root strength, shoot and root development, and seedling survival rates were all significantly decreased by alkaline stress. Additionally, there was significant root cell damage, as seen by enhanced membrane injury, a buildup of malondialdehyde, and Evans Blue staining in alkaline soil. A substantial buildup of superoxide anions ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) was induced by alkaline stress. The build-up of reactive oxygen species (ROS) is intimately related to root cell damage under alkaline conditions. Moreover, under this stress, the antioxidant activities of catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), and peroxidase (POX) increased in the roots, most likely in response to cellular damage caused by oxidative stress.¹²

Recent studies have shown that endophytic fungi (which live in the leaves, stems, and roots of plants) play a critical role in controlling plant growth and stress tolerance by forming mutualistic associations that are known to be advantageous for both species. Fungal endophytes are actively involved in agriculture as biostimulants to increase crop protection and productivity. This will give farmers environmentally friendly methods to improve yield and quality without sacrificing plant health or disrupting the ecosystem.¹³ Endophytes are known for their ability to promote plant development and synthesize natural compounds under stressful conditions. These microorganisms live within healthy plant tissues, such as bacteria and fungi.² Endophytes, mainly fungi and bacteria, spend all or part of their lives inside living plant tissues with no detrimental side effects. They are essential for structuring and shaping plant activities such as competition, colonization, coexistence, and soil nutrient dynamics. Several endophytes boost host plant development by improving nitrogen and phosphate absorption. Endophytic fungi have recently acquired prominence in agriculture due to their capacity to provide tolerance to various abiotic stress conditions such as cold, light intensity, drought, temperature, salinity, etc., as well as biotic stress conditions, such as pathogens attack and insects herbivory, by assisting various processes of plant development.¹⁴

Endophytic fungi live in harmony with their host plants without causing infections, and produce bioactive secondary metabolites that protect host plants against environmental stress.¹⁵ Endophytic fungi can be employed as biofertilizers and antimicrobial agents to promote crop protection and productivity.¹⁶ As a result, endophytes have evolved two

different mechanisms to combat abiotic stress: (a) activating the host response system in response to stress to overcome its effects and (b) synthesizing anti-stress metabolites.²

Through the synthesis of reactive oxygen species (ROS), scavengers under stress conditions (like ascorbate, catalase, and glutathione) are crucial for the endophytic elimination of ROS.¹⁷ SOD, CAT, and APX are involved in ROS elimination directly, while ascorbate and glutathione are regenerated in the cell to remove ROS indirectly. Another endophytic enzyme that promotes stress tolerance is 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which cleaves the plant's ACC ethylene precursor.¹⁸ Other endophytic processes involved in mitigating salt stress in plants include nitrogen fixation, phytohormone synthesis, and the generation of compatible solutes.^{19,20} As osmotic and salt stresses develop, reactive oxygen species are produced in plants. Catalase, ascorbate peroxidase, and superoxide dismutase are scavenging enzymes that inhibit oxidation of membrane proteins, lipids, and DNA. Microorganisms employ similar strategies to combat oxidative stress.²¹

This study aimed to isolate and identify endophytic fungi from wild xerophytic plants collected from western deserts of Egypt. We also investigated the effects of soil alkalinity and salinity stress on the variability, tolerance, and antioxidant enzymes of the isolated fungi.

Materials and Methods

Sampling Site and Plant Species

In July 2020 and in November 2020, 3 different plant species, including *Kochia indica*, *Zygophyllum coccineum*, and *Amaranthus viridis* L, were collected from the side of Alexandria desert road with graphical coordinate 30° 45' 52" N 29° 58' 52" E. Another plant species named *Dodonaea viscosa* was collected in December 2020 from Giza desert land with graphical coordinate 30° 07' 48.6" N 31° 00' 23.4" E. Sample from Nile shore agricultural soil with graphical coordinate 29° 53' 42" N 31° 15' 47" E was collected and used as a control. These plant species were identified in the Cairo University Herbarium.

Assessment of pH and Salinity in Soil Samples

Soil samples were collected from the plant collection sites at a depth of 20 cm. The samples were placed in sterilized plastic bags and brought to the laboratory in an insulated container where they were air-dried for 48 h. The pH and electrical conductivity of each soil sample were then assessed. Soil pH was estimated by setting a digital pH meter (Mettler pH Meter, S220, B21959388) into a soil water suspension at a ratio of 1:2.5 (w/v) clarification solution.²² However, the electrical conductivity (EC, $dS\ m^{-1}$) of the soil was estimated according to Hardie and Doyle,²³ in which a 1:5 (w/v) soil water suspension was prepared and shaken for 30 min at 25 °C to dissolve soluble salts. The

sample was allowed to settle for 15 min and was analyzed using a Mettler Toledo conductivity meter (S230). Each measurement was repeated thrice.

Isolation of Indigenous Fungi from Xerophytic Plants

Fresh, infection-symptom-free plant samples were used to isolate the endophytic fungi. Plant materials were transferred to the laboratory for preparation within 12 h of collection in sterilized bags. Tissue samples were cleaned with distilled water several times to remove surface soil before being surface sterilized by immersion in 70% ethanol for 2 min, followed by 4% sodium hypochlorite for 90 s and 70% ethanol for 2 min. Finally, it was rinsed three times in sterile distilled water for 1 min each. The lack of microbial growth on the culture media after plating the last water-washed samples proved the efficacy of the surface-sterilizing approach. Young stem cuttings, and root pieces ranging from 0.5 to 1 cm in length were made. The inner portions of the tissues were plated in Petri dishes (9 cm diameter) containing different media (Potato Dextrose Agar (PDA), Czapek-Dox's agar, Martin's agar and Sabouraud dextrose agar) treated with chloramphenicol (50 mg/ml) and rose bengal. Plates were incubated at 28 ± 2 °C in the dark for four weeks and monitored daily for hyphal growth. Hyphal tips from the developing fungi were separated and sub-cultured to produce pure cultures for subsequent identification. Purified fungi were maintained at 4 °C.²⁴

Identification of the Indigenous Fungi

The developed fungal isolates were identified morphologically and microscopically, according to Moubasher.²⁵ Cultures of 4 days old were observed for phenotypic characteristics, such as color and shape of colonies. A light microscope (5, 10, and 40X) was used for spores and conidiophores examination. Furthermore, amplification and analysis of internal transcribed spacer (ITS) rDNA sequences for molecular identification were limited to more tolerant endophytes.

Molecular Identification

Whole genomic DNA was isolated for DNA-based analysis.²⁶ The universal primers ITS1 (ITS1-5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (ITS4-5'-TCC TCC GCT TATTGA TAT GC-3') were used to amplify the ITS region.²⁷ Each 25 µl MyTaq Red Mix contained 1 µl of each primer (20 pmol), 15 µl of nuclease-free water, and 8 µl of DNA template. Polymerase chain reaction (PCR, ArKTIK) amplification cycles included an initial denaturation for 6 min at 94 °C, denaturation for 45 s at 94 °C, annealing at 56 °C for 45 s (35 cycles), extension for 1 min at 72 °C, and a final extension for 5 minutes at 72 °C. The PCR products were purified and sequenced using the ITS1 primer. The BLAST tool from the National Center for Biotechnology

Information (NCBI) GenBank database was used to identify the obtained sequences. Using MEGA X, the endophyte sequences derived from NCBI were utilized for phylogenetic analysis based on the initial ITS sequence BLAST results from the GenBank database. All consensus sequences were uploaded to the GenBank database (<http://www.ncbi.nlm.nih.gov/BLAST>) to obtain the accession numbers of the fungal strains.

Assaying Salt Tolerance of the Indigenous Fungi

PDA medium containing five different concentrations of sodium chloride (1, 2, 3, 4, and 5 molar) was used to assess the salt tolerance of the fungal species. Three replicates were performed for each NaCl concentration tested. After inoculation and incubation at 25 ± 2 °C for 7 days, the capacity of the fungal isolates to proliferate in the presence of NaCl was recorded daily as radial growth (mm/plate).²⁸

Assaying pH Tolerance of Indigenous Fungi

A set of 250-ml Erlenmeyer flasks containing 100 ml of Dox's broth medium was prepared. After sterilization, the assay was conducted at pH values ranging from (3 to 13), which were adjusted using a pH meter (Mettler pH Meter, S220, B21959388). The flasks were inoculated with a fungal culture suspension (0.5 ml) and incubated statically for 7-10 days at 25 ± 2 °C.^{16,29} Growth at the specified pH was assessed by measuring the dry weight (mg/ml). Mycelia from the cultures were filtered using filter papers that had already been weighed and then dried at 70 °C to a constant weight to obtain dry biomass.³⁰ The experiments were repeated three times to ensure reliability and reproducibility.

Screening of Antioxidant Enzymes of the Most Tolerant Fungi

Qualitative Assay of the Antioxidant Enzymes Using H₂O₂

Fungal hyphae of the most tolerant species were grown on a PDA medium at 28 °C for 7 days. A disc of fungal growth (5 mm in diameter) of each fungus was placed inside a test tube containing 1-2 ml of 0.3% H₂O₂ solution to test the catalytic activity. Visual detection of enzyme synthesis was accomplished by observing the immediate bubbling.³¹

Extraction of Fungal Enzyme

A set of 250-ml Erlenmeyer flasks containing 100 ml broth Dox's medium was prepared for induction of alkalinity stress, where the flasks were adjusted at pH 9 and 11. Concerning salinity stress, another set of PDB flasks was prepared with 2 and 3M NaCl, which are the sublethal concentrations in saline tolerance). After inoculation with the selected endophytes, all cultures were shaken for 5 minutes then incubated at 28 °C for 7 days statically. Control samples for both stresses (without addition of NaCl or adjusted pH at 7) were prepared and inoculated under the

same conditions. After incubation, the media were centrifuged at 5000 rpm for 10 min, and peroxidase and catalase activities were tested in the supernatant.³²

Determination of Peroxidase and Catalase Activities

Peroxidase activity (POX; EC 1.11.1.7) was determined using guaiacol as substrate. A 50 mM potassium phosphate buffer (pH 7.0) containing 1.5mM guaiacol was added to the fungal supernatant (0.1 ml). The reaction was initiated by adding 60 mM H₂O₂ to the total reaction volume. The POX activity was estimated by monitoring the increase in absorbance at 470 nm every 30 s. The amount of enzyme required to oxidize 1 mmol of guaiacol per minute was defined as one unit of POX activity (extinction coefficient, 25.5 mM⁻¹cm⁻¹).³³

Catalase activity (CAT, EC 1.11.1.6) was measured by estimating the H₂O₂ breakdown. The catalase assay was determined using 0.1 ml culture supernatant mixed with 30 mM H₂O₂ and 50 mM phosphate buffer (pH 7.0). The reduction in the absorbance at 240 nm (A₂₄₀) was recorded for 3 min at 20 °C. A molar extinction coefficient of 43.6 M⁻¹cm⁻¹, was applied to calculate the amount of H₂O₂. The amount of enzyme needed to decompose 1 μmol H₂O₂ per minute of 30 mM initial H₂O₂ concentration at pH 7.0 and 25 °C was used to define one unit of CAT activity.³⁴

Determination of Extracellular Protein Concentration

The concentration of extracellular total proteins was measured

using Lowry's method for selected fungal samples.³⁵

Statistical Analysis

All tests were run in triplicate. A one-way analysis of variance (ANOVA) test was used to evaluate the data using Statistical Package of Social Science (SPSS) software version 20. Microsoft Office Excel (2013) represented the data. The Origin 2022b program was used for drawing heat maps. The results were expressed as mean ± standard deviations (mean ± SD). The differences between mean values were evaluated at $p \leq 0.05$ with Duncan's multiple range test.³⁶

Results

Assessment of Alkalinity and pH in Soil Samples

Desert soils had higher salinity and pH values than the Nile control soil (Table 1). The plant species *Kochia indica*, *Zygophyllum coccineum*, and *Amaranthus viridis L* tolerated and grew in highly saline and alkaline soils (samples 1, 2, and 3), respectively. *Dodonaea viscosa* tolerated and grew in moderately saline but highly alkaline soil (sample 4).

Diversity of the Indigenous Fungi Isolated from Xerophytic Plants

Total fungal count and heat map (Tables 2 & 3 and Figures 1 & 2) indicated that, from four types of media and four species of xerophytic plants, the total screened fungal isolates were 36 CFU/plate, comprising four endophytic species.

Table 1. Salinity and pH Determination at Isolation Sites of each Collected Plant

Soil sample	Collected plant from soil samples	Salinity EC _s (dS/m)*	pH
1	<i>Kochia indica</i>	0.990	8.80
2	<i>Zygophyllum coccineum</i>	0.920	9.08
3	<i>Amaranthus viridis L</i>	0.913	8.62
4	<i>Dodonaea viscosa</i>	0.427	8.30
5	Nile soil (control)	0.192	6.80

*Electrical conductivity (deci-Siemens per meter)

Table 2. Endophytic Fungi Isolated from Xerophytic Plants using Different Media

Medium	Plant sp	Total fungal count and frequency %									
		<i>Kochia indica</i>		<i>Zygophyllum coccineum</i>		<i>Amaranthus viridis L</i>		<i>Dodonaea viscosa</i>		Total / all	
		Tc*	Freq%**	Tc*	Freq%**	Tc*	Freq%**	Tc*	Freq%**	Tc/medium*	Freq%**
Potato Dextrose Agar		3	30.0	2	28.6	1	25.0	2	13.3	8	22.2
Czapek-Dox's agar		3	30.0	5	71.4	3	75.0	7	46.7	18	50.0
Martin's agar		2	20.0	0	0	0	0	3	20	5	13.9
Sabouraud dextrose agar		2	20.0	0	0	0	0	3	20	5	13.9
Total count/plant sp		10	27.8	7	19.4	4	11.1	15	41.7	36	100

*Total count = CFU/Plate; ** Frequency % = No. of isolated species/total isolates x 100

Table 3. Diversity of Indigenous Fungal Species Isolated from Xerophytic Plants Grown in Czapek-Dox's Agar Media

Fungal isolate	Plant sp	Total fungal count and frequency %									
		<i>A. terreus</i>		<i>A. flavus</i>		<i>A. fumigatus</i>		<i>A. aculeatus</i>		Total / all	
		Tc*	Freq%**	Tc*	Freq%**	Tc*	Freq%**	Tc*	Freq%**	Tc/plant*	Freq%**
<i>Kochia indica</i>		2	66.7	2	66.7	0	0	6	28.6	10	27.8
<i>Zygophyllum coccineum</i>		1	33.3	1	33.3	3	33.3	2	9.5	7	19.4
<i>Amaranthus viridis L</i>		0	0	0	0	1	11.1	3	14.3	4	11.1
<i>Dodonaea viscosa</i>		0	0	0	0	5	55.6	10	47.6	15	41.7
Total count/fungal sp		3	8.3	3	8.3	9	25.0	21	58.4	36	100

*Total count = CFU/Plate; ** Frequency % = No. of isolated species/total isolates x 100

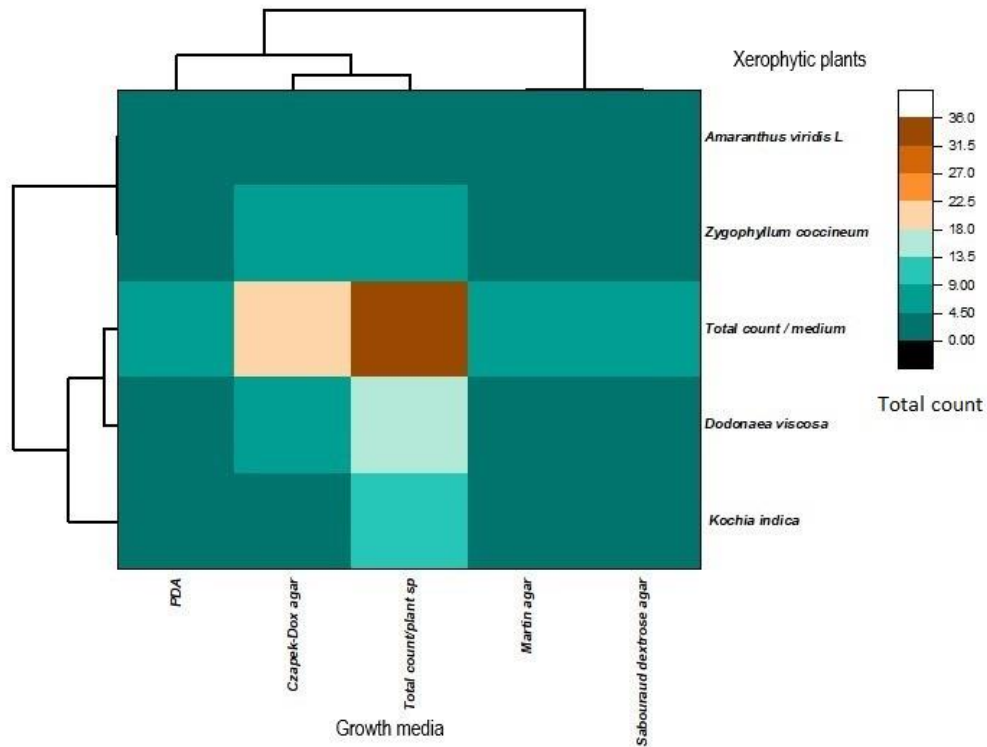


Figure 1. Heat Map of a Total Count of Indigenous Fungi Isolated from Xerophytic Plants using 4 Different Media.

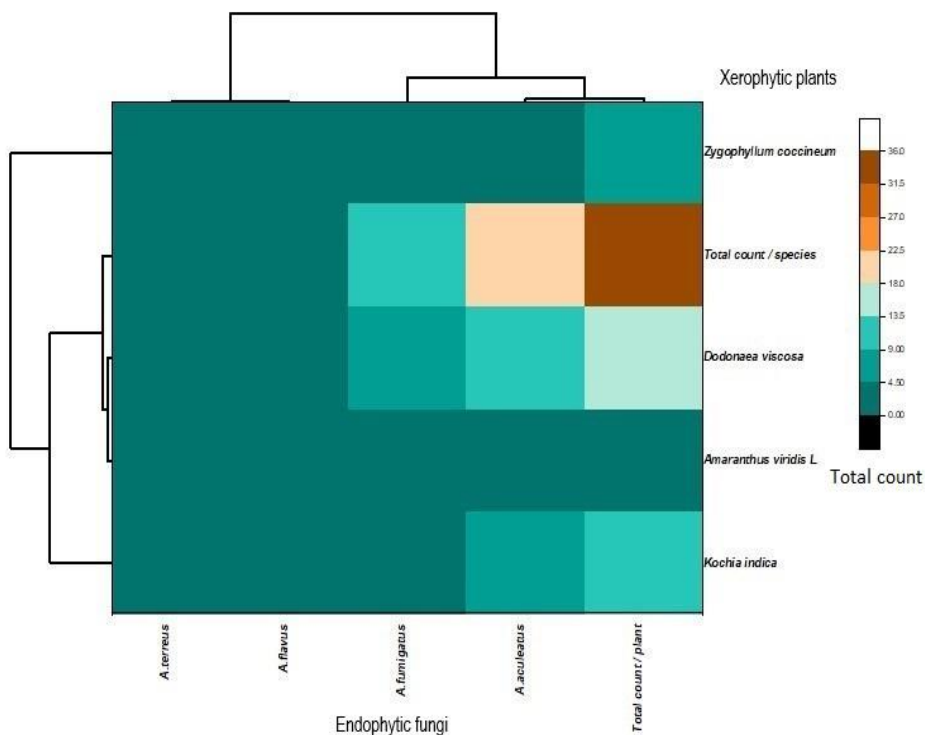


Figure 2. Heat Map of Indigenous Fungi Isolated from Four Xerophytic Plants.

The highly colonized plant with indigenous fungi was *D. viscosa* where fifteen isolates with frequency (41.7%) were isolated, 10 isolates with frequency (27.8%) from *K. indica*, 7 isolates with frequency (19.4%) from *Z. coccineum* and

only 4 isolates with frequency (11.1%) from *A. viridis L.*

Considering the media, the most suitable medium for isolating fungi was Czapek-Dox's agar medium, where 18 isolates were screened with a frequency of 50.0%, 8 isolates

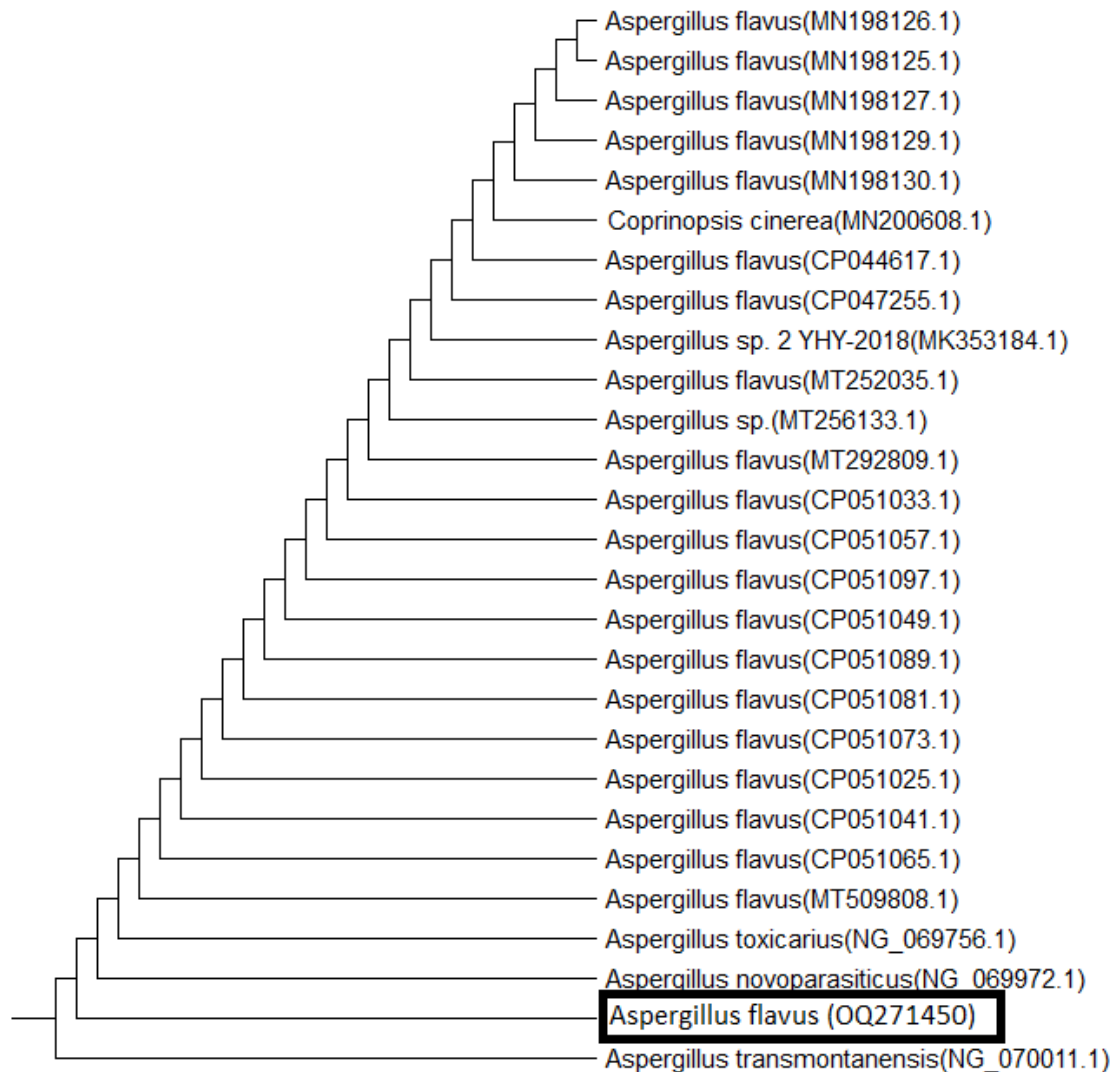


Figure 4. The Phylogenetic Tree Showing Relationship between Endophytic Fungal Species *Aspergillus flavus* OQ271450 and other Closely related NCBI GenBank Reference Taxa Sequences.

Salinity Tolerance of the Endophytic Fungi

The results showed that all fungal species were tolerant to salt concentrations and were capable of growing up to 3 M NaCl. *Aspergillus flavus* and *Aspergillus terreus* showed the highest growth at 3M with a minimum reduction percentage. The greatest reduction in growth was achieved with *A. fumigatus*. Salt concentration of 3M were considered sublethal while at 4M complete growth inhibition was recorded (Figure 5).

pH Tolerance of Endophytic Fungi

The endophytic fungi isolated from xerophytic plants proved to be alkali-tolerant and could grow up to pH 11, which displayed a sublethal value, where no growth was recorded at pH 13. The order of tolerance was *A. terreus* > *A. fumigatus* > *A. flavus* > *A. aculeatus* (Figure 6).

Qualitative Assay of the Antioxidant Enzymes Using H₂O₂

As shown in Table 4, *A. flavus* and *A. terreus* possessed

antioxidant enzyme activities, where H₂O₂ evolved in the reaction media. *A. terreus* being more active than *A. flavus*.

Quantitative Assay of the Antioxidant Enzymes

From previous results, *A. flavus* was the most tolerant to salinity stress, and *A. terreus* was the most tolerant to alkalinity stress. The data in Tables 5 and 6 indicate that alkalinity or salinity stress conditions significantly activated the production of antioxidant enzymes CAT and POX in the tested endophytic fungi.

A significant increase in peroxidase activity and specific activity was detected at 2M and 3M NaCl concentrations in the growth medium of *A. flavus*. The peroxidase activity and the specific activity at the sublethal NaCl concentration (3M) were 72.7 Units and 129.82 Units/mg, respectively, with about 6 folds increase than control in both the activity and the specific activity of the enzyme (Table 5).

Peroxidase activity also increased with an increase of pH to reach maximum activity and specific activity at the

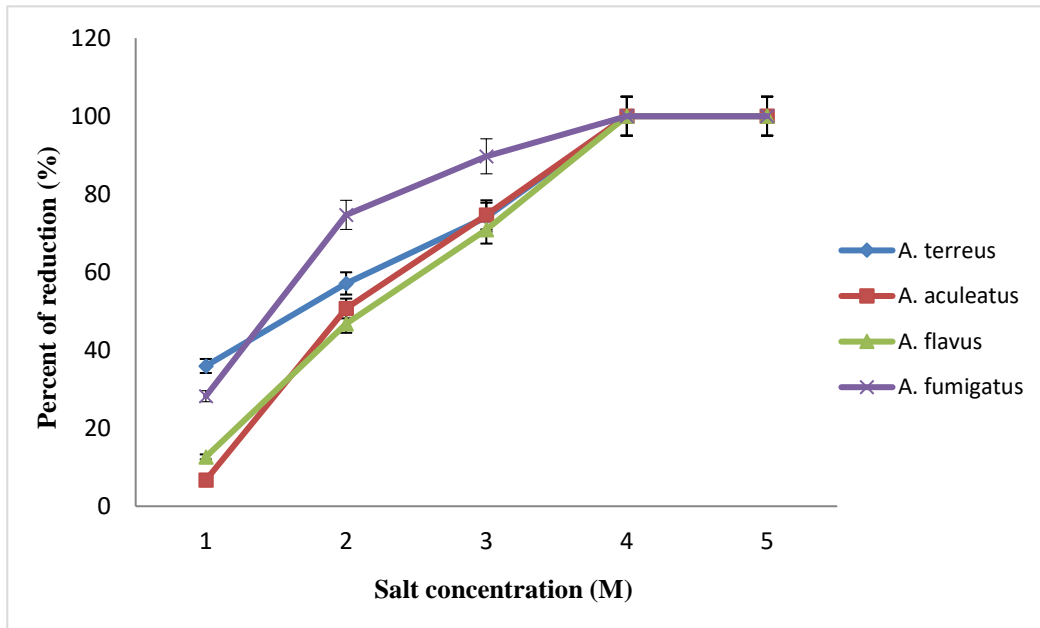


Figure 5. Reduction Percent in Radial Growth of Indigenous Fungi under Salinity Stress. The Results were Expressed as Mean ± SD.

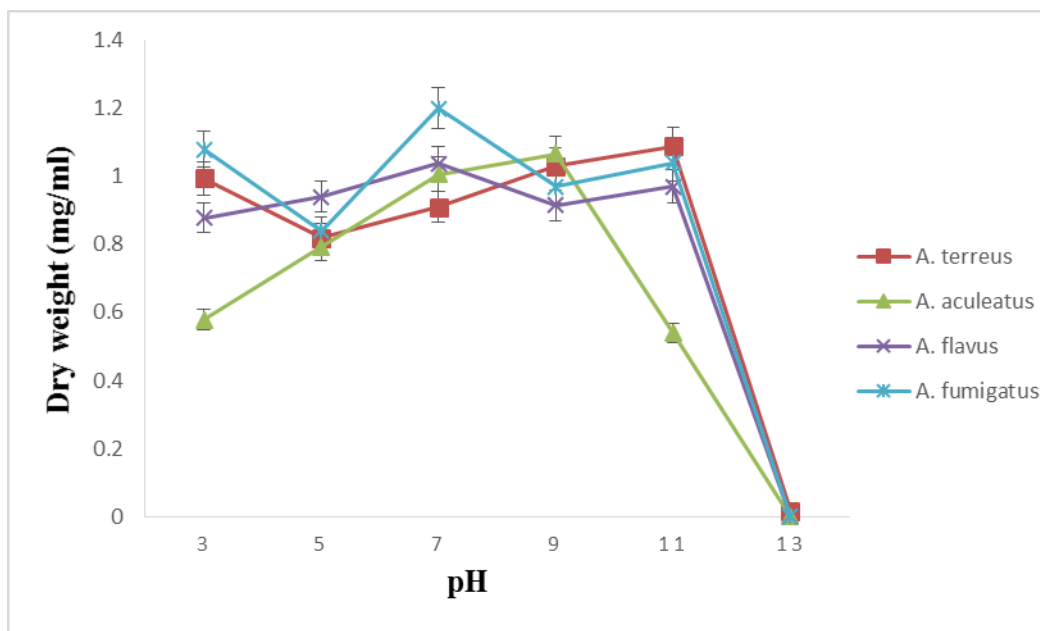


Figure 6. Effect of pH on the Dry Biomass Gain (mg/ml) of the Endophytic Fungi. The Results were Expressed as Mean ± SD.

sublethal value (pH 11) in the growth media of *A. terreus* with 43.7 Units and 78.1 Units/mg, respectively. This increase represents about 14 folds increase than control in both the activity and the specific activity of the enzyme (Table 5).

Table 6 revealed that catalase exhibited the same trend as peroxidase, where its activity increased with sublethal concentration (3M) in *A. flavus* growth media. This increase was approximately 3 folds than that of the control in both the activity and the specific activity of the enzyme.

Table 4. Qualitative Assay on Antioxidant Enzymes

Species	Antioxidant enzyme activity
<i>A. flavus</i>	++
<i>A. terreus</i>	++++

++ Moderate Activity; ++++ High Activity

Concerning the high alkalinity in the growth media of *A. terreus*, catalase showed a significant increase in activity and specific activity, especially at the sublethal value (pH 11), where an increase of approximately 6 folds to the control value was attained (Table 6).

Table 5. Assay of Peroxidase (POX) Activity under High Salinity (for *A. flavus*) and High Alkalinity (for *A. terreus*) Stress Conditions

Enzyme	Salinity			pH		
	Control (0M)	2M	3M	Control (pH 7)	pH 9	pH 11
POX activity (Units)	12.7 ^b ±0.38	20.94 ^c ±0.38	72.7 ^f ±0.18	3.2 ^a ±0.11	32.6 ^d ±0.12	43.76 ^e ±0.08
Protein content (mg/ml)	0.591 ^d ±0.01	0.45 ^a ±0.01	0.56 ^c ±0.03	0.58 ^d ±0.01	0.51 ^b ±0.01	0.56 ^c ±0.02
Specific activity (Units/ mg)	21.31 ^b ±0.75	46.53 ^d ±1.17	129.82 ^f ±0.60	5.52 ^a ±0.16	43.91 ^c ±1.22	78.14 ^e ±3.11

The results are expressed as mean ± SD. Mean values with different letters are considered significant at $p \leq 0.05$, according to Duncan's multiple range test.

Table 6. Assay of Catalase (CAT) Activity under High Salinity (for *A. flavus*) and High Alkalinity (for *A. terreus*) Stress Conditions

Enzyme	Salinity			pH		
	Control (0M)	2M	3M	Control (pH 7)	pH 9	pH 11
CAT activity (Units)	24.77 ^b ±0.03	59.72 ^d ±0.025	72.38 ^e ±0.11	13.9 ^a ±0.34	49.05 ^c ±0.07	79.04 ^f ±0.25
Protein content (mg/ml)	0.59 ^c ±0.03	0.45 ^a ±0.006	0.56 ^{b,c} ±0.01	0.58 ^c ±0.026	0.53 ^b ±0.071	0.56 ^{b,c} ±0.01
Specific activity (Units/ mg)	41.56 ^b ±1.91	132.7 ^d ±1.50	129.25 ^d ±2.20	23.97 ^a ±0.561	92.54 ^c ±0.925	141.14 ^e ±1.45

The results are expressed as mean ± SD. Mean values with different letters are considered significant at $p \leq 0.05$, according to Duncan's multiple range test.

Discussion

More attention is needed to study fungal endophytic species in stressed environments such as arid and semi-arid deserts. Endophytic fungi are necessary biotechnological tools for arid lands to improve agriculture and other activities under extreme environmental conditions. Consequently, microbial identification, diversity, community distribution, and variability are of significant importance.³⁷ The recent study reported high alkalinity and salinity in soil samples from which wild xerophytic plants were collected. Low variability and diversity were observed where only four endophytic fungal species were isolated from the four tested xerophytic plants. The ascomycetous fungi *A. terreus* and *A. flavus* (with accession numbers OQ271446 and OQ271450, respectively) were the most tolerant species to alkalinity and salinity stresses. In relation to this study, Moghaddam et al.³⁸ reported that endophytic composition and variation were influenced by host species, soil type, and electrical conductivity. Endophytes adapted to severe abiotic stress may transfer this tolerance to their host plants. However, El-Zayat et al.³⁹ found that endophytes proliferation and diversity were significantly inhibited in arid deserts. *Aspergillus* was the most frequent genus in *Hyoscyamus muticus* L., a desert-adapted medicinal plant. Other endophytic microbiomes isolated from arid soils, including those of *Alternaria* sp.,⁴⁰⁻⁴² *Penicillium*, and *Fusarium* genera.⁴³ They were isolated from asymptomatic halophytes. The variability of endophytic fungi isolated from xerophytic shrubs was significantly lower than that in temperate zones.⁴⁴⁻⁴⁶ Jalili et al.²⁸ studied the enzymatic activity and the salt tolerance of fungal species isolated from halophyte plants. They reported that *Acremonium sclerotigenum* and *Aspergillus terreus* obtained from *Rubia tinctorum* were isolated as halo-tolerant species with strong antibacterial and enzymatic activities. They also showed that *Microascus pyramidus* and *Monosporascus ibericus* were salt-tolerant isolates of *Salsola tomentosa* and *Anabasis iranica*. They revealed that the phylum *Ascomycota* contains strains that may be

involved in salt tolerance in plants. Irshad et al.⁴⁷ aimed to improve plant salt tolerance by estimating the sustainability of bio-priming salt tolerant endophytes. *Paecilomyces lilacinus* and *Trichoderma hamatum* tolerated high salt concentrations of up to 500 mM NaCl. García-Latorre et al.⁴⁸ evaluated the ability of 11 endophytic fungi isolated from plants growing in a severe environment in Spanish dehesas to grow under salt stress conditions (100, 200, and 500 mM NaCl). The eleven species were able to grow under salt stress conditions. The growth response differed broadly according to the fungal species. *Acremonium implicatum* and *Microdiplodia hawaiiensis* grew in a significantly higher rate at NaCl concentration of 500 mM than those which were grown without salt stress. In addition, *Fusarium avenaceum* and *Colletotrichum cereale* showed similar results, but only at lower concentrations of NaCl. Eighty eight percent of the endophytic fungi isolated from *Nigella sativa* L seeds were able to survive at 3% NaCl concentration with a maximum colony growth diameter of 7.5-8.0 cm.¹⁶ Among the 48 fungal endophytes screened, only four were found to be salt tolerant (*Chaetomium* sp., *Fusarium avenaceum*, *F. tricinctum*, and *Ulocladium* sp.). *Fusarium avenaceum* were found to be superior to other endophytes.⁴⁹ The endophytic fungus, *Bipolaris* sp. CSL-1 screened for NaCl stress tolerance can grow with up to 200 mM NaCl in Czapek medium.⁵⁰ Among the 130 fungal isolates screened *in vitro* for their tolerance to NaCl (1.0 M), 27 isolates were highly tolerant, 39 isolates were tolerant, and 58 isolates were moderately tolerant.⁵¹ Alkaliphobic fungal species could not withstand pH 10, but an alkali-tolerant species could grow at this value and less or more, while alkaliphilic species could grow up to pH 10.⁵² In the recent study, the isolated endophytes were all alkali-tolerant and halotolerant. They can tolerate a wide range of pH (3-11) and salinity (1-3 M). Gopane et al.¹⁶ found that 12% of isolated endophytes survived at high pH values. *Acremonium* sp. (ENF 31), and *Penicillium simplicissum* (ENF22) could grow at pH values ranging from 2-12 and 3-12, respectively.²⁹ Xiao et al.⁵³

isolated and screened stress-resistant endophytic fungal strains and found that there were differences in growth between different strains at the same acidic or basic environments, when compared with the control (pH 7). At pH 3, the growth of the seven strains was greater than that of the control. At pH 9, the growth of the two strains was higher than that of the control strain. The *in vitro* assay on the induction of catalase and peroxidase in the growth media of the endophytes *A. terreus* and *A. flavus* grown under high salinity or alkalinity indicated a significant increase in both activities. Endophytes such as fungi and bacteria are an acceptable tool for mediating tolerance in plants not only by stimulating growth but also by releasing hormones, growth-regulating and synthesizing ROS scavengers such as catalase and superoxide dismutase, which help plant survival under conditions of high salinity stress.⁵⁴ Reactive oxygen species (ROS) are produced and increased under salinity stress. Plants respond by an increase in antioxidant enzymes such as peroxidase, catalase, and superoxide dismutase and buildup of osmolytes such as proline.⁵⁵ However, producing antioxidants is essential to protect plants against oxidative stresses under biotic or abiotic stresses. Endophytes promote the induction of antioxidants and phenolic compounds in plants, where ROS are produced under any stress.¹⁴ *A. ochraceus* was able to withstand 200 g L⁻¹ of sodium chloride, which exhibited increased antioxidant activity under salt stress.^{56,57} Biochemical data demonstrated that under extreme salinity conditions, *Aspergillus sydowii* cells were protected against peroxidant molecules by the combined action of catalase, superoxide dismutase, and glutathione reductase, which act as a primary antioxidant defense system. To counteract the oxidative damage to cells caused by excessively high Na⁺ concentrations, *A. sydowii* elicited a broad antioxidant response. At 5.13 M NaCl, there was a significant increase in both catalase and superoxide dismutase activities, where superoxide dismutase significantly increased, roughly 197-fold, in the supernatant, contrary to the optimal 1.0 M NaCl concentration, whereas catalase activity was doubled in the supernatant and mycelium exposed.⁵⁸

Conclusion

This study clearly showed that the indigenous fungal species *A. flavus* OQ271456 and *A. terreus* OQ271446 isolated from wild xerophytic plants were highly tolerant to salinity and alkalinity stress. It also proved that these stress conditions significantly stimulated the activities of the antioxidant enzymes CAT and POX in these fungal species. In future studies, we aim to use these fungal species to improve the growth and productivity of wheat plants suffering from high alkalinity, salinity, or both.

Authors' Contributions

ARTMA, AEAM, and ADMI contributed to the study's

conception and design. Methodology, data collection, and analysis were performed by SNHF. Statistical analyses of the results were performed by AEAM. The first draft of the manuscript was written by SNHF. Reviewing and editing were performed by ARTMA, AEAM, and ADMI. All authors read and approved the final manuscript.

Authors' Contributions

All authors contributed equally to this study.

Data Availability

The manuscript includes all the data generated in this study. Sequences resulting from molecular identification are deposited in the GenBank database (accessible at: <http://www.ncbi.nlm.nih.gov/BLAST>).

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

References

- Chaves MM, Oliveira MM. Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. *J Exp Bot.* 2004;55(407):2365-84. doi:10.1093/jxb/erh269
- Lata R, Chowdhury S, Gond SK, White JF. Induction of abiotic stress tolerance in plants by endophytic microbes. *Lett Appl Microbiol.* 2018;66(4):268-76. doi:10.1111/lam.12855
- Nanda S, Mohanty B, Joshi RK. Endophyte-mediated host stress tolerance as a means for crop improvement. *Endophytes and secondary metabolites.* 2019:677-701. doi:10.1007/978-3-319-76900-4_28-1
- Meena KK, Sorty AM, Bitla UM, Choudhary K, Gupta P, Pareek A, et al. Abiotic stress responses and microbe-mediated mitigation in plants: the omics strategies. *Front Plant Sci.* 2017;8:172. doi:10.3389/fpls.2017.00172
- Numan M, Bashir S, Khan Y, Mumtaz R, Shinwari ZK, Khan AL, et al. Plant growth promoting bacteria as an alternative strategy for salt tolerance in plants: a review. *Microbiol Res.* 2018;209:21-32. doi:10.1016/j.micres.2018.02.003
- Jones A, Panagos P, Barcelo S, Bouraoui F, Bosco C, Dewitte O, et al. The state of soil in Europe. *JRC Reference Reports.* 2012;78. doi:10.2788/77361
- Stolbovoy V, Montanarella L. Application of Soil Organic Carbon Status Indicators for policy-decision making in the EU. *Threats to soil quality in Europe.* 2008:87. doi:10.2788/8647
- Jamil A, Riaz S, Ashraf M, Foolad MR. Gene expression profiling of plants under salt stress. *Crit Rev Plant Sci.* 2011;30(5):435-58. doi:10.1080/07352689.2011.605739
- Munns R. Genes and salt tolerance: bringing them together. *New Phytol.* 2005;167(3):645-63. doi:10.1111/j.1469-8137.2005.01487.x
- Manchanda G, Garg N. Salinity and its effects on the functional biology of legumes. *Acta Physiol Plant.* 2008;30:595-618. doi:10.1007/s11738-008-0173-3
- Minasny B, Hong SY, Hartemink AE, Kim YH, Kang SS. Soil pH increase under paddy in South Korea between 2000 and 2012. *Agric Ecosyst Environ.* 2016;221:205-13. doi:10.1016/j.agee.2016.01.042
- Zhang H, Liu XL, Zhang RX, Yuan HY, Wang MM, Yang

- HY, et al. Root damage under alkaline stress is associated with reactive oxygen species accumulation in rice (*Oryza sativa* L.). *Front Plant Sci.* 2017;8:1580. doi:10.3389/fpls.2017.01580
13. Toppo P, Subba R, Roy K, Mukherjee S, Mathur P. Elucidating the strategies for isolation of endophytic fungi and their functional attributes for the regulation of plant growth and resilience to stress. *J Plant Growth Regul.* 2023;42(3):1342-63. doi:10.1007/s00344-022-10638-w
 14. Dey P, Datta D, Saha D, Parida S, Panda D. Plant-endophyte interaction and its application to abiotic stress management of crop plants. *Int J Curr Microbiol App Sci.* 2019;8(7):2708-16. doi:10.20546/ijcmas.2019.807.332
 15. Manganyi MC, Regnier T, Kumar A, Bezuidenhout CC, Ateba CN. Biodiversity and antibacterial screening of endophytic fungi isolated from *Pelargonium sidoides*. *S Afr J Bot.* 2018;116:192-9. doi:10.1016/j.sajb.2018.03.016
 16. Gopane B, Tchatchouang CK, Regnier T, Ateba CN, Manganyi MC. Community diversity and stress tolerance of culturable endophytic fungi from black seed (*Nigella sativa* L.). *S Afr J Bot.* 2021;137:272-7. doi:10.1016/j.sajb.2020.10.026
 17. Rouhier N, San Koh C, Gelhaye E, Corbier C, Favier F, Didierjean C, et al. Redox based anti-oxidant systems in plants: biochemical and structural analyses. *Biochim Biophys Acta - Gen Subj.* 2008;1780(11):1249-60. doi:10.1016/j.bbagen.2007.12.007
 18. Glick BR. Bacteria with ACC deaminase can promote plant growth and help to feed the world. *Microbiol Res.* 2014;169(1):30-9. doi:10.1016/j.micres.2013.09.009
 19. Hardoim PR, Van Overbeek LS, Berg G, Pirttilä AM, Compant S, Campisano A, et al. The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiol Mol Biol Rev.* 2015;79(3):293-320. doi:10.1128/mmbr.00050-14
 20. Hassan SE. Plant growth-promoting activities for bacterial and fungal endophytes isolated from medicinal plant of *Teucrium polium* L. *J Adv Res.* 2017;8(6):687-95. doi:10.1016/j.jare.2017.09.001
 21. Hediye Sekmen A, Türkan İ, Takio S. Differential responses of antioxidative enzymes and lipid peroxidation to salt stress in salt-tolerant *Plantago maritima* and salt-sensitive *Plantago media*. *Physiol Plant.* 2007;131(3):399-411. doi:10.1111/j.1399-3054.2007.00970.x
 22. Zuo Y, Hu Q, Liu J, He X. Relationship of root dark septate endophytes and soil factors to plant species and seasonal variation in extremely arid desert in Northwest China. *Appl Soil Ecol.* 2022;175:104454. doi:10.1016/j.apsoil.2022.104454
 23. Hardie M, Doyle R. Measuring soil salinity. *Plant salt tolerance: methods and protocols.* 2012:415-25. doi:10.1007/978-1-61779-986-0_28
 24. Hosseyni-Moghaddam MS, Soltani J. Bioactivity of endophytic Trichoderma fungal species from the plant family Cupressaceae. *Ann Microbiol.* 2014;64:753-61. doi:10.1007/s13213-013-0710-1
 25. Moubasher AH. Soil fungi in Qatar and other Arab countries. The Centre for Scientific and Applied Research, University of Qatar. *Econ Bot.* 1993;50:242. doi:10.1007/BF02861455
 26. Salehi M, Moieni A, Safaie N. Elicitors derived from hazel (*Corylus avellana* L.) cell suspension culture enhance growth and paclitaxel production of *Epicoccum nigrum*. *Sci Rep.* 2018;8(1):12053. doi:10.1038/s41598-018-29762-3
 27. White TJ, Bruns T, Lee SJ, Taylor J. Amplification and Direct Sequencing of Fungal Ribosomal Rna Genes for Phylogenetics. *PCR protocols: a guide to methods and applications.* 1990;18(1):315-22. doi:10.1016/b978-0-12-372180-8.50042-1
 28. Jalili B, Bagheri H, Azadi S, Soltani J. Identification and salt tolerance evaluation of endophyte fungi isolates from halophyte plants. *Int J Environ Sci Technol.* 2020;17:3459-66. doi:10.1007/s13762-020-02626-y
 29. Potshangbam M, Devi SI, Sahoo D, Strobel GA. Functional characterization of endophytic fungal community associated with *Oryza sativa* L. and *Zea mays* L. *Front Microbiol.* 2017;8:325. doi:10.3389/fmicb.2017.00325
 30. Broekaert WF, Terras FR, Cammue BP, Vanderleyden J. An automated quantitative assay for fungal growth inhibition. *FEMS Microbiol Lett.* 1990;69(1-2):55-9. doi:10.1111/j.1574-6968.1990.tb04174.x
 31. Bansal N, Kanwar SS. Peroxidase (s) in environment protection. *Sci World J.* 2013;2013(1):714639. doi:10.1155/2013/714639
 32. Asemoloye MD, Ahmad R, Jonathan SG. Transcriptomic responses of catalase, peroxidase and laccase encoding genes and enzymatic activities of oil spill inhabiting rhizospheric fungal strains. *Environ Pollut.* 2018;235:55-64. doi:10.1016/j.envpol.2017.12.042
 33. Nakano Y, Asada K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.* 1981;22(5):867-80. doi:10.1093/oxfordjournals.pcp.a076232
 34. Aebi H. catalase in vitro. *Methods in Enzymology.* 1984;105:121-6. doi:10.1016/s0076-6879(84)05016-3
 35. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem.* 1951;193(1):265-75. doi:10.1016/s0021-9258(19)52451-6
 36. Duncan DB. Multiple range and multiple F tests. *Biometrics.* 1955;11(1):1-42. doi:10.2307/3001478
 37. Moghaddam MS, Safaie N, Soltani J, Hagh-Doust N. Desert-adapted fungal endophytes induce salinity and drought stress resistance in model crops. *Plant Physiol Biochem.* 2021;160:225-38. doi:10.1016/j.plaphy.2021.01.022
 38. Moghaddam MS, Safaie N, Tedersoo L, Hagh-Doust N. Diversity, community composition, and bioactivity of cultivable fungal endophytes in saline and dry soils in deserts. *Fungal Ecol.* 2021;49:101019. doi:10.1016/j.funeco.2020.101019
 39. El-Zayat SA, Nassar MS, El-Hissy FT, Abdel-Motaal FF, Ito SI. Mycoflora associated with *Hyoscyamus muticus* growing under an extremely arid desert environment (Aswan region, Egypt). *J Basic Microbiol.* 2008;48(2):82-92. doi:10.1002/jobm.200700107
 40. Zuo Y, Hu Q, Zhang K, He X. Host and tissue affiliations of culturable endophytic fungi associated with xerophytic plants in the desert region of northwest china. *Agronomy.* 2022;12(3):727. doi:10.3390/agronomy12030727
 41. Gond SK, Mishra A, Sharma VK, Verma SK, Kumar J, Kharwar RN, et al. Diversity and antimicrobial activity of endophytic fungi isolated from *Nyctanthes arbor-tristis*, a well-known medicinal plant of India. *Mycoscience.* 2012;53(2):113-21. doi:10.1007/s10267-011-0146-z
 42. Muhsin TM, Booth T. Fungi associated with halophytes of an inland salt marsh, Manitoba, Canada *Canad J. Bot.* 1987;65(6):1137-51. doi:10.1139/b87-159
 43. Gautam AK. Diversity of fungal endophytes in some medicinal plants of Himachal Pradesh, India. *Arch Phytopathol Plant Prot.* 2014;47(5):537-44. doi:10.1080/03235408.2013.813678
 44. Luo J, Walsh E, Miller S, Blystone D, Dighton J, Zhang N. Root endophytic fungal communities associated with

- pitch pine, switchgrass, and rosette grass in the pine barrens ecosystem. *Fungal Biol.* 2017;121(5):478-87. doi:10.1016/j.funbio.2017.01.005
45. Bonfim JA, Vasconcellos RL, Baldesin LF, Sieber TN, Cardoso EJ. Dark septate endophytic fungi of native plants along an altitudinal gradient in the Brazilian Atlantic forest. *Fungal Ecol.* 2016;20:202-10. doi:10.1016/j.funeco.2016.01.008
46. Loro M, Valero-Jiménez CA, Nozawa S, Márquez LM. Diversity and composition of fungal endophytes in semiarid Northwest Venezuela. *J Arid Environ.* 2012;85:46-55. doi:10.1016/j.jaridenv.2012.04.009
47. Irshad K, Shaheed Siddiqui Z, Chen J, Rao Y, Hamna Ansari H, Wajid D, et al. Bio-priming with salt tolerant endophytes improved crop tolerance to salt stress via modulating photosystem II and antioxidant activities in a sub-optimal environment. *Front Plant Sci.* 2023;14:1082480. doi:10.3389/fpls.2023.1082480
48. García-Latorre C, Rodrigo S, Santamaría O. Potential of fungal endophytes isolated from pasture species in Spanish dehesas to produce enzymes under salt conditions. *Microorganisms.* 2023;11(4):908. doi:10.3390/microorganisms11040908
49. Pandit AG, Vendan KT, Earanna N. Fungal endophyte mediated salinity stress tolerance in mung bean (*Vigna radiata* L. Wilczek). *Mysore J Agric Sci.* 2023;57(2):40-7.
50. Lubna, Khan MA, Asaf S, Jan R, Waqas M, Kim KM, et al. Endophytic fungus *Bipolaris* sp. CSL-1 induces salt tolerance in *Glycine max.* L via modulating its endogenous hormones, antioxidative system and gene expression. *J Plant Interact.* 2022;17(1):319-32. doi:10.1080/17429145.2022.2036836
51. Manjunatha N, Manjunatha N, Li H, Sivasithamparam K, Jones MG, et al. Fungal endophytes from salt-adapted plants confer salt tolerance and promote growth in wheat (*Triticum aestivum* L.) at early seedling stage. *Microbiology.* 2022;168(8):001225. doi:10.1099/mic.0.001225
52. Nagai K, Sakai T, Rantiatmodjo RM, Suzuki K, Gams W, Okada G. Studies on the distribution of alkalophilic and alkali-tolerant soil fungi I. *Mycoscience.* 1995;36(3):247-56. doi:10.1007/BF02268598
53. Xiao JL, Sun JG, Pang B, Zhou X, Gong Y, Jiang L, et al. Isolation and screening of stress-resistant endophytic fungus strains from wild and cultivated soybeans in cold region of China. *Appl Microbiol Biotechnol.* 2021;105:755-68. doi:10.1007/s00253-020-11048-2
54. Fouda A, Hassan SE, Eid AM, El-Din Ewais E. The interaction between plants and bacterial endophytes under salinity stress. *Endophytes and secondary metabolites. Reference Series in Phytochemistry.* Springer, Cham. 2019. doi:10.1007/978-3-319-90484-9_15
55. Wang J, Zhu J, Zhang Y, Fan F, Li W, Wang F, et al. Comparative transcriptome analysis reveals molecular response to salinity stress of salt-tolerant and sensitive genotypes of indica rice at seedling stage. *Sci Rep.* 2018;8(1):2085. doi:10.1038/s41598-018-19984-w
56. Gupta S, Schillaci M, Walker R, Smith PM, Watt M, Roessner U. Alleviation of salinity stress in plants by endophytic plant-fungal symbiosis: Current knowledge, perspectives and future directions. *Plant Soil.* 2021:219-44. doi:10.1007/s11104-020-04618-w
57. Zhang F, Wang Y, Liu C, Chen F, Ge H, Tian F, et al. *Trichoderma harzianum* mitigates salt stress in cucumber via multiple responses. *Ecotoxicol Environ Saf.* 2019;170:436-45. doi:10.1016/j.ecoenv.2018.11.084
58. Jiménez-Gómez I, Valdés-Muñoz G, Moreno-Perlin T, Mouriño-Pérez RR, Sánchez-Carbente MD, Folch-Mallol JL, et al. Haloadaptive responses of *Aspergillus sydowii* to extreme water deprivation: morphology, compatible solutes, and oxidative stress at NaCl saturation. *J Fungi.* 2020;6(4):316. doi:10.3390/jof6040316