Research Square

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Salinity Stress Alleviation in Maize Using Endophytic Fungi Isolated from Desert Trees: Physiological and Biochemical Responses

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Research Article

Keywords: Antioxidant system, Endophytic fungi, Biochemical traits, Bio-inoculants, Desert trees, Growth parameters, Ion homeostasis, Salt tolerance

Posted Date: June 3rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9371056/v1

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Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at BMC Plant Biology on August 11th, 2026. See the published version at https://doi.org/10.1186/s12870-026-09695-0.

Abstract

Background

This study investigated the effects of two desert root-endophytic fungi, *Trichoderma harzianum* (T) and *Fusarium solani* (F), applied alone and in combination (TF), on maize growth under salinity stress (levels S0 to S3).

Results

Salinity significantly reduced vegetative traits, including fresh biomass (− 35.92%), dry biomass (− 44.38%), tissue moisture content (− 33.97%), root-to-shoot ratio (− 66.96%), and greenness index (− 13.16%), while increasing electrolyte leakage (+ 124.19%), malondialdehyde (+ 19.91%), and ion accumulation (Na and Cl). Inoculation with endophytes mitigated salinity-induced damage, leading to significant increases in growth parameters, such as fresh biomass (+ 10.76%), dry biomass (+ 18.04%), tissue moisture content (+ 9.33%), root-to-shoot ratio (+ 26.34%), relative water content (+ 5.34%), membrane stability index (+ 19.30%), and greenness index (+ 7.74%). Additionally, endophyte inoculation enhanced antioxidant enzyme activities (CAT + 28.87%, POD + 39.55%, APX + 37.01%, SOD + 18.98%) and DPPH radical scavenging activity (+ 21.69%). It also reduced electrolyte leakage (− 25.81%) and malondialdehyde (− 20.29%), while improving ion homeostasis by decreasing Na^+ and Cl^- accumulation in leaves and roots.

Conclusions

The protective effects of endophytes were more pronounced under higher salinity stress (S3), particularly with the *Fusarium solani* and *Trichoderma harzianum* combination. These fungi enhance the salt tolerance of maize and demonstrate strong potential as bio-inoculants for saline agriculture.

Introduction

Global food security is increasingly threatened by rapid population growth and climate change. According to the Food and Agriculture Organization of the United Nations (FAO), the global population is projected to exceed 9.3 billion by 2050, necessitating at least a 60% increase in food production to meet future demand [1]. Achieving this target is particularly challenging under ongoing climate change, which exacerbates abiotic stress in agricultural systems and substantially limits crop productivity worldwide [2].

Maize (*Zea mays* L.) is one of the most widely cultivated cereal crops globally, ranking third after wheat and rice in terms of production volume. It plays a critical role in human nutrition, livestock feed production and biofuel production [3, 4]. Despite its economic and nutritional importance, maize exhibits moderate sensitivity to salinity stress, with yield losses ranging from 20 to 46% in saline and sodic soils [5]. Given that maize is cultivated on more than 170 million hectares worldwide, much of it in developing and semi-arid regions, the expansion of soil salinization poses a serious threat to its sustainable production [1, 6].

Salinity stress adversely affects plant growth through a biphasic mechanism involving osmotic stress in the early stages and ionic toxicity in the later stages. Reduced water uptake caused by elevated soil osmotic pressure limits cell expansion, whereas excessive accumulation of Na^+ and Cl^- ions disrupt ionic homeostasis, impairs nutrient acquisition, and inhibits photosynthetic and metabolic processes [7, 8]. Salinity also induces oxidative stress by promoting the overproduction of reactive oxygen species (ROS). Although ROS function as signaling molecules at low concentrations, their excessive accumulation damages lipids, proteins, nucleic acids, and enzymes, ultimately compromising cell viability and plant performance [9, 10].

To cope with salinity stress, plants activate a complex network of physiological and biochemical responses, including osmotic adjustment, maintenance of ionic balance, and activation of antioxidant defense systems. Enzymatic antioxidants, such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), along with non-enzymatic antioxidants, including ascorbate, glutathione, carotenoids, and compatible osmolytes such as proline, play central roles in mitigating oxidative damage and maintaining cellular redox homeostasis [11, 12]. Among these, proline is particularly important because of its multifunctional role in osmotic regulation, membrane stabilization, ROS detoxification, and stress signaling [8, 13, 14].

In recent years, the exploitation of beneficial microorganisms has emerged as a sustainable and environmentally friendly strategy for enhancing plant tolerance to abiotic stresses. Endophytic fungi that colonize internal plant tissues without causing disease have gained increasing attention because of their ability to modulate host physiology and improve stress resilience [2, 15]. Endophytes can enhance plant performance under stress by improving root architecture, regulating osmotic balance, activating antioxidant systems, modulating phytohormone levels, and improving nutrient uptake [16–19].

In this context, desert ecosystems, characterized by extreme environmental constraints such as severe water scarcity, high salinity, intense ultraviolet radiation, and pronounced temperature fluctuations, are known as unique habitats and reservoirs for the discovery of stress-adapted and functionally efficient microorganisms [20–22]. Native plants inhabiting these harsh environments have evolved an array of morphological, physiological, and molecular adaptations to endure extreme conditions, a significant proportion of which is attributed to their symbiotic association with salinity- and drought-tolerant endophytic fungi [23]. These fungal endophytes have undergone intense selective pressure, resulting in distinctive traits such as high salinity tolerance, the ability to produce stress-resistant enzymes, and robust antioxidant activity, which can be transferred to sensitive crop species [18, 19, 24]. Despite this considerable potential, the biodiversity, functional mechanisms, and agronomic applicability of desert-adapted native endophytes remain insufficiently understood. This knowledge gap is a serious obstacle to the practical exploitation of these resources in the future.

Previous studies have demonstrated that endophytic fungi can enhance plant tolerance to abiotic stress by regulating antioxidant activity, osmolyte accumulation, membrane stability, and photosynthetic efficiency [16, 19]. In our earlier study, inoculation of maize with *Trichoderma harzianum* and *Fusarium solani*, isolated from native desert plants, significantly improved drought tolerance by enhancing growth, photosynthetic performance, osmolyte accumulation, and membrane integrity [25]. However, the potential of these desert-derived endophytes to enhance maize tolerance to salinity stress and the associated physiological and biochemical mechanisms remain largely unexplored. Given the high capacity of these strains to tolerate osmotic conditions, it is hypothesized that these endophytes can also be effective in reducing the detrimental effects of salinity stress.

Therefore, the present study aimed to evaluate the capacity of native desert root-associated endophytic fungi to enhance salinity tolerance in maize plants. From a collection of 640 fungal isolates obtained from desert plants, 109 isolates were screened for salinity tolerance under in vitro conditions, and two superior isolates, *Trichoderma harzianum* (81Y1) and *Fusarium solani* (19K3), were selected for greenhouse experiments. The effects of these endophytes, applied individually and in combination, were assessed on maize morphological traits, physiological status, osmotic adjustment, antioxidant responses, photosynthetic performance, and mineral nutrient balance under salinity stress. This study provides mechanistic insights into the role of desert-derived endophytic fungi in improving maize salinity tolerance and highlights their potential application in sustainable agriculture under saline conditions.

Materials and methods

Isolation of fungal strains and evaluation of their salt tolerance under in-vitro conditions

To recover endophytic fungi associated with the roots of common tree species native to Iran's hyper-arid deserts, root samples were collected between 2018 and 2021 from healthy, asymptomatic individuals representing 12 desert tree and shrub species: *Prosopis spicigera*, *P. stephaniana*, *Zizyphus spina-christi*, *Calotropis procera*, *Tamarix sp.*, *T. aphylla*, *Amygdalus scoparia*, *Haloxylon aphyllum*, *H. ammodendron*, *H. persicum*, *Seidlitzia rosmarinus*, and *Lycium shawii*. Sampling was conducted across 61 desert locations in Bushehr, Kerman, Yazd, Isfahan, and Fars provinces, with sites selected randomly based on the dominant vegetation in each desert area (Fig. 1, Table 1- supplementary files).

Endophytic fungi were isolated from fresh root tissues according to the protocol described by Zhou et al. [26]. Roots were first sectioned into 1.5 cm segments, washed under running tap water for 20 min, and surface sterilized by sequential immersion in 70% ethanol (1 min), 4% sodium hypochlorite (2–5 min, depending on root thickness), and again in 70% ethanol (1 min). The segments were then rinsed three times in sterile distilled water, blotted dry on sterile filter paper, and aseptically cut into 1 cm pieces. The inner tissue was placed on potato dextrose agar (PDA) supplemented with 250 µL surfactant (Sigma TERGITOL-type TMN-6), ampicillin (50 µg mL^{-1}), and rifampin (10 µg mL^{-1}) and incubated at 26 ± 2°C in the dark for 1–8 weeks. Emerging fungal colonies were regularly examined under a dissecting microscope and subcultured until pure cultures were obtained. Purified isolates were grouped into morphotypes based on colony characteristics and reproductive structures and preserved on sterile filter paper at -20°C and on PDA slants at 4°C for future use.

To assess salinity tolerance, representative isolates were cultured on PDA amended with varying NaCl concentrations (unpublished data). Based on in vitro performance, two endophytic isolates each recovered from different host species and geographic origins and displaying strong salinity tolerance were selected for greenhouse trials.

For molecular identification, total DNA was extracted from fresh mycelia grown on PDA, as described by Zhong and Steffenson [27]. The internal transcribed spacer (ITS) region of 18S rDNA was amplified via PCR using the universal primers ITS1 and ITS4 [28]. The amplicons were purified and sequenced at the Cardiogenetic Research Center in Tehran, Iran. The resulting sequences were deposited in GenBank and compared with reference sequences using BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Greenhouse experiment

The study was conducted as a pot experiment in the Plant Pathology greenhouse (29°72'68.94"N; 52°59'09.18"E, 1795 m) from September to November 2021. A factorial experiment was arranged in a completely randomized design (CRD) with three replicates, including fungal inoculation and salinity as factors, as described in detail below. The greenhouse was equipped with heating and cooling systems to control temperature. During the day–night cycle, the maximum and minimum temperatures were maintained at 32 ± 2°C and 18°C, respectively, with a photoperiod of 16 h of light and 8 h of darkness. Relative humidity was maintained between 65–75%, and sunlight exposure was recorded from 06:30 to 17:30 throughout the growth period

To prevent unwanted effects from microorganisms, the soil used for potting was sterilized by steam twice at 100°C for 4 h each, with a 48-hour interval between sterilizations, 24 h prior to the start of the experiment to eliminate any microbial contaminants. Six-kg polyethylene pots (21.5 × 21.5 cm) were disinfected with a 2% sodium hypochlorite solution. Each pot was then filled with 5.5 kg of a sterilized soil–sand mixture in a 2:1 (v/v) ratio.

After preparing the soil for potting, 2 kg of the soil was air-dried, passed through a 2-mm sieve, and sent to the Soil Science Laboratory at Shiraz University for chemical analysis. Various physical and chemical properties of soil were measured. The investigated soil had a texture of loamy sand (sand:82.1%, silt: 6.5%, clay: 11.4%, organic matter: 0.8%, organic carbon: 0.24%, nitrogen: 0.16%, FC: 12.42%, Sp: 15.97%, PWP: 5.14%, available K: 63.3 mg kg-1, available P: 7.5 mg kg-1, available Fe: 8.43 mg kg-1, available Zn: 0.65 mg kg-1, available Mn: 7.09 mg kg-1, available Cu: 0.85 mg kg-1, electrical conductivity: 1.29 dS m-1, and hydrogen potential: 7.74).

Plant material

Certified seeds of maize (*Zea mays* L. cv. Simon) were supplied by the Zarghan Research Institute. To eliminate surface contaminants, seeds were first immersed in 70% (v/v) ethanol for 2 min and subsequently treated with 2.5% (v/v) sodium hypochlorite for 10 min. The sterilized seeds were then thoroughly rinsed several times with sterile distilled water. Thereafter, they were transferred onto Petri dishes containing 1% water agar and maintained in darkness at 26 ± 2°C for three days to allow germination. Uniform seedlings free from visible fungal growth and exhibiting radicles approximately 1-1.5 cm in length were selected and subjected to inoculation with the prepared fungal suspension.

Preparation of fungal inoculum and inoculation of seedlings

Four fungal treatments were applied: non-inoculated control, inoculation with selected fungal isolate 1, inoculation with selected fungal isolate 2, and inoculation with a mixture of the two selected isolates. The selected fungi were grown in PDB liquid medium for 4–7 days on a shaker at 120 rpm. Fungi were cultured on PDA plates and incubated at 25°C for 10 days. The fungal biomass was collected using a scalpel, suspended in 10 mL sterile distilled water, and filtered through double-layered muslin to remove debris. The final suspension volume was adjusted to 100 mL, supplemented with 0.1% Tween 80, and the spore concentration was determined using a Neubauer hemocytometer. The suspension was adjusted to 10^7 spores mL^{-1}. For the dual-isolate treatment, equal volumes of each isolate were mixed in a 1:1 ratio. To improve seed adhesion, the germinated maize seeds were soaked for 15 min in 1.5% carboxymethyl cellulose (CMC) under a laminar–flow hood. The spore suspension was then supplemented with 2% CMC. Control seeds were soaked in a sterile water–Tween–CMC solution. The seeds were then shaken on a rotary shaker at 80 rpm for 3 h. Four maize seeds were sown per pot, and an additional 2 mL of spore suspension was applied directly to each seed before covering it with soil and lightly irrigating. (Note: pots had been pre-watered to field capacity two days prior to sowing) [25].

Confirmation of endophytic fungal colonization

To confirm endophytic colonization, inoculated and mock-inoculated plants were assessed 14 days post-inoculation and again at harvest. Root subsamples were plated on PDA [16] and stained with acid fuchsin [29] for microscopic verification of fungal structures.

Salinity Stress

Stress treatments were initiated 21 days after successful confirmation of endophytic fungal colonization in the roots, beginning on September 21, 2021. Maize plants were harvested on November 23, 2021. Fertilizer application was carried out twice monthly, with each plant receiving 100 mL of a 2% (w/v) Megaflor EC solution (20–20–20 NPK supplemented with trace elements; Mugavero Teresa, Italy).

For the first three weeks after sowing maize and transplanting citrus seedlings, the pots were irrigated with regular water to the field capacity. Salinity stress was imposed using NaCl at four concentrations: 0, 50, 75, and 100 mM. The electrical conductivities (EC) of the solutions after dissolving the salt in water were 0.5 (S0 = 0 mM), 4.56 (S1 = 50 mM), 6.84 (S2 = 75 mM), and 9.13 dS m-1 (S3 = 100 mM). Salinity was applied gradually: NaCl concentrations were increased stepwise to 25, 50, 75, and finally to 100 mM for S2 and S3 treatments. Irrigation with saline solution was continued until the end of the stress period (60 d). The control plants were irrigated with non-saline water only. Irrigation was applied such that 15–20% of the solution drained from the bottom of the pot. After every four saline irrigations, a leaching event was performed along with 1–2 g L^{-1} of (MEGAFLOR 20:20:20 + TE) fertilizer.

Measured parameters

Sampling for biochemical parameter

One week before harvest, 1 g of leaf tissue was sampled from each pot, immediately wrapped in foil, frozen in liquid nitrogen for subsequent metabolomic and enzymatic analyses, and stored at − 80°C. At the end of the stress period (60 days for maize), the shoots and roots were harvested separately and carefully washed with tap water to remove soil and debris. At harvest, 0.5 g of root tissue from all treatments was preserved in 50% ethanol for root staining and determination of the colonization percentage.

Plant growth indices

The fresh weights of shoots and roots were measured using a digital balance (A&D Co., Ltd., Japan) with a precision of 0.01 g. Fresh biomass was calculated as the sum of the fresh shoot and root biomasses. For dry biomass, plant tissues were oven-dried at 70°C for 72 h, and the sum of the dry root and shoot biomasses was calculated. Plant water content was determined based on the difference between the fresh and dry weights. The root-to-shoot ratio (RSR) was calculated as the ratio of dry root weight to dry shoot weight [26]. Dried leaf tissue (2 g) was separately ground to obtain a homogeneous sample for physiological analyses, including phenol, proline, soluble sugar, and starch content. Additionally, 2 g of leaf and root tissue from the salinity treatments were ground for mineral ion concentration analyses.

Plant physiological indices

Leaf relative water content (RWC)

RWC is a simple and classical approach for evaluating the hydration status of plants, and was determined following Badr and Brueggemann [30]. At the end of the experimental period, ten leaf discs (1 cm diameter) were sampled from the youngest fully expanded leaves of plants in each pot. The leaf discs were immediately weighed to obtain the fresh weight (FW) using a digital balance (Sartorius Basic BA110; precision, 0.0001 g). The discs were then soaked in 20

mL of sterile double-distilled water at 4°C in the dark for 24 h. After blotting excess water with paper towels, the turgid weight (TW) was recorded. The leaf discs were then oven-dried at 60°C for 48 h to determine their dry weight (DW).

relative water content (RWC) was calculated using the following formula:

$$RWC\ (\%) = (FW-DW)/(TW-DW) \times 100$$

Electrolyte leakage (EL) and membrane stability index (MSI)

Cell membrane stability was evaluated by measuring electrolyte leakage (EL) and the membrane stability index (MSI) following the method described by Jespersen and Huang [31]. Two weeks before harvest, 10 leaf discs (0.5 cm diameter) were collected from the leaves of all the treatments. The collected leaf discs were gently washed with sterile double-distilled water to remove surface contaminants. Subsequently, they were transferred into sealed test tubes containing 10 mL of sterile double-distilled water and maintained on a rotary shaker (100 rpm) at 25°C for 24 h. After incubation, the initial electrical conductivity (EC_1) of the bathing solution was determined using a digital conductivity meter (Metrohm Conductometer 644, Switzerland). Thereafter, the tubes were autoclaved at 121°C for 20 min to achieve complete tissue disruption, allowed to cool to room temperature, and the final electrical conductivity (EC_2) was measured. Electrolyte leakage (EL) and membrane stability index (MSI) were subsequently calculated from EC_1 and EC_2 values to evaluate the integrity of cellular membranes.

Starch content (SC)

Starch content was determined following the anthrone–sulfuric acid protocol as outlined by Kuai et al. [32] with slight modifications. After removal of ethanol from the residue remaining after soluble sugar extraction, 200 µL of cold distilled water was added to each tube and thoroughly mixed. For starch extraction and hydrolysis, 52% perchloric acid ($HClO_4$) was added in two sequential steps (260 µL followed by 130 µL), and the mixture was diluted to a final volume of 2 mL with deionized water. The samples were then incubated at 100°C for 15 min to ensure complete hydrolysis. An aliquot (100 µL) of the resulting extract was reacted with 200 µL of freshly prepared anthrone reagent (0.2% anthrone dissolved in 95% sulfuric acid) and heated in a boiling water bath for 15 min to allow color development. After cooling rapidly in an ice bath, absorbance was recorded at 620 nm using a spectrophotometer. Starch content was quantified using a calibration curve generated with standard glucose solutions and expressed as mg g^{-1} dry weight (DW), applying a conversion factor of 0.92.

Total soluble sugars (TSS)

The total soluble sugar (TSS) content in dried and powdered leaf samples was measured following the procedure of Fox and Robyt [33]. In brief, 10 mg of ground leaf material was placed into a microtube, and 1 mL of 80% (v/v) ethanol was added. The mixture was incubated at room temperature for 24 h and then centrifuged at 10,000 rpm for 10 min. The supernatant was collected in a separate 2-mL microtube. The extraction step was repeated on the remaining pellet with an additional 1 mL of 80% ethanol, centrifuged at 10,000 rpm for 5 min, and combined with the previous supernatant. The residual pellet was reserved for starch determination. For the sugar assay, 100 µL of the combined extract was mixed with 125 µL of sulfuric acid and 25 µL of 5% phenol. After gentle mixing, the samples were incubated in an oven for 30 min, cooled on ice, and transferred to a 96-well plate. Absorbance was recorded at 490 nm using a spectrophotometer. The TSS concentration was calculated from a D-glucose standard curve and expressed as mg g^{-1} dry weight (DW) of the leaf tissue.

Total phenolic content (TPC)

For total phenol determination, 100 mg of dried and powdered leaf tissue from each sample was mixed with 5 mL of 80% (v/v) methanol and incubated in the dark at 4°C for 48 h. After centrifugation at 12,000 rpm for 10 min, the supernatant was transferred to a new microtube and used for total phenol estimation and free radical scavenging activity (% inhibition, I). For total phenol measurement, 200 µL of the supernatant was mixed with 40 µL of freshly prepared 50% Folin–Ciocalteu reagent (diluted 1:1 with distilled water) in the dark, followed by the addition of 200 µL of 2% (w/v) saturated sodium carbonate solution. The mixture was vortexed and incubated for 1 h in the dark at room temperature. A standard curve was prepared using gallic acid at concentrations ranging from 100 to 1000 mg L^{-1} (Sigma-Aldrich, St. Louis, MO, USA). After incubation, absorbance of the samples and standards was measured at 650 nm against a blank using a spectrophotometer. The total phenol content was expressed as mg gallic acid equivalents per 100 g dry weight (mg GAE 100 g^{-1} DW) [34].

Proline content (PC)

Proline, a key osmolyte involved in osmotic adjustment [35], was quantified following the method of Carillo and Gibon [36]. Briefly, 100 mg of dried, ground leaf tissue was homogenized in 5 mL of 80% methanol and incubated overnight at 4°C in the dark. The homogenate was centrifuged at 5,000 rpm for 20 min, and 100 µL of the supernatant was mixed with 250 µL of freshly prepared acidic ninhydrin solution and 450 µL of distilled water. The ninhydrin solution was prepared by dissolving 1.25 g of ninhydrin in a mixture of 30 mL of 60% acetic acid and 20 mL of 6 M phosphoric acid (8 mL phosphoric acid + 12 mL distilled water). The reaction mixture was incubated in a boiling water bath at 100°C for 1 h until a brick-red color appeared, then rapidly cooled on ice and centrifuged at 5,000 rpm for 10 min. A 200 µL aliquot of each sample was transferred to a 96-well plate, and absorbance was measured at 520 nm using a spectrophotometer. Methanol and the reaction mixture without sample served as blanks. Proline content was determined from a standard curve generated with pure L-proline and expressed as µmol g^{-1} dry weight.

Extraction for antioxidant enzyme activity and total protein in leaves

Frozen leaf tissue (0.25 g, stored at − 80°C) was ground thoroughly in liquid nitrogen and homogenized in 1,000 µL of cold 50 mM potassium phosphate buffer (pH 7.0) containing 2 mM EDTA and 1% (w/v) polyvinylpyrrolidone (PVP), referred to as the extraction buffer. The homogenate was centrifuged at 12,000 rpm for 20 min at 4°C, and the supernatant was collected on ice. It was then aliquoted into six 0.5 mL microtubes and stored at -80°C for subsequent analyses. To prepare 100 mL of 50 mM potassium phosphate buffer (pH 7.0), 38.5 mL of 50 mM monobasic potassium phosphate was mixed with 61.5 mL of 50 mM dibasic potassium phosphate, as described by Ozden et al. [37].

Total protein (TP)

The total protein content in leaves was determined using the Bradford assay [38]. Briefly, 30 µL of enzyme extract was mixed with 1,500 µL of Bradford reagent in a microtube. For the blank, 30 µL of extraction buffer was mixed with 1,500 µL of Bradford reagent. Absorbance was measured at 595 nm against a blank using a spectrophotometer within 60 min. The Bradford reagent was prepared by dissolving 100 mg of Coomassie Brilliant Blue G-250 in 50 mL of 95% ethanol and 100 mL of 85% phosphoric acid, followed by dilution with distilled water. A standard curve was prepared using bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, MO, USA). The protein concentration in the samples was calculated from the standard curve and expressed as mg g^{-1} of fresh leaf weight.

Catalase (CAT) activity

CAT activity was determined as described by Sun et al. [39]. The reaction mixture (3 mL) consisted of 50 µL of enzyme extract, 2,750 µL of 50 mM potassium phosphate buffer (pH 7.0), and 200 µL of hydrogen peroxide (H2O2). Immediately after adding the enzyme extract to the reaction mixture, the decrease in absorbance at 240 nm due to H_2O_2 decomposition was recorded over 1 min using a spectrophotometer (Epoch Microplate, Biotech, Winooski, VT, USA). Enzyme activity was calculated using an extinction coefficient of 39.14 mM^{-1} cm^{-1} and expressed as µmol H_2O_2 decomposed per min per mg of protein (µmol H_2O_2 min^{-1} mg^{-1} protein).

Peroxidase (POD) activity

POD activity was determined as described by Sun et al. [39]. The reaction mixture contained 1,830 µL of 50 mM potassium phosphate buffer (pH 7.0), 100 µL of 20 mM guaiacol, 20 µL of enzyme extract, and 50 µL of 40 mM hydrogen peroxide (H_2O_2). The reaction was initiated by adding H_2O_2, and the increase in absorbance at 470 nm was measured. POD activity was calculated using an extinction coefficient of 26.6 mM^{-1} cm^{-1} and expressed as µmol guaiacol oxidized per min per mg of protein.

Superoxide dismutase (SOD) activity

SOD activity was measured as described by He et al. [40]. For each assay, 50 µL of enzyme extract was mixed with 940 µL of SOD assay solution, which contained 50 mM potassium phosphate buffer (pH 7.15), 75 µM nitroblue tetrazolium (NBT, 0.061 g), 13 mM L-methionine (0.194 g), 0.1 mM EDTA (0.037 g), and 4 µM riboflavin (0.001 g in 10 mL potassium phosphate buffer). The riboflavin solution was freshly prepared and stored in the dark until use. After combining the enzyme extract with the assay solution, the mixture was transferred to cuvettes, gently mixed, and illuminated under a fluorescent lamp for 20 min. The absorbance of each sample was measured at 560 nm using a spectrophotometer. SOD activity was expressed as units per gram of fresh leaf tissue weight.

Ascorbate peroxidase (APX) activity

APX activity was measured by mixing 50 µL of the enzyme extract with 1 mL of APX assay solution containing 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.5 mM ascorbic acid, and 0.15 mM hydrogen peroxide. Enzyme activity was determined by monitoring the decrease in absorbance at 290 nm due to ascorbate oxidation over one minute using a spectrophotometer. The activity was expressed as µmol of ascorbate oxidized per minute per milligram of protein [39].

Malondialdehyde (MDA) content

To the enzyme extract (250 µL), 1 mL of malondialdehyde (MDA) assay solution containing 20% trichloroacetic acid (TCA) and 0.5% thiobarbituric acid (TBA) was added. The mixture was then incubated in a water bath for 30 min, immediately cooled, and centrifuged at 10,000 × g for 5 min. Finally, 200 µL of each sample was transferred to a microplate, and the absorbance was measured at 532 and 600 nm using a spectrophotometer. For the blank, 0.1% TCA was used instead of the plant extract. MDA content was expressed as µmol per gram of fresh weight (µmol g^{-1} FW) using an extinction coefficient of 155 Mm^{-1} cm^{-1} [41].

Free radical scavenging activity (I %)

The methanolic leaf extract (23 µL, prepared in the phenol analysis section) was added to 200 µL of 0.1 mM DPPH methanolic solution. After 30 min of incubation in the dark, the absorbance of the solution was measured at 517 nm using a spectrophotometer. The control sample contained of 23 µL methanol and 200 µL of DPPH solution. The percentage of free radical scavenging activity (I %) was calculated based on the measured absorbance of the samples.

Greenness index (GI)

The total chlorophyll content of fully expanded young leaves was measured one week before plant harvest using a chlorophyll meter (SPAD-502 Plus, Konica Minolta Sensing Inc., Osaka, Japan) between 9:00 and 11:00 a.m. Three young mature leaves were selected from each plant, and three readings were taken for each leaf using the device. The mean of these readings was then calculated. The Greenness Index (GI) was determined based on chlorophyll measurements obtained using a chlorophyll meter, as described by Khan et al. [42].

Sodium and potassium concentrations in roots and shoots

One gram of ground dry root and one gram of ground dry shoot samples were separately weighed and placed in 25-mL porcelain crucibles. The crucibles were then transferred to a muffle furnace at 550°C for 5 h to ash the plant material. After cooling, 5 mL of 2 N hydrochloric acid (prepared by adding 83 mL HCl to 500 mL of distilled water) was added to each crucible. The resulting solutions were filtered through Whatman No. 42 filter paper into volumetric flasks,

and the final volume was adjusted to 50 mL using boiling distilled water. Standard solutions of sodium and potassium at concentrations of 0, 10, 20, 30, 40, and 50 mg L-1 were prepared using stock solutions of sodium chloride (0.254 g) and potassium chloride (0.191 g) dissolved in 100 mL of distilled water. The sodium and potassium concentrations in the samples and standards were determined by flame photometry using a flame photometer (Jenway PEP7, ELE Instrument Co. Ltd.). Element concentrations were calculated from standard curves, corrected for dilution factors, and expressed as mg element per gram of plant dry weight. The potassium-to-sodium (K^+/Na^+) ratio was calculated according to Horneck and Hanson [43].

Phosphorus concentration in roots and shoots

Phosphorus concentration was determined spectrophotometrically using the molybdate–vanadate yellow colorimetric method, according to Olsen et al. [44]. First, 22.5 g of ammonium molybdate was dissolved in 400 mL of distilled water (Reagent A). Then, 1.25 g of ammonium metavanadate was dissolved in 300 mL of boiling distilled water and used after cooling (Reagent B). Subsequently, Reagents A and B were mixed, and 250 mL of nitric acid was added. The mixture was then brought to a final volume of 1 L with distilled water to prepare the working solution (Reagent T). Next, 10 mL of Reagent T was added to 20 mL of the extract prepared for sodium and potassium analysis, and the final volume was adjusted to 50 mL in a volumetric flask. Finally, 200 µL of each sample was transferred into a 96-well microplate, and absorbance was measured at 470 nm using a spectrophotometer. For phosphorus standard preparation, 0.4394 g of potassium dihydrogen phosphate (KH_2PO_4) was dissolved in 1 L of distilled water, and a series of standard dilutions was then prepared.

Chloride concentration in roots and shoots

To determine the chloride concentration, 1 g of plant powder was mixed with 250 mg of calcium oxide and distilled water to form a paste. The mixture was then ashed in a muffle furnace at 550°C for 5 h. After cooling, 15 mL of boiling distilled water was added to the crucibles, and the contents were filtered and transferred to a volumetric flask for further analysis. After cooling, 3–4 drops of phenolphthalein indicator were added, and the solution turned a faint pink. If necessary, the pH was adjusted to 6–7 using a few drops of 0.1 N nitric acid or dilute acetic acid. Subsequently, five drops of 5% potassium chromate (K_2CrO_4) solution (prepared by dissolving 2.5 g potassium chromate in distilled water and making up to 50 mL) were added as an indicator. Each sample solution was titrated with a 0.05 N silver nitrate ($AgNO_3$) solution (prepared by dissolving 2.12 g of silver nitrate in 250 mL of distilled water) until a brick-red precipitate appeared, indicating the endpoint.

$$\%Cl = (V\ AgNO_3\text{-}VC)(\%5 \times 35.5 \times 100/\ WS)$$

Data analysis

Data were analyzed using SAS statistical software (version 9.4; SAS Institute, Cary, NC, USA). Before performing the analysis of variance, the normal distribution of residuals was examined through the Shapiro–Wilk, Cramér–von Mises, Kolmogorov–Smirnov, and Anderson–Darling tests. Treatment effects were evaluated using two-way analysis of variance (ANOVA). When the F-test was significant, mean comparisons were carried out using Tukey's HSD test at a probability level of 0.05. Relationships among measured traits were determined based on Pearson's correlation coefficients. In addition, principal component analysis (PCA) was conducted in R (version 4.2.2; R Core Team, 2022). Figures were prepared using Microsoft Excel 2016 (Microsoft, Redmond, WA, USA).

Results

Sample collection, Fungal isolates and root colonization

A total of 99 root samples were collected from trees growing in the desert regions of Bushehr, Fars, Isfahan, Kerman, and Yazd provinces during 2018–2021. Samples were obtained from 12 different plant hosts (Table 1, supplementary files).

Two desert-derived endophytic fungi were identified based on BLASTn searches against the NCBI database, showing over 97% sequence similarity to known species. Isolate 81Y1, obtained from roots of *Haloxylon aphyllum* in Ardakan, Yazd (234981.31 E, 3549529.62 N), was classified as *Trichoderma harzianum* (GenBank accession OP710046). Isolate 19K3, recovered from *Tamarix* sp. in Tikdar, Kerman (488443.96 E, 3393352.75 N), was identified as *Fusarium solani* (GenBank accession OP710067). Both isolates were applied individually and in combination in the present study. Among the identified fungi, *Fusarium* was the most prevalent taxon. Successful colonization of maize roots by *T. harzianum* (T), *F. solani* (F), and their consortium was confirmed through re-isolation and microscopic root staining. Importantly, these fungal species were absent in the roots of non-inoculated control plants.

Salinity stress in maize

The analysis of variance revealed that the interaction between salinity stress and microorganisms had significant effects (at the 1% and 5% probability levels) on many of the measured traits in maize plants, including PFB, PDB, TMC, RSR, SC, TSS, TPC, CAT, SOD, POD, APX, MDA, DPPH, Na (leaf), Na (root), Cl (leaf), P (leaf), and P (root). In contrast, the interaction between salinity stress and microorganisms did not significantly affect RWC, EL, MSI, PC, TP, GI, K (leaf), K (root), or Cl (root) (Table 1).

Growth indices of maize under salinity stress

The results of the mean comparisons showed that increasing salinity stress levels in maize significantly reduced the vegetative traits PFB (35.92%), PDB (44.38%), TMC (33.97%), and RSR (66.96%). In contrast, inoculation with endophytes significantly increased PFB (10.76%), PDB (18.04%), TMC (9.33%), and RSR (26.34%) compared with the non-inoculated control at different salinity levels (Fig. 1, A–D). The highest RSR was observed in the TF fungal treatment under non-saline conditions (S0). At the lowest salinity level (TS0), endophyte T had the greatest effect on increasing PFB, PDB and TMC. Control plants at the highest salinity level (CS3) exhibited the lowest PFB, PDB, and RSR values. However, at this salinity level, treatment T did not show a significant difference from the control in terms of PFB. Under S3 conditions, endophyte T had the least effect on TMC (Fig. 1, A–D).

With increasing salinity stress, the effects of endophytes on PFB and TMC were further enhanced. Accordingly, at S0, S1, S2, and S3, endophyte application increased PFB by 5.38%, 6.95%, 15.60%, and 21.23%, and TMC by 2.18%, 5.09%, 15.75%, and 22.17%, respectively, compared to the control. Among the endophytes, F, TF, and T increased PFB by 14.83%, 13.19%, and 4.23%, and TMC by 12.99%, 12.30%, and 2.06%, respectively, relative to the control (Fig. 1, A, B).

Endophyte inoculation increased PDB by 21.14%, 17.46%, 14.86%, and 16.44% at salinity levels S0, S1, S2, and S3, respectively, compared with that of the control. Among the endophytes, F, T, and TF increased PDB by 24.25%, 15.35%, and 14.58%, respectively, relative to that in the control (Fig. 1, C).

With increasing salinity stress, the effect of endophytes on RSR increased. At salinity levels S0, S1, S2, and S3, endophyte treatments enhanced RSR by 18.77%, 24.67%, 36.89%, and 46.44%, respectively, compared to the control. Among the endophytes, TF, F, and T contributed to increases in RSR of 42.72%, 20.22%, and 16.06%, respectively, compared with that of the control (Fig. 1, D).

Physiological indices of maize under salinity stress

The results of the mean comparisons indicated that RWC and MSI significantly decreased with increasing salinity stress levels in maize, by 18.43% and 27.01%, respectively. In contrast, EL increased significantly with increasing salinity stress, showing a 124.19% increase. Inoculation with endophytes significantly increased RWC and MSI compared with the non-inoculated control across the different salinity levels S0, S1, S2, and S3 by 5.34% and 19.30%, respectively. In addition, endophyte inoculation significantly reduced EL relative to that in the control (– 25.81%).

The interaction between endophytes and salinity stress in maize was not significant for RWC, MSI, or EL (Fig. 2, A–C). The results showed that endophytes T and TF caused a significant increase in RWC, whereas endophyte F had a negative effect, although it did not differ significantly from that of the control. Moreover, with increasing salinity stress, endophyte inoculation led to a marked increase in the RWC of maize plants. At salinity levels S0, S1, S2, and S3, RWC increased by 1.09%, 4.11%, 6.42%, and 11.91%, respectively, compared to the control. The highest RWC values were observed with the application of endophytes T and TF under non-stress conditions (TS0 and TFS0), whereas the lowest RWC values were recorded for endophyte F and the control at the highest salinity level (FS3 and CS3) (Fig. 2, A).

MSI was significantly enhanced by endophyte inoculation, with the greatest effects observed as salinity stress increased. At salinity levels S0, S1, S2, and S3, endophytes increased the MSI by 11.33%, 20.21%, 22.44%, and 28.63%, respectively. The highest MSI values were associated with the application of endophytes F and TF under non-saline conditions (FS0 and TFS0), whereas the lowest MSI was observed in the control plants at the highest salinity level (CS3) (Fig. 2, B).

Different responses were observed for EL following endophyte inoculation; however, overall EL was reduced compared to that of the control. The highest EL was recorded in the control treatment at the highest salinity level (CS3), whereas the lowest EL occurred with the application of endophyte F under non-stress conditions (FS0) (Fig. 2, C).

Osmotic indices of maize under salinity stress

The results of mean comparisons showed that SC, TPC, and PC significantly increased with increasing salinity stress levels in maize by 62.86%, 28.57%, and 36.83%, respectively. In contrast, TSS decreased by 24.96% under increasing salinity stress. Inoculation with endophytes significantly increased SC, TSS, TPC, and PC by 29.21%, 28.17%, 16.51%, and 11.70%, respectively, compared with the non-inoculated control across various salinity levels. The highest SC, TSS, and TPC values were observed with endophyte T at the highest salinity level (TS3), whereas the highest PC was recorded with the TF treatment at the highest salinity level (TFS3). The lowest SC, TPC, and PC values were recorded in the control treatment under non-stress conditions (CS0), whereas the lowest TSS was observed in the control at salinity levels S2 and S3 (CS2 and CS3) (Fig. 3, A–D).

Endophyte inoculation increased SC by 42.94%, 30.31%, 26.54%, and 24.29% at salinity levels S0, S1, S2, and S3, respectively, compared with that in the control. The effect of endophytes on SC was greater at lower salinity levels. Across all salinity treatments, endophytes F, T, and TF increased SC by 38.34%, 28.71%, and 20.57%, respectively, relative to the non-inoculated control, with endophyte F showing the greatest effect (Fig. 3, A).

With increasing salinity stress, the effects of endophytes on TSS and TPC increased. At salinity levels S0, S1, S2, and S3, TSS increased by 20.22%, 26.87%, 30.62%, and 39.18%, respectively, whereas TPC increased by 8.96%, 11.07%, 19.56%, and 24.31%, respectively, compared with the control. Among the endophytes, T, F, and TF increased TSS by 46.16%, 28.31%, and 10.04%, and TPC by 21.12%, 17.68%, and 10.70%, respectively, compared to the non-inoculated control. However, TF treatment at S3 reduced TSS by 13.34% (Fig. 3, B–C).

The interaction between endophytes and salinity stress on PC was not significant. Endophytes T, F, and TF had similar effects on increasing PC; however, fungal treatments T and TF increased PC compared with the control (Fig. 3, D).

Biochemical indices of maize under salinity stress

The mean comparisons showed that TP, CAT, POD, APX, and SOD significantly increased by 9.18%, 66.47%, 83.67%, 61.36%, and 86.66%, respectively, with increasing salinity stress. Inoculation with endophytes significantly enhanced TP (6.54%) and the activities of CAT (28.87%), POD (39.55%), APX (37.01%), and SOD (18.98%) compared to the non-inoculated control across different salinity levels.

With increasing salinity stress, the effect of endophytes on TP became more pronounced, showing increases of 2.35%, 4.88%, 7.89%, and 10.67% at S0, S1, S2, and S3, respectively, compared with that of the control. Overall, endophyte F had the greatest effect on TP (8.19%), followed by TF (6.13%), and T (5.31%). At S3, the maximum TP increase was observed with T, F, and TF, showing 14.27%, 8.73%, and 9.01% higher TP than that of the control, respectively. The effects of fungi and their interaction with salinity were not significant; although protein content increased with stress, no significant difference was observed at S1, whereas TP significantly increased at S2 and S3 compared with S0 (Fig. 4, A).

The minimum CAT, POD, APX, and SOD activities were recorded in the control under non-stress conditions (CS0). The highest CAT levels were observed in the T and TF treatments at the highest salinity (TS3 and TFS3), and the highest POD and APX levels were observed in the TF treatment at S3. The effects of TF on APX at S2 and S3 were not significantly different. The highest SOD was recorded for endophyte T at S2 (Fig. 4, B–E).

At lower salinity levels, endophytes had greater effects on CAT and SOD, whereas their impact sharply declined at S3. Across salinity levels S0, S1, S2, and S3, endophytes increased CAT by 49.04%, 39.87%, 27.88%, and 17.66%, and SOD by 37.68%, 29.10%, 20.96%, and 2.59%, respectively, compared to the control. Among the endophytes, TF, T, and F increased CAT by 45.89%, 23.03%, and 17.70%, and T, TF, and F increased SOD by 31.92%, 21.04%, and 14.78%, respectively (Fig. 4, B, E).

At S2, endophytes had the greatest effect on POD, with T, F, and TF increasing POD by 96.35%, 45.27%, and 65.39%, respectively, compared to the control. TF was the most effective endophyte on POD, and across all salinity levels, TF, T, and F increased POD by 54.83%, 39.77%, and 24.05%, respectively (Fig. 4, C). A similar trend was observed for APX, with the highest effect at S2. T, F, and TF increased APX by 57.94%, 52.99%, and 83.78% compared to the control, respectively. Across all stress levels, F, TF, and T increased APX by 47.49%, 38.91%, and 24.09%, respectively (Fig. 4, D).

MDA significantly increased by 19.91% with increasing salinity stress and was significantly reduced by endophyte inoculation by 20.29% compared to the control. The greatest reduction in MDA levels occurred at S3. At different stress levels, T, TF, and F reduced MDA by 24.08%, 20.95%, and 15.83%, respectively (Fig. 4, F).

DPPH decreased significantly by 9.78% with increasing salinity stress, whereas endophyte inoculation significantly increased DPPH by 21.69% relative to the control. The highest DPPH was observed in the combined fungal treatment under non-stress conditions (TFS0), whereas the lowest DPPH was observed in the control at the highest salinity level (CS3). With increasing stress, the effect of endophytes on DPPH increased, showing increases of 16.79%, 18.08%, 22.05%, and 31.007% at S0, S1, S2, and S3, respectively. At S3, T, F, and TF increased DPPH levels by 24.21%, 32.42%, and 36.38%, respectively. Overall, TF, F, and T increased DPPH by 25.96%, 22.02%, and 17.02%, respectively, compared to the control (Fig. 4, G).

Greenness index of maize under salinity stress

The results of the mean comparisons showed that increasing salinity stress led to a decrease in the greenness index (GI) of maize plants by 13.16%. In contrast, inoculation with endophytes significantly increased this trait by 7.74% compared with the non-inoculated control at different stress levels (Fig. 5).

With increasing salinity intensity, GI decreased; however, no significant difference was observed between the S1 and S2 treatment groups. The effects of endophytes became more pronounced with increasing stress levels. At S0, S1, S2, and S3, the GI increased by 2.65%, 6.25%, 11.24%, and 12.39%, respectively, compared to that of the control. Among the different endophytic treatments, inoculation with T and TF significantly enhanced GI compared with the control, whereas F did not show a significant difference (Fig. 5).

Mineral elements in maize under salinity stress

Mean comparisons showed that Na in leaves (Na_leaf, 91.95%) and roots (Na_root, 228.57%), and Cl in leaves (Cl_leaf, 37.57%) and roots (Cl_root, 26.58%) increased significantly with increasing salinity. In contrast, endophyte inoculation significantly reduced Na_leaf (− 31.35%), Na_root (− 24.12%), Cl_leaf (− 22.09%), and Cl_root (− 16.28%) compared to the non-inoculated control (Fig. 6, A–H).

Under non-stress conditions (S0), plants inoculated with F (FS0) showed the lowest Na levels in leaves and roots, whereas T also reduced root Na levels similarly to F. The highest leaf and root Na + concentrations were observed in the control group at the highest stress level (CS3), and the maximum root Na + concentration was observed in TS3 (Fig. 6, A, B).

Endophytes reduced Na_leaf by 24.19%, 42.54%, 38.49%, and 22.49% at S0, S1, S2, and S3, respectively, with the greatest effect at S1. T, F, and TF reduced Na_leaf by 20.87%, 46.61%, and 26.55%, respectively. F was most effective at S1, reducing leaf Na by 34.54%, 55.83%, 45.93%, and 46.05% at S0–S3, while T had no significant effect at S0 and S3 but reduced leaf Na by 30.45% and 31.15% at S1 and S2. TF had no effect at S0 but significantly reduced leaf Na + at S1–S3. Overall, T alone at S3 had no effect, but in combination with F (TF), it significantly reduced leaf Na. Endophytes had the greatest effect on reducing Na_root at S0 (− 56.37%), with the effect declining at higher salinity levels (S1: −35.98%, S2: −19.33%, and S3: −5.68%). TF showed no significant effect across all stress levels, whereas F had the greatest reduction (− 35.46%), followed by T (− 27.67%) and TF (− 9.22%) (Fig. 6, A, B).

The interaction between salinity and endophytes on root Cl content was not significant. The highest leaf Cl content occurred in the control and T-inoculated plants at S3 (CS3 and TS3), while the lowest leaf Cl content was observed in T-treated plants at S0. Root Cl was highest in the control at S2 and S3 and lowest in F-treated plants at S0 (Fig. 6, C, D). Leaf Cl was reduced by F, TF, and T by 30.47%, 19.82%, and 15.09%, respectively, with F being most effective at

all salinity levels. T significantly reduced Cl_leaf only at S0, whereas TF had no significant effect at S0 but reduced Cl_leaf at S1–S3. Root Cl reduction by endophytes was not significant in the interaction with salinity. Across S0–S3, F, T, and TF reduced Cl_root by 12.06%, 13.68%, 17.94%, and 19.52%, respectively. F was more effective than T and TF, and at S3 all three significantly reduced root Cl (− 15%, − 24.6%, − 19.52%) (Fig. 6, C, D).

The interaction between endophytes and salinity on leaf and root K was not significant. Maximum K in leaves and roots was observed in T-, F-, and TF-inoculated plants at S0, whereas the lowest values were recorded in the control at S3 (CS3) (Fig. 6, E, F). Salinity stress decreased K_leaf and K_root by 9.42% and 31.45%, respectively, whereas endophyte inoculation increased K_leaf and K_root by 10.20% and 16.64%, respectively, compared with the control (Fig. 6, E, F). The effect of endophytes on K_leaf increased with increasing salinity. At S0–S3, K_leaf increased by 5.42%, 6.27%, 8.58%, and 24.26%, respectively, relative to the control. F, TF, and T increased K_leaf by 14.77%, 9.17%, and 6.67%, respectively, compared to CK. T and TF had no significant effect on S0–S2 but increased K_leaf at S3. F had a significant effect across all levels (S0–S3:8.99%, 8.75%, 12.88%, 33.41%). The interaction between salinity and endophytes on K_root was not significant in this study. With increasing stress, endophytes increased root K by 11.62%, 15.45%, 18.22%, and 27.18% at S0–S3. F, TF, and T increased K_root by 20.88%, 16.79%, and 12.25%, respectively. F was most effective, with significant effects at S0–S1, but not at higher stress levels, whereas TF combined with T at S3 increased K_root by 39.36% (Fig. 6, E, F).

The phosphorus content in leaves and roots decreased by 8.52% and 5.411%, respectively, under salinity, but endophyte inoculation increased the P content in leaves and root P by 6.86% and 4.81%, respectively (Fig. 6, G, H). Endophytes had the greatest effect on increasing leaf P at S0, with diminishing effects at higher salinity. Leaf P increased by 12.31%, 8.37%, 5.37%, and 1.18% at S0–S3. TF, T, and F increased leaf P by 13.78%, 4.54%, and 2.25%, respectively, with TF being the most effective at S0–S2 (12.23%, 20.97%, and 7.98% increase, respectively) and no significant difference at S3. Root P increased with salinity, with endophytes enhancing uptake by 2.78%, 3.41%, 6.14%, and 7.22% at S0–S3. TF, T, and F increased root P by 5.83%, 4.49%, and 3.89%, respectively, with the combination (TF) having the greatest effect (Fig. 6, G, H).

Pearson correlation and principal component analysis (PCA) of maize under salinity stress in maize

Pearson correlation analysis revealed a strong negative correlation between CAT, APX, SOD, MDA, EL, Cl_leaf, Cl_root, Na_leaf, and Na_root with most other measured traits. In contrast, TSS showed a weak correlation with the other indices. A strong positive correlation was observed between the growth index RSR and the physiological trait RWC (Fig. 7).

To investigate the interrelationships among treatments and measured traits, principal component analysis (PCA) was conducted. The first two components explained more than 81.9% of the total variance, with the first and second components accounting for 68.4% and 13.5% of the variation, respectively. The first component (PC1) was positively correlated with growth and physiological traits, such as PFB, PDB, TMC, RWC, MSI, GI, P_leaf, and K_root, and negatively correlated with stress and ionic traits, including EL, MDA, Na_leaf, Na_root, Cl_leaf, and Cl_root. The second component (PC_2) showed positive correlations with TSS, DPPH, P_leaf, P_root, and K_leaf, and negative correlations with osmotic and biochemical traits, including PC, SC, TPC, CAT, SOD, POD, APX, and TP (Fig. 7). The PCA biplot revealed strong positive relationships among growth traits, whereas physiological (EL), osmotic (SC, PC, TPC), biochemical (CAT, POD, SOD, APX, TP, MDA), and nutritional traits (Na_leaf, Na_root, Cl_leaf, and Cl_root) exhibited strong negative correlations with most other traits. These results were consistent with the Pearson correlation analysis (Fig. 7).

Discussion

The successful isolation and identification of stress-adapted fungal endophytes from extreme environments, such as arid and saline deserts, is a promising strategy for enhancing crop resilience. In this study, two endophytic strains, *Trichoderma harzianum* (81Y1) and *Fusarium solani* (19K3), were isolated from the roots of desert plants (*Haloxylon aphyllum* and *Tamarix* sp., respectively) and selected based on their inherent salinity tolerance. Molecular identification confirmed their taxonomy, and subsequent inoculation experiments demonstrated their ability to successfully colonize maize roots, even under saline conditions, as verified by re-isolation and staining methods. The absence of these specific strains in the control plants confirmed the establishment of the introduced symbiotic relationship. This successful colonization is the critical first step for the potential benefits of endophytes, as it forms the basis for direct and indirect plant growth-promoting mechanisms [45]. The prevalence of *Fusarium* among the isolated taxa is noteworthy; while some species are pathogenic [46, 47], many are beneficial endophytes capable of enhancing host plant tolerance to abiotic stresses [48–50], highlighting the importance of functional characterization over taxonomic identity alone in selecting microbial inoculants [17]. The choice of *Trichoderma* strain is equally strategic, as members of this genus are widely recognized for their plant growth-promoting and stress alleviating properties [5, 51, 52]. The deliberate selection of these desert-adapted isolates, followed by confirmation of their colonization competence under stress, forms a solid foundation for investigating their efficacy in mitigating salinity induced damage in maize, as explored in subsequent physiological and biochemical assessments.

Quantitative assessment of vegetative growth parameters clearly demonstrated the dual impact of salinity stress and the mitigating role of endophytic inoculation in maize. As anticipated, increasing salinity levels induced a severe decline in key growth indices, including plant fresh biomass (PFB, -35.92%), dry biomass (PDB, -44.38%), total mineral content (TMC, -33.97%), and root-to-shoot ratio (RSR, -66.96%). This pervasive suppression aligns with the established paradigm that salinity primarily disrupts water and ion homeostasis, leading to osmotic stress, ionic toxicity, and ultimately, a profound reallocation of resources away from growth and toward survival mechanisms [53].

In contrast, inoculation with the desert-adapted endophytes *T. harzianum*, *F. solani*, and their consortium significantly alleviated these adverse effects. Across all salinity treatments, inoculated plants exhibited higher PFB (up to + 21.23%), PDB (up to + 24.25%), TMC (up to + 22.17%), and RSR (up to + 46.44%) compared with non-inoculated controls. Notably, the promotive effects particularly on PFB and TMC were more pronounced at elevated salinity levels (S2 and S3), suggesting that the contribution of these endophytes becomes increasingly important under severe stress conditions [54]. Mechanistically, these improvements may be explained by enhanced root system development and greater nutrient acquisition, which likely increased mineral uptake and biomass

production under saline environments. Endophytic colonization may also facilitate better ionic balance and osmotic adjustment, enabling plants to maintain cellular homeostasis. Furthermore, stimulation of antioxidant defense systems and modulation of phytohormonal balance could help preserve photosynthetic efficiency, thereby sustaining growth despite salt induced constraints.

The positive role of plant–endophyte symbiosis under stressful environments has been widely reported [16, 25, 55]. Similarly, Ali et al. [14] showed that *S. lycopersici* enhanced maize biomass under salinity, while Jan et al. [56] demonstrated that *Meyerozyma caribbica* improved growth and mitigated oxidative damage in salt-stressed maize.

The superior performance of the F and TF treatments in many parameters and the context-dependent effectiveness of T (e.g., strongest effect on biomass at S0 but lesser effect on TMC at S3) highlights a critical insight: different endophytes or their combinations may employ distinct or complementary strategies. Endophytes may possess genes involved in growth-related signaling [57] and can activate new gene clusters in microorganisms to produce stress-alleviating compounds [58]. The consortium did not always yield additive effects, implying potential complex interactions that warrant further investigations.

The restoration of a healthier RSR in inoculated plants is particularly significant, as it indicates a reversal of the stress-induced preferential biomass allocation and points toward improved root system architecture and function, which is a key factor for water and nutrient foraging under saline conditions [13]. These growth promoting effects can be attributed to the multifaceted biochemical arsenal of beneficial endophytes, which includes the production of phytohormones (e.g., IAA and gibberellins) to stimulate cell division and elongation, synthesis of osmoprotectants (e.g., proline and glycine betaine) to maintain cellular turgor, and enhanced nutrient solubilization and uptake [51]. By mitigating primary osmotic and ionic stresses, these microbial functions allow the plant to redirect energy back into growth processes, thereby explaining the observed resilience in terms of biomass accumulation and mineral nutrition.

Physiological assessments of leaf relative water content (RWC), membrane stability index (MSI), and electrolyte leakage (EL) provide crucial insights into the mechanisms by which endophytes confer salinity tolerance to maize. As expected, salinity stress severely compromised plant water status and membrane integrity, as evidenced by significant reductions in RWC (-18.43%) and MSI (-27.01%), alongside a pronounced increase in EL (+ 124.19%). This triad of responses is classically indicative of osmotic stress induced dehydration and the subsequent escalation of oxidative damage, where reactive oxygen species (ROS) attack membrane lipids, leading to peroxidation and loss of semi-permeability [59]. The nonsignificant interaction between endophytes and salinity levels for these parameters suggests that the beneficial effects of inoculation are robust and consistent across the stress gradient, functioning as a general enhancer of cellular homeostasis rather than a stress level specific response. Notably, endophyte inoculation effectively counteracted these deleterious effects, significantly increasing the RWC (by up to 11.91% at S3) and MSI (by up to 28.63% at S3), while reducing the EL (-25.81%). The progressive enhancement of MSI with increasing salinity underscores the growing importance of the protective role of endophytes under conditions of severe stress.

The differential performance of the isolates was particularly revealing in this study. While *T. harzianum* and the consortium consistently improved RWC, *F. solani* had a negative effect on this parameter; however, it was among the most effective treatments for enhancing MSI under non-saline conditions and reducing EL. This divergence indicates that distinct endophytic strains may employ different physiological strategies. Some may excel at improving root water uptake or regulating stomatal conductance to bolster RWC, whereas others may emphasize direct cellular protection by strengthening antioxidant systems or stabilizing membrane architecture. The marked reduction in EL across all inoculated treatments was a strong indicator of preserved membrane integrity. This can be attributed to the endophytes' ability to mitigate oxidative stress by upregulating the host's antioxidant enzyme activities (e.g., superoxide dismutase, catalase, and peroxidase), producing antioxidant metabolites, and improving ion selectivity (e.g., favoring K^+ over Na^+ uptake), thereby minimizing the ionic toxicity that drives membrane damage [14]. Consequently, the maintenance of higher MSI and lower EL in inoculated plants directly translates to sustained cellular function, protecting the photosynthetic apparatus and other organelles, thereby providing the physiological basis for the improved growth and biomass accumulation observed under salinity stress.

The analysis of key biochemical metabolites revealed a strategic reprogramming of plant metabolism under salinity stress, which is further optimized by endophytic symbiosis to enhance tolerance. Salinity stress triggered a significant accumulation of osmoprotectants and antioxidants, including starch (SC, + 62.86%), total phenolic content (TPC, + 28.57%), and proline (PC, + 36.83%), indicating a classical adaptive response to mitigate osmotic and oxidative stresses. The concurrent decrease in total soluble sugars (TSS, -24.96%) likely reflects their rapid utilization as both an energy source for stress responses and as precursors for the synthesis of other protective compounds. Inoculation with the endophytes *T. harzianum*, *F. solani*, and their consortium significantly amplified this adaptive metabolic profile, elevating SC, TPC, TSS, and PC by 29.21%, 16.51%, 28.17%, and 11.70% on average, respectively, compared to the stressed controls. The highest values for SC, TSS, and TPC were recorded in the T treatment at the highest salinity level (TS3), underscoring the potent role of this endophyte in modulating host metabolism under severe duress. The differential effects of the endophytes are instructive: while endophyte F had the greatest impact on starch accumulation, endophyte T was most effective in boosting soluble sugars and phenolic compounds levels.

This specialization suggests that microbial partners can fine-tune different branches of the host metabolic network. The increased accumulation of soluble sugars and proline in inoculated plants serves a dual purpose: acting as compatible osmolytes to maintain cell turgor and water uptake (thereby supporting the higher RWC observed earlier) and functioning as potent antioxidants or redox buffers to quench reactive oxygen species (ROS), thereby preserving membrane integrity (as evidenced by improved MSI and reduced EL) [8, 60]. Similarly, the enhanced phenolic content indicates a reinforced secondary antioxidant system, in which these compounds scavenge ROS and inhibit lipid peroxidation chain reactions [61]. The endophyte-induced increase in starch content, particularly at lower salinity levels, may represent an enhanced carbon reserve, providing sustained energy and carbon skeletons for the synthesis of other defense compounds under prolonged stress. Importantly, this coordinated upregulation of diverse osmoprotective and antioxidant metabolites by endophytes creates a robust cellular defence matrix. This matrix not only buffers the immediate physicochemical insults of salinity but also stabilizes proteins and membranes, safeguards photosynthetic machinery, and ultimately channels resources toward sustained growth and biomass production, effectively linking the biochemical resilience documented here with the physiological and agronomic improvements demonstrated in the preceding sections.

A cornerstone of the salinity tolerance conferred by the fungal endophytes in this study was the profound enhancement of the plant's antioxidant defense system. Salinity stress triggered a significant increase in malondialdehyde (MDA) content, a definitive marker of lipid peroxidation and oxidative membrane damage. Inoculation with *T. harzianum* and *F. solani* effectively counteracted this, leading to a marked reduction in the MDA levels. This protective effect was underpinned by a substantial upregulation of the activity of key antioxidant enzymes, including catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), and ascorbate peroxidase (APX), as well as an increase in soluble protein content. Elevated enzyme activities signify an increased capacity to scavenge and detoxify reactive oxygen species (ROS), thereby preventing the cascade of oxidative damage that targets lipids, proteins, and nucleic acids. Concurrently, the increase in soluble proteins likely includes both protective enzymes and other stress-responsive proteins, contributing to cellular osmotic adjustment and stability. The synergy between reduced MDA levels and enhanced DPPH radical scavenging capacity further confirmed the overall bolstering of the antioxidant buffering capacity in colonized plants. This coordinated enzymatic and non-enzymatic reinforcement effectively safeguards cellular integrity, particularly of chloroplast membranes, which is directly linked to the preservation of chlorophyll content (SPAD values) and photosynthetic function under stress conditions. Thus, beyond merely mitigating damage, endophytes proactively amplify the host's inherent biochemical resilience, transforming its metabolic state to one of proactive defense, a critical adaptation that supports sustained physiological performance and growth under saline conditions [14, 62].

Based on the obtained results, salinity stress significantly impaired the ionic homeostasis of maize plants, primarily through the disruption of potassium (K^+) and phosphorus (P) uptake and translocation. The decreased K^+/Na^+ ratio under salinity, particularly in leaves, indicates competitive inhibition at uptake sites where Na interferes with K absorption, leading to osmotic and metabolic imbalances. However, endophyte inoculation, particularly the dual combination, effectively mitigated this effect. It enhanced K^+ retention in roots and improved its translocation to shoots at higher salinity levels, thereby maintaining a more favorable K^+/Na^+ ratio, which is crucial for enzymatic activity, stomatal regulation, and overall plant vigor. Concurrently, the decline in leaf and root P content under salinity, likely due to precipitation and reduced mobility, was alleviated by endophyte inoculation. The enhanced P uptake, particularly with the combined inoculants, can be attributed to mechanisms such as phosphate solubilization and improved root system architecture. These findings collectively demonstrate that the applied endophytes enhance salinity tolerance by concurrently modulating two critical nutritional pathways: maintaining potassium homeostasis to counteract sodium toxicity and improving phosphorus acquisition under ionic stress [13, 59].

Conclusion

In conclusion, this study provides compelling evidence that inoculation with adapted root-endophytic fungi is a potent and sustainable strategy for mitigating salinity stress in crops. Symbionts enhance plant resilience through a multi-pronged mechanism involving improved osmotic adjustment, reinforced antioxidant defense systems, and critical restoration of ionic homeostasis by modulating Na^+/K^+ ratios and enhancing phosphorus uptake. These coordinated physiological and biochemical responses culminated in significantly improved growth and biomass production under saline conditions. These findings strongly advocate for integrating of tailored microbial inoculants into agricultural practices to enhance crop productivity in salinity-affected areas. However, translating this promise from controlled environments to real-world settings remains an essential step. Future research should prioritize extensive field-scale validation across diverse agroecosystems and growing seasons to evaluate the efficacy, consistency, and ecological impact of these endophytic partnerships under complex environmental conditions. Elucidating the underlying molecular signaling and genetic regulation of these symbioses will be key to optimizing their application and developing next-generation bio-solutions for sustainable agriculture in the face of escalating abiotic stresses. Furthermore, elucidating the underlying molecular signaling and genetic regulation of these symbioses through advanced techniques like RNAi, CRISPR-Cas, metabolomics, NGS, and machine learning-based predictive models will be key to unraveling plant-endophyte interactions and developing next-generation bio-solutions for sustainable agriculture in the face of escalating abiotic stresses

Abbreviations

Abbreviations

APX: ascorbate peroxidase	K leaf: potassium in leaf	POD: peroxidase
BSA: bovine serum albumin	K root: potassium in root	ROS: reactive oxygen species
CAT: catalase	MDA: malondialdehyde	RSR: root-to-shoot ratio
Cl-leaf: chlorine in leaf	MSI: membrane stability index	RWC: relative water content
Cl-root: chlorine in root	Na leaf: Sodium in leaf	SC: starch concentration
CMC: carboxymethyl cellulose	Na root: Sodium in root	SOD: superoxide dismutase
CV: coefficient of variation	NBT: nitro blue tetrazolium	T: *Trichoderma harzianum*
DF: degree of freedom	ns: non-significant	TF: *T. harzianum* + *F. solani*
DPPH: 2, 2-diphenyl-1-picrylhydrazyl	P leaf: phosphorus in leaf	TMC: total moisture content
EDTA: ethylene diamine tetraacetic acid	P root: phosphorus in root	TP: total protein
EI: electrolyte leakage	PC: proline content	TPC: total phenolic content
F: *Fusarium solani*	PCA: principal component analysis	TSS: total soluble sugars
GI: greeness index	PDB: plant dry biomass	
HSD: tukey's honestly significant difference test	PFB: plant fresh biomass	

Declarations

Acknowledgments

The authors would like to acknowledge all the team members.

Author contributions

Conceptualization S.B., S.E., Z.B., and M.M.; data curation, S.B., and S.E.; formal analysis and methodology, S.B., S.E., and Z.B.; project administration, S.E., and Z.B.; visualization, S.B., S.E., and M.A.; writing original draft, S.B., and M.A.; writing-review and editing. S.B., M.A., S.E., Z.B and M.M. All authors have read and agreed to the published version of the manuscript.

Funding

This work was supported by Shiraz University and Iran National Science Foundation (INSF), grant number 99005565.

Data availability

Data will be made available on request.

Ethics approval and consent to participate

All procedures were conducted following the relevant institutional, national, and international guidelines and legislations.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Table

Table 1. Two-way analysis of variance (ANOVA) of the effects of desert endophytic fungal treatments under different salinity stress levels on the measured t
maize plants grown in the greenhouse.

Variables	DF	PFB	PDB	TMC	RSR	RWC	MSI	EL	SC	TSS	TPC	PC
Fungi	3	8855.542**	471.046**	5898.412**	0.014**	442.76**	387.48**	387.48**	17.63**	29.55**	0.43**	98
Salinity	3	143849.7**	7351.61**	86535.28**	0.29**	1225.6**	2285.60**	2285.60**	69.77**	26.56**	1.50**	67
Fungi* Salinity	9	6078.872**	106.49**	4984.52**	0.01**	12.23ns	15.67ns	15.67ns	0.72**	10.26**	0.15**	57
Residuals	32	1289.00	7.85	1264.08	0.00	32.38	16.72	16.72	0.21	0.28	0.02	27
CV		8.70	3.96	10.41	5.04	7.31	6.24	11.85	5.09	5.69	5.49	12.

Variables	TP	CAT	POD	APX	SOD	MDA	DPPH	GI	Leaf Na	Root Na	Leaf Cl	Ro
Fungi	0.136ns	98.05**	0.076**	6069.64**	79.72**	0.023**	611.39**	34.39**	0.14**	2.62**	4.79**	2.9
Salinity	0.46**	641.78**	0.32**	18096.56**	721.054**	0.02**	320.12**	107.93**	0.46**	15.21**	8.9**	5.0
Fungi* Salinity	0.02ns	7.511*	0.006**	1498.81**	10.51**	0.005**	6.57**	2.39ns	0.013**	0.22*	0.85**	0.1
Residuals	0.06	2.50	0.00	174.29	0.43	0.00	1.93	3.73	0.003	0.073	0.172	0.2
CV	7.82	8.60	7.26	9.53	2.37	3.45	1.91	5.81	12.87	11.55	9.942	11.

Variables	Leaf K	Root K	Leaf P	Root P
Fungi	0.61**	0.09**	0.00064**	0.00009**
Salinity	1.55**	1.05**	0.0006**	0.0002**
Fungi* Salinity	0.056ns	0.011ns	0.00011**	0.000008**
Residuals	0.033	0.017	0.00002	0.0000004
CV	4.56	12.13	3.155	0.51

*, ** and ns indicate significance at the 5% and 1% probability levels and non-significant differences, respectively, according to Tukey's multiple range test.

Figures

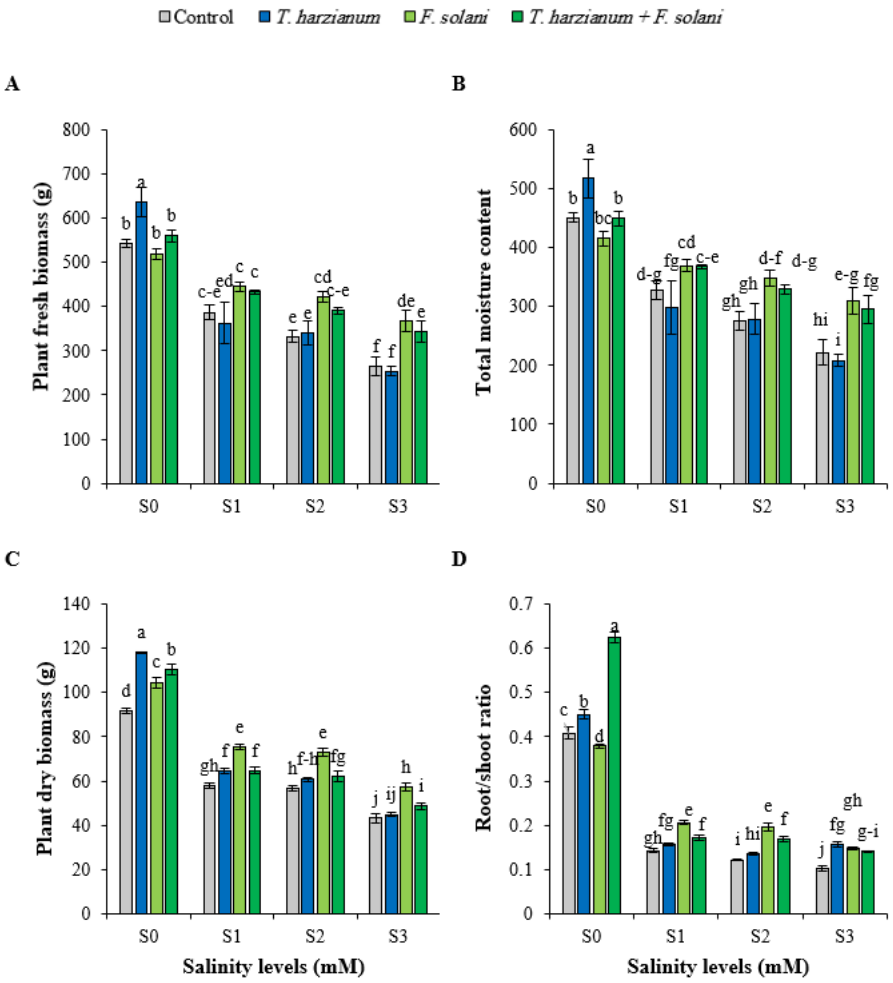


Figure 1

Mean comparison plots of the data obtained from the effects of endophytic fungal treatments (Control (C), *T. harzianum* (T), *F. solani* (F), *T. harzianum* + *F. solani* (TF)) under different salinity stress levels (0.5 (S0 = 0 mM), 4.56 (S1 = 50 mM), 6.84 (S2 = 75 mM), and 9.13 dS m^{-1} (S3 = 100 mM)) on the plant fresh biomass (A), total moisture content (B), plant dry biomass (C) and root/shoot ratio (D) of maize plants. Treatments sharing the same letters are not significantly different according to Tukey's test at the 5% probability level.

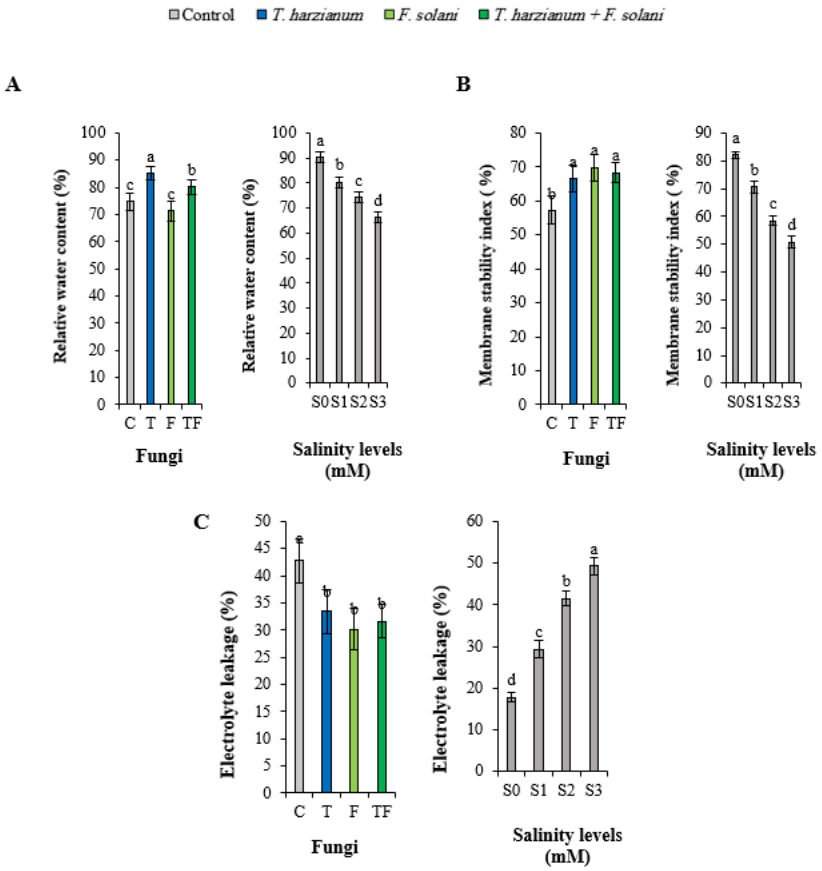


Figure 2

Mean comparison plots of the data obtained from the effects of endophytic fungal treatments (Control (C), *T. harzianum* (T), *F. solani* (F), *T. harzianum* + *F. solani* (TF)) under different salinity stress levels (0.5 (S0 = 0 mM), 4.56 (S1 = 50 mM), 6.84 (S2 = 75 mM), and 9.13 dS m^{-1} (S3 = 100 mM)) on the relative water content (A), membrane stability index (B) and electrolyte leakage (C) of maize plants. Treatments sharing the same letters are not significantly different according to Tukey's test at the 5% probability level.

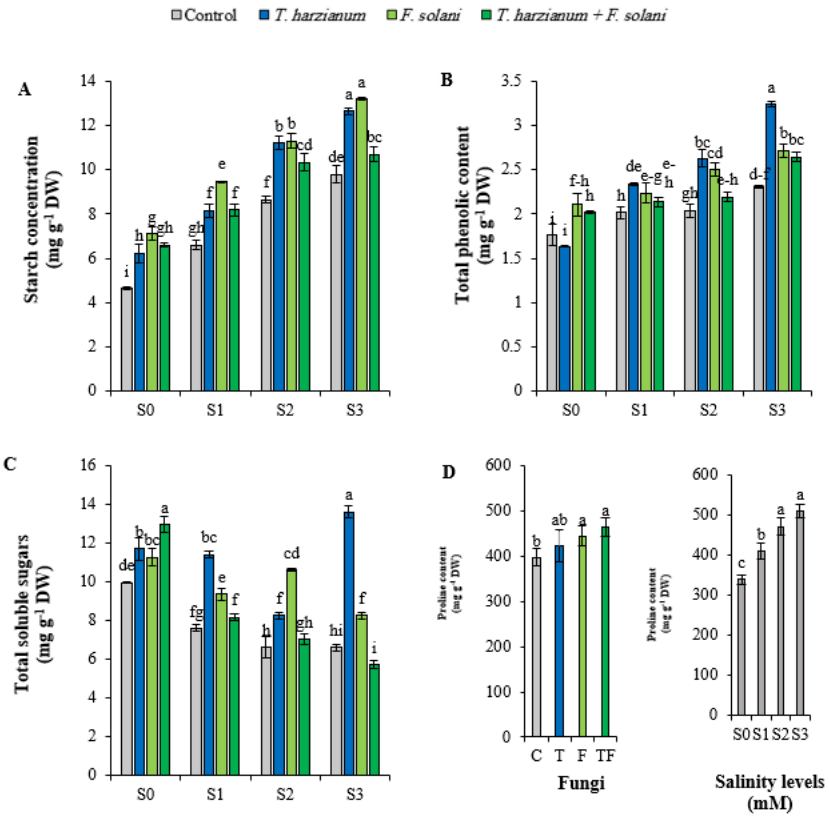


Figure 3

Mean comparison plots of the data obtained from the effects of endophytic fungal treatments (Control (C), *T. harzianum* (T), *F. solani* (F), *T. harzianum* + *F. solani* (TF)) under different salinity stress levels (0.5 (S0 = 0 mM), 4.56 (S1 = 50 mM), 6.84 (S2 = 75 mM), and 9.13 dS m^{-1} (S3 = 100 mM)) on the starch concentration (A), total phenolic content (B), total soluble sugars (C) and proline content (D) of maize plants. Treatments sharing the same letters are not significantly different according to Tukey's test at the 5% probability level.

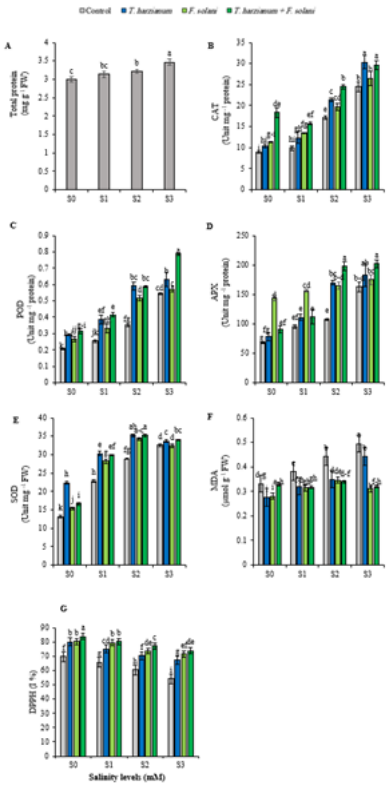


Figure 4

Mean comparison plots of the data obtained from the effects of endophytic fungal treatments (Control (C), *T. harzianum* (T), *F. solani* (F), *T. harzianum + F. solani* (TF)) under different salinity stress levels (0.5 (S0 = 0 mM), 4.56 (S1 = 50 mM), 6.84 (S2 = 75 mM), and 9.13 dS m^{-1} (S3 = 100 mM)) on the total protein (A), CAT (B), POD (C), APX (D), SOD (E), MDA (F) and DPPH (G) of maize plants. Treatments sharing the same letters are not significantly different according to Tukey's test at the 5% probability level.

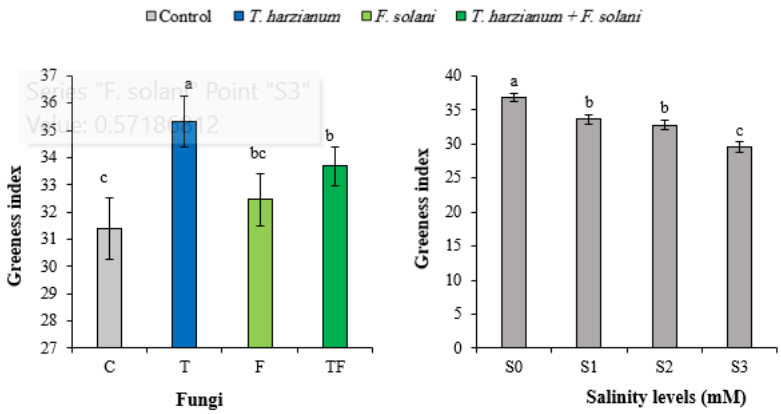


Figure 5

Mean comparison plots of the data obtained from the effects of endophytic fungal treatments (Control (C), *T. harzianum* (T), *F. solani* (F), *T. harzianum + F. solani* (TF)) under different salinity stress levels (0.5 (S0 = 0 mM), 4.56 (S1 = 50 mM), 6.84 (S2 = 75 mM), and 9.13 dS m^{-1} (S3 = 100 mM)) on the greenness index of maize plants. Treatments sharing the same letters are not significantly different according to Tukey's test at the 5% probability level.

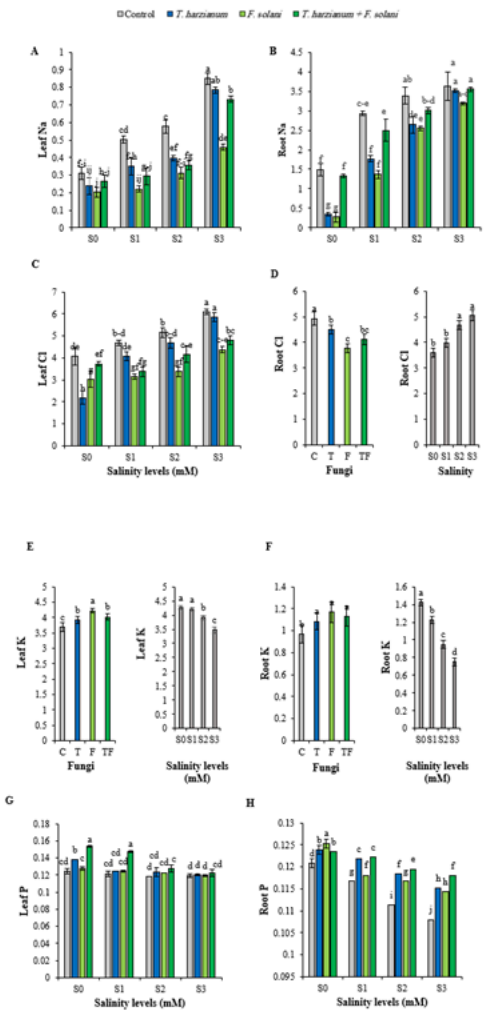


Figure 6

Mean comparison plots of the effects of endophytic fungal treatments (Control (C), *T. harzianum* (T), *F. solani* (F), *T. harzianum* + *F. solani* (TF)) under different salinity stress levels (0.5 (S0 = 0 mM), 4.56 (S1 = 50 mM), 6.84 (S2 = 75 mM), and 9.13 dS m^{-1} (S3 = 100 mM)) on the nutrient indices of maize plants. Treatments sharing the same letters are not significantly different according to Tukey's test at the 5% probability level.

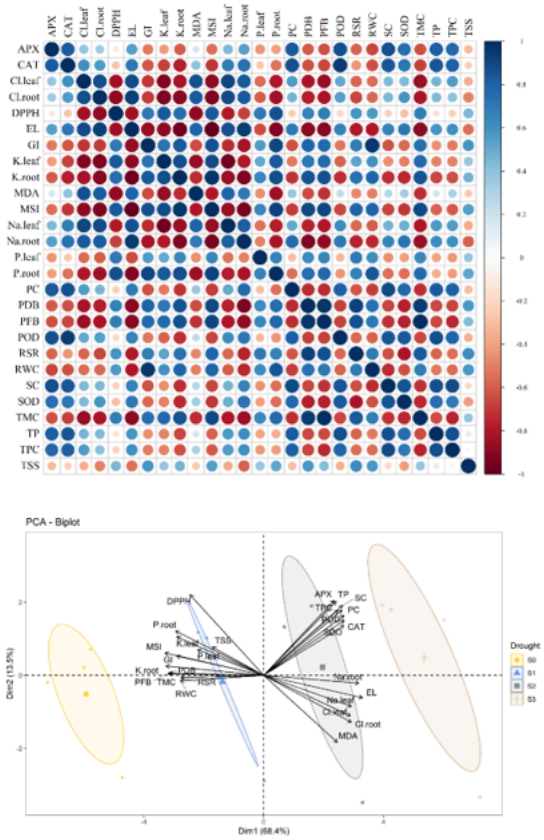


Figure 7

Pearson correlation plot (Corr plot) and Principal component analysis (PCA) biplot showing the relationships between the effects of endophytic fungi *T. harzianum* (T), *F. solani* (F), and their combination (TF) on various measured traits of maize under four salinity levels: S0 (0 mM), S1 (50 mM), S2 (75 mM), and S3 (100 mM).

Supplementary Files

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- Fig.docx