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Endophytic Fungus *Aspergillus terreus* Nf1 Enhances Salt Tolerance and Reactive Oxygen Species (ROS) Defense in Maize

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ABSTRACT

Salinization is associated with a high content of sodium chloride (NaCl) in the soil, which affects adverse plant growth and agricultural yield. In this study, the role of endophytic fungus *Aspergillus terreus* Nf1 in the alleviation of NaCl stress in maize seedlings was investigated. The fungus was grown in Czapek medium with 100 mM and 200 mM NaCl. It resulted in growth promotion and an increase in flavonoids, salicylic acid (SA), and phenols; however, there was a decrease in the concentration of IAA and soluble sugars. Exposure to 70 mM and 140 mM NaCl in maize seedlings led to decreased growth in terms of length of roots and shoots as well as fresh and dry weights. Growth parameters were significantly improved in the case of treatment with *A. terreus* Nf1. In treated plants, there were increases in flavonoids, SA, phenolics, proline, and chlorophyll content as well as in MDA content reduction. The endophyte enhances maize's salt resilience, demonstrating that NF1 promotes growth by mitigating salt stress effects.

Keywords: chlorophyll content, crop quality, endophytic fungi, flavonoid, osmolytes, salt tolerance.

INTRODUCTION

Extreme environmental conditions increasingly threaten plant survival in semi-arid and arid regions. Abiotic stress arises from environmental factors such as salinity, drought, temperature extremes, and water scarcity (Muhammad et al., 2024). Among these factors, salinity and drought are significant constraints limiting agricultural productivity worldwide. Soil salinity, particularly due to excessive sodium chloride (NaCl), adversely affects crop growth, yield, and germination. High levels of Na^+ disrupt ionic balance by inhibiting the uptake of essential nutrients such as potassium (K^+) and calcium (Ca^{2+}), impairing physiological and photosynthetic processes. This disruption leads to stomatal closure, reduced leaf growth, enzyme inhibition, and oxidative stress, ultimately restricting plant development (Jha et al., 2026). Although plants employ adaptive mechanisms like osmolyte accumulation, hormonal regulation, antioxidant defense, and gene expression control, these responses are often insufficient under severe stress conditions (Gul et al., 2023).

To address these challenges, sustainable and cost-effective strategies are needed. Plant-associated microorganisms, particularly endophytic fungi, have emerged as promising biological tools for enhancing plant stress

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tolerance. These fungi establish beneficial symbiotic relationships with host plants, improving nutrient uptake, phytohormone balance, and stress resilience, thereby enhancing overall plant performance under adverse conditions (Jha and Mohamed, 2024). Maize (*Zea mays* L.), one of the most important cereal crops globally, is highly sensitive to salinity stress (Jha and Mohamed, 2023). Exploring the role of endophytic fungi in improving maize tolerance to salt stress can significantly contribute to sustainable agriculture. Therefore, this study aims to evaluate the potential of endophytic fungi to mitigate NaCl-induced stress and promote maize growth.

MATERIAL AND METHODS

Acquisition of Fungal Strain

A strain of endophytic fungus, which is known to promote plant growth, was acquired from the PMI Lab, Botany Department, AWKUM. The strain was maintained on potato dextrose agar (PDA) plates at 28°C. Regular subculture of the strain was carried out to maintain its viability.

Screening of Endophytic Fungi for Salt (NaCl) Tolerance

The salt tolerance ability of the acquired fungal strain was examined using Czapek broth with different concentrations of salt (NaCl) at 100 mM and 200 mM. Czapek medium was prepared according to the standard protocol described by Carranza et al. (2014). The spores from the acquired fungal strain were inoculated into autoclaved broth, and the flasks were maintained at 28°C with 120 rpm using a shaking incubator for seven days. The growth of the fungus was determined based on both fresh weight and dry weight. The isolates showing resistance to salt stress were used for further studies.

Detection of Metabolites and Phytohormones in Fungal Culture Filtrate

Salicylic Acid (SA) Determination

The concentration of salicylic acid in fungal culture filtrates was determined by a slightly modified procedure of Warrier et al. (2013). This involves mixing 0.1 mL of fungal filtrate with 0.1% ferric chloride solution to a total volume of 3 mL. The development of a purple color, a result of the reaction of ferric ions and salicylic acid, was measured by determining the absorbance at 540 nm. A blank solution consisting of ferric chloride solution only was used as a reference, and a standard curve was constructed by using a range of SA concentrations (100 to 500 µg/mL).

Flavonoid Determination

Flavonoid content in the fungal filtrates was determined by adding 0.5 mL of fungal filtrate to 4.3 mL methanol, 0.1 mL aluminum chloride, and 0.1 mL potassium acetate. The mixture was well shaken to enable color development, and absorbance was measured at 415 nm. The results were determined by using a standard curve constructed by using a range of 10 to 100 µg/mL of quercetin as described by Pan et al. (2017).

Phenol Determination

Phenols were quantified by reacting 0.5 mL of the fungal filtrate with 0.5 mL of 80% methanol and 0.5 mL of Folin-Ciocalteu reagent (Pan et al., 2017). After three minutes of incubation, 2 mL of 20% sodium carbonate solution was added, and the mixture was heated for 1-2 minutes using a water bath at 40°C. The development of a deep blue color indicated the presence of phenols. The mixture was then subjected to measurement of absorbance at 650 nm. Gallic acid (100-900 µg/mL) was used for the preparation of a standard curve.

Indole Acetic Acid (IAA) Determination

IAA content of the fungal filtrates was quantified by using Salkowski's reagent, as proposed by Benizri et al. (1998). The mixture of 1 mL of the filtrate and 2 mL of Salkowski's reagent was incubated for 30 minutes in darkness. The absorbance of the mixture was then measured at 540 nm. The standard curve for IAA determination was

prepared by using IAA solutions containing 10-100 µg/mL.

Total Sugar Determination

The total sugar content was determined using the phenol sulfuric acid method, as described by Dubois et al. (1956). A mixture of 2 mL of fungal filtrate and 5 mL of 80% phenol solution was prepared, followed by the addition of 5 mL of concentrated sulfuric acid. The mixture was then heated for a period of 1 hour. The absorption spectrum was then measured at a wavelength of 490 nm.

Microscopy of Endophytic Fungal Strain

A small quantity of fungal mycelia was placed on a water drop on a microscope slide. A coverslip was then placed on the drop, gently pressing it to spread the fungal mycelia. Staining with lactophenol cotton blue enabled clear visualization of the fungal cell walls under a microscope at a magnification of 400×.

Plant Growth Experiment

Zea mays L. seeds were surface sterilized and germinated under standardized conditions. Uniform seedlings were transplanted into plastic pots (diameter=15 cm) filled with 300 g of garden soil. Characterization of the soil was done prior to the experiment. Soil had a pH of 7.2, an electrical conductivity (EC) of 1.8 dS m^{-1} and was a sandy loam type of soil. The pots were arranged randomly with 5 biological replicates per treatment. The experiment was carried out with six major treatments to determine the role of the fungus *Aspergillus terreus* Nf1 in alleviation of salt stress in the plants:

Control: Without any treatment.

NF1: Inoculation of plants with *A. terreus* Nf1 only.

70 mM: NaCl stress (70 mM) without inoculation of the fungus.

140 mM: NaCl stress (140 mM) without inoculation of the fungus.

NF1+70 mM: Fungal inoculation followed by 70 mM NaCl stress.

NF1+140 mM: Fungal inoculation followed by 140 mM NaCl stress.

In the case of fungal inoculation, 1 g of fungal biomass was combined with 300 g of soil prior to transplantation. The control group was provided with 300 g of soil without any addition of fungal biomass. After establishment, seedlings were exposed to salt stress. Salt stress was slowly induced using NaCl solutions in order not to cause any osmotic shock to the plants. Plantlets were grown under natural illumination at 28/22°C (day/night), 65% humidity, and natural day length. The moisture of the soil was kept at 70% of the field capacity during the experiment. The duration of growing was 21 days following the addition of NaCl solution; then, shoots and roots were collected. They were immediately frozen in liquid nitrogen and stored at -80°C until further biochemical and physiological investigations, such as chlorophyll content determination, antioxidants (catalase), stress indicators (MDA and electrolyte leakage), and secondary metabolites (phenols, flavonoids, SA, proline).

Photosynthetic Pigments

Total chlorophyll and carotenoids from 0.3 g of fresh leaf tissue of the plants were extracted using 90% acetone. The homogenate was centrifuged at 10,000 rpm, and the extract was then diluted to 7 ml with acetone. The absorbance of the extract at 480, 645, and 663 nm was measured to determine the concentration of carotenoids, chlorophyll A, and chlorophyll B using the standard equations provided by Maclachlan and Zalik (1963).

Quantification of Leaf Metabolites and Phytohormones

Leaf tissue homogenate (0.3-0.5 g) was used with appropriate solvents according to the target metabolite. The homogenate was then centrifuged to get a clear solution. IAA and SA were analyzed using the same methods described for fungal filtrates. Flavonoids, phenolic compounds, total sugars, and lipids (Vogel, 1956) were assayed using standard colorimetric methods. The absorption was measured at appropriate wavelengths. Proline content was assayed using the following methods: homogenization

of leaf tissue in 3% sulfosalicylic acid, incubation with ninhydrin and glacial acetic acid, and then using toluene as a solvent (Bates et al., 1973). The absorption was measured at 520 nm. Catalase activity was assayed using the following methods: homogenization of leaf tissue in sodium phosphate buffer, incubation with H_2O_2, and measurement of decrease in absorption at 240 nm for 2 min at intervals of 30 sec (Chandlee and Scandalios, 1984). Malondialdehyde (MDA) content was used as a marker for lipid peroxidation. Leaf tissue homogenate was incubated with thiobarbituric acid and trichloroacetic acid, then incubated at 95°C. The absorption was measured at 450, 532, and 600 nm (Dhindsa et al., 1981). Electrolyte leakage was assayed using the following methods: measurement of initial and final conductivity of leaf pieces immersed in distilled water and autoclaved to release total electrolytes. EL was then calculated as follows:

$EL = (EC1/EC2) \times 100$ (Mao et al., 2007)

Root Colonization by Endophytic Fungi

Roots of *Z. mays* L. were washed with distilled water, stained with lactophenol cotton blue for 1 minute, destained, and mounted on slides. Endophytic colonization was observed using a light microscope (Higashi and Abe, 1980).

3,3′-Diaminobenzidine (DAB) Staining

DAB staining was conducted to observe the reactive oxygen species in the maize leaves. The leaves of the seedlings were immersed in 2 mL of DAB solution for 4-5 hours under gentle shaking at a speed of 80-100 rpm. After that, the leaves were subjected to destaining in a solution containing ethanol, acetic acid, and glycerol in the ratio of 3:1:1 at 40°C. The presence of ROS was indicated by the brown color under the light microscope.

Statistical analysis

A comparison of means was conducted using the Statistical Package for the Social Sciences (SPSS) for Windows to assess the significance between treatments. The quantitative tests performed encompassed Duncan's multiple range test and analysis of variance (ANOVA). Graphics were created using the GraphPad Prism software.

RESULTS AND DISCUSSION

The endophytic fungus

In this study, the endophytic fungal strain used was obtained from the culture collection of PMI laboratory, Abdul Wali Khan University Mardan (AWKUM), and demonstrated plant growth–promoting potential. The strain was found to form a brown-colored colony covering the entire plate in five days (Figure 1a). Under controlled conditions and at 40x magnification, microscopic studies on fungi show healthy growth in terms of conidiophores and hyphae (Figure 1b). However, in the presence of 100 mM NaCl, there was found to be moderate growth inhibition in terms of a decrease in conidiophores and thin hyphae (Figure 1c). At a concentration of 200 mM NaCl, severe growth inhibition was observed in terms of sparse and fragmented hyphae and a decrease in conidiophores (Figure 1d). These observations indicate a progressive decline in fungal structural integrity with increasing salinity stress (Sodhi and Saxena 2023).

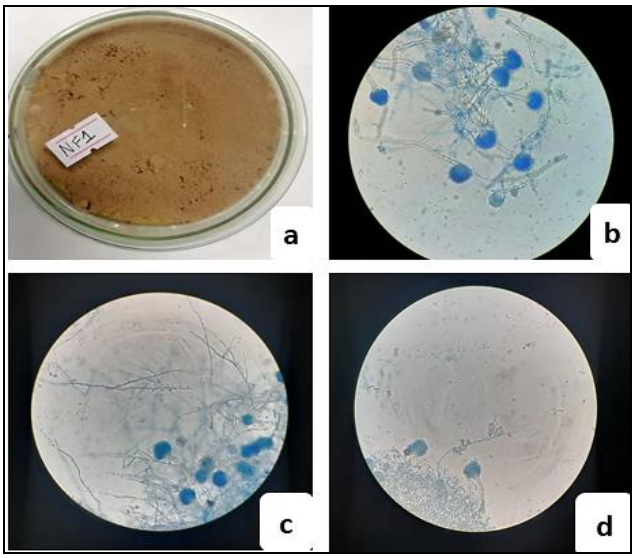

Figure 1. Colony morphology (a) and microscopy of fungal cultures under control (b), 100 mM NaCl (c), and 200 mM NaCl (d). A 400x light microscope magnified lactophenol cotton blue-stained fungal mycelia.

Screening of endophytic fungal strain on Czapek medium under NaCl stress

The subcultures of NF1 were maintained in 250 ml flasks with 50 ml of Czapek medium and were subjected to 100 mM and 200 mM concentrations of NaCl stress (Figure 2). The endophytic fungal strain showed good biomass production and was able to exhibit resistance to 100 mM NaCl stress. However, it was able to produce biomass in only 43.6% of the culture medium containing 200 mM NaCl.

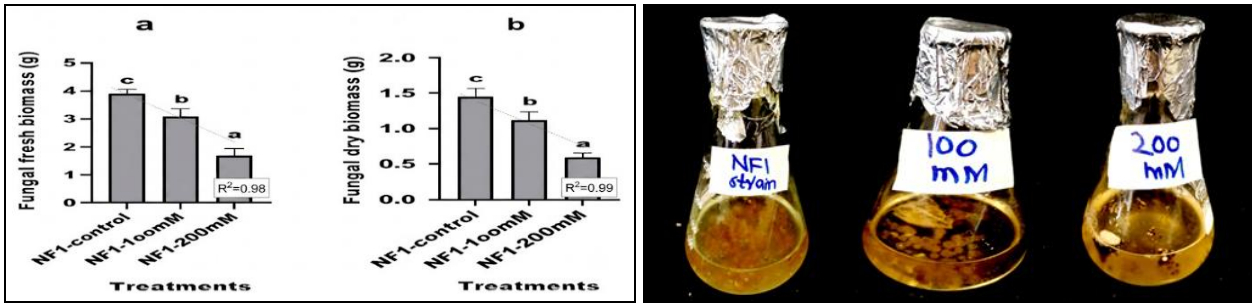

Figure 2. Effect of NaCl (100 mM and 200 mM) on the growth of endophytic fungus (NF1) in Czapek medium. Bars show the meaning of three replicates, with ± SE and significance labels (ANOVA-Duncan MRT; $p \leq 0.05$).

IAA, flavonoids, SA, sugar, and phenol in cultural filtrates

Salt exposure significantly influenced the production of stress-related metabolites in the endophytic fungus, including indole-3-acetic acid (IAA), salicylic acid (SA), flavonoids, sugars, and phenolic compounds (Figure 3). For example, the endophyte has released significantly lower amounts of IAA and sugars in response to salt exposure than in the control. It has been found that the decrease in the release of IAA and sugars in response to 100 and 200 mM concentrations of NaCl in the culture medium is 27.3% and 54.4% and 10.4% and 23.3%, respectively, compared to the control. The release of flavonoids, SA, and phenol has been enhanced in response to salt exposure in cultures compared to the control, i.e., 10.7% and 20.6%, 9.8% and 19.4%, and 15.2% and 21.3%, respectively. It has been found that higher amounts of flavonoids, phenols, proline, and SA are released by endophytes in response to salt exposure, which may be related to stress adaptation. Proline acts as an osmolyte not only within the living system but also in the external environment to maintain optimal water potential. The release of proline

enhances symbiosis with other plant root systems. Phenols can be used to chelate (Ali et al., 2022a, b). In addition, phenols, like flavonoids, can scavenge ROS present in the surrounding environment, which could have accumulated as a result of stress. Internal stress can be alleviated by the release of excess SA, which can improve the colonization potential of the endophytic fungi (Gupta et al., 2021). Overall, this shows the potential to survive under salt stress, improve soil health, and synergistically interact with the host plant.

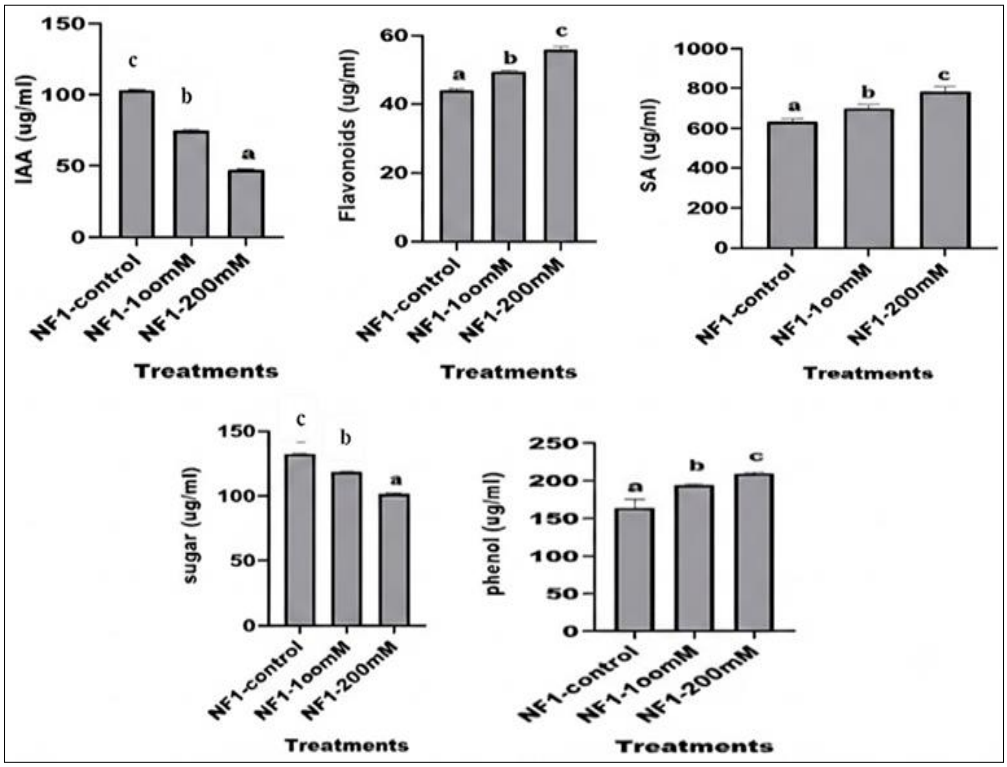

Figure 3. Concentration of indole-3-acetic acid (IAA), flavonoids, SA, sugar, and phenol in the culture filtrate of selected endophytic fungi under salt exposure. The bars exhibit the mean values of three replicates and are accompanied by the standard error (± SE) and labels indicating statistical significance (ANOVA-Duncan MRT; $p \leq 0.05$).

Effects of salinity and endophytic fungi on shoot and root length and fresh, dry weight

The seedlings colonized with endophytes showed a significant increase in terms of shoot length, root length, and fresh and dry weight compared with the control (Figure 4). The NF1-associated seedlings showed a remarkable increase of 12.5% and 20.8% in terms of shoot length and root length and a 15.3% and 18.4% increase in terms of fresh and dry weight compared with the control. In terms of NaCl concentrations of 70 mM and 140 mM, the shoot length decreased by 18.2% and 33.3%, the root length decreased by 16.6% and 30.5%, the fresh weight decreased by 22.7% and 38.3%, and the dry weight decreased by 12.5% and 22.5% compared with the control. The positive changes in growth parameters in terms of shoot length, root length, and biomass due to endophytic infection indicate the ability of these fungi to regulate plant hormonal signal transduction and ion balance. In the absence of stress, endophytes usually trigger the tryptophan metabolic pathway, leading to auxin (indole-3-acetic acid, IAA) biosynthesis (Chauhan et al., 2024). The decrease in morphological parameters under salt stress (70 mM and 140 mM) is due to the proven effect of salinity in disturbing osmotic pressure and mineral uptake. However, colonization by endophytes greatly reduces these negative effects. Previous studies have revealed that *A. terreus* strains promote transcriptional reprogramming, thereby promoting the activity of antioxidant enzymes such as catalase. Antioxidant enzymes prevent oxidative stress by breaking down reactive oxygen species (ROS) (Jiao et al., 2026).

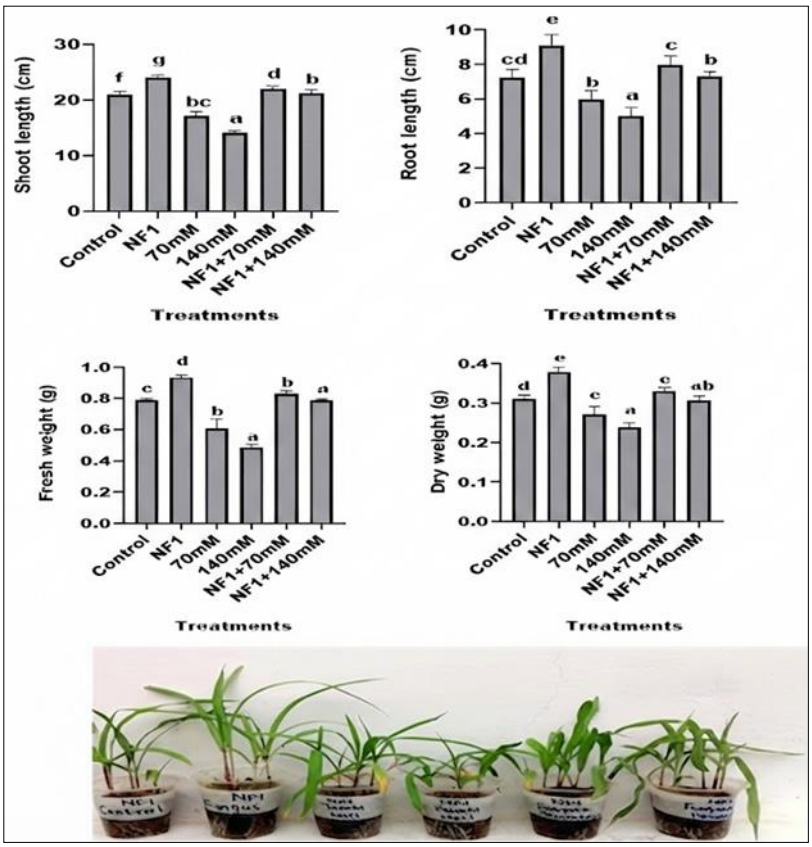

Figure 4. Effect of endophytic fungi (NF1) on growth parameters of maize under salinity stress. The data are presented as mean ± SE (n=3). The superscripted lowercase letters in each bar denote statistical differences between treatments determined by one-way ANOVA and post hoc DMRT test at P≤0.05.

Effects of salinity and endophytic fungi on chlorophyll content

The photosynthetic pigment levels of the plants were found to be affected by salinity stress and NF1 inoculation (Table 1). In the absence of salinity stress, NF1 inoculation significantly increased the pigments as compared to the control, resulting in an increase of 13.3% in chlorophyll a, 50.0% in chlorophyll b, 15.5% in total chlorophyll, and 46.2% in carotenoids. Salinity stress caused a marked reduction in the levels of all photosynthetic pigments. Exposure of the plants to 70 mM NaCl reduced chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids by 33.3%, 28.6%, 31.0%, and 23.1%, respectively, compared to the control. At 140 mM NaCl, the reductions in all pigments were more marked, with chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids being reduced by 37.8%, 42.9%, 39.7%, and 46.2%, respectively. Inoculation with NF1 significantly ameliorated the adverse impacts of salinity on the photosynthetic pigments. As compared to the plants treated with 70 mM NaCl alone, the plants inoculated with NF1 under 70 mM NaCl treatment showed considerable increments of 53.3%, 70.0%, 47.5%, and 60.0% in the amounts of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids, respectively. Furthermore, in the case of extreme salinity, the inoculation of NF1 greatly enhanced the amounts of photosynthetic pigments as compared to plants that were treated with 140 mM NaCl alone. In this regard, there was an increase of 57.1%, 87.5%, 60.0%, and 85.7% in the amounts of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids, respectively. In conditions of salt stress, considerable accumulation of Na+ ions occurs within the chloroplast, where it affects the functioning of photosystem II and impedes photosynthesis and consequently the growth of the plant. It appears that considerable restoration of the content of chlorophyll a, b, total chlorophyll, and

carotenoids in NF1-inoculated plants relative to salt-stressed non-inoculated controls can indicate that the endophyte is successful in strengthening the light-harvesting mechanism (Chauhan et al., 2024). Destruction of chlorophyll caused by salt stress due to the accumulation of Na+ and Cl- ions usually results in ion toxicity and generation of excessive ROS, leading to the damage of chloroplast lamellae. Presumably, through stimulating the formation of photosynthetic pigments, the endophyte stabilizes the structure of the chloroplast and improves its functioning as a light-harvesting complex, which is vital for biomass formation. In addition, increased carotenoid content plays a special role since these are not only accessory pigments but also strong antioxidants protecting the photosynthetic system from photo-oxidative damage under conditions of salinity stress (Jiao et al., 2026).

Table 1. Effect of endophytic fungi (NF1) on photosynthetic pigments of maize under salinity stress

Treatment	Chlorophyll A (mg g^{-1})	Chlorophyll B (mg g^{-1})	Total Chlorophyll (mg g^{-1})	Carotenoids (mg g^{-1})
Control	4.5 ± 0.2^{e}	1.4 ± 0.1^{d}	5.8 ± 0.4^{e}	$1.3 \pm SE^{d}$
NF1	5.1 ± 0.2^{f}	2.1 ± 0.1^{e}	6.7 ± 0.5^{f}	$1.9 \pm SE^{e}$
NaCl 70 mM	3.0 ± 0.1^{b}	1.0 ± 0.1^{b}	$4.0 \pm SE^{b}$	$1.0 \pm SE^{b}$
NaCl 140 mM	2.8 ± 0.1^{a}	0.8 ± 0.1^{a}	$3.5 \pm SE^{a}$	$0.7 \pm SE^{a}$
NF1 + 70 mM	4.6 ± 0.2^{de}	1.7 ± 0.1^{c}	$5.9 \pm SE^{d}$	$1.6 \pm SE^{bc}$
NF1 + 140 mM	4.4 ± 0.2^{c}	1.5 ± 0.1^{b}	$5.6 \pm SE^{c}$	$1.3 \pm SE^{b}$

The data are presented as mean ± SE (n=3). The superscripted lowercase letters in each column denote statistical differences between treatments determined by one-way ANOVA and post hoc DMRT test at $P \leq 0.05$.

Effects of salinity and endophytic fungi on antioxidant enzymes, phytohormones, and metabolites

The levels of metabolites, phytohormones, and antioxidant enzymes were assessed in leaf extracts from *Zea mays* L. seedlings under both normal and stressed conditions. Seedlings colonized by endophytes exhibited significantly higher levels of total sugars, flavonoids, phenols, proline, lipids, salicylic acid, indole-3-acetic acid (IAA), and catalase. In contrast, they showed lower electrolyte leakage and malondialdehyde (MDA) content compared to the control group (Figure 5). Seedlings associated with the endophyte NF1 demonstrated a significant increase of 21% in leaf sugars and 10% in flavonoids, as well as increases of 8%, 58%, and 12% in leaf phenols, proline, and lipids, respectively, compared to the control group. However, seedlings exposed to NaCl stress at concentrations of 70 mM and 140 mM exhibited reductions in leaf sugars (22.7% and 48.4%, respectively) and lipids (5.2% and 16.4%). Conversely, there were increases in leaf flavonoids (2% and 5.7%), phenols (17% and 26%), and proline (83% and 86%) compared to the control group. While the endophytes associated with seedlings under stress conditions led to increased levels of sugars, flavonoids, phenols, proline, and lipids compared to their controls, salt stress resulted in decreased sugar and lipid levels but increased flavonoids, phenols, and proline levels. Seedlings linked to NF1 also showed notable increases of 13%, 29.2%, and 2.6% in leaf salicylic acid, IAA, and catalase concentrations when compared to the control group. Under 70 mM and 140 mM NaCl concentrations, leaf salicylic acid levels increased by 12% and 17%, respectively, while catalase levels rose by 10.2% and 14.5%. However, IAA levels decreased by 13.9% and 22.6%, respectively, compared to the control. Overall, seedlings growing under stress conditions along with endophytes exhibited elevated levels of salicylic acid, IAA, and catalase compared to their respective controls.

Electrolyte leakage and malondialdehyde (MDA) levels from the leaves of endophyte-colonized seedlings were lower than those in the control group. In seedlings associated with the NF1 endophyte, the reduction in leaf electrolyte leakage was 53%, and the MDA concentration was reduced by 36% compared

to the control group. When exposed to solutions containing 70 and 140 millimolar (mM) of sodium chloride (NaCl), the electrolyte leakage levels in the leaves of these seedlings increased by 64% and 66%, respectively. Similarly, the concentration of MDA in the leaves increased by 53% and 63%. Overall, the endophytes present in the stressed seedlings displayed reduced levels of electrolyte leakage and MDA compared to their corresponding control group.

To evaluate the hypothesis that endophytes NF1 help mitigate NaCl stress by releasing metabolites that reduce salt toxicity in host seedlings, we analyzed the endogenous metabolites in both inoculated and non-inoculated seedlings. Exposure to salt significantly reduced the levels of indole-3-acetic acid (IAA) in maize seedlings. This reduction may be due to decreased biosynthesis, increased breakdown, disrupted transport, and hormonal interactions, particularly with abscisic acid (ABA), as a mechanism for coping with stress (Chauhan et al., 2024). In contrast, salt exposure had a negligible effect on IAA accumulation in maize seedlings associated with endophytes, which showed higher levels of this phytohormone compared to their non-endophyte counterparts. The enhanced levels of IAA in endophyte-colonized seedlings could result directly from the endophyte or indirectly from reduced salt toxicity (Gul et al., 2023).

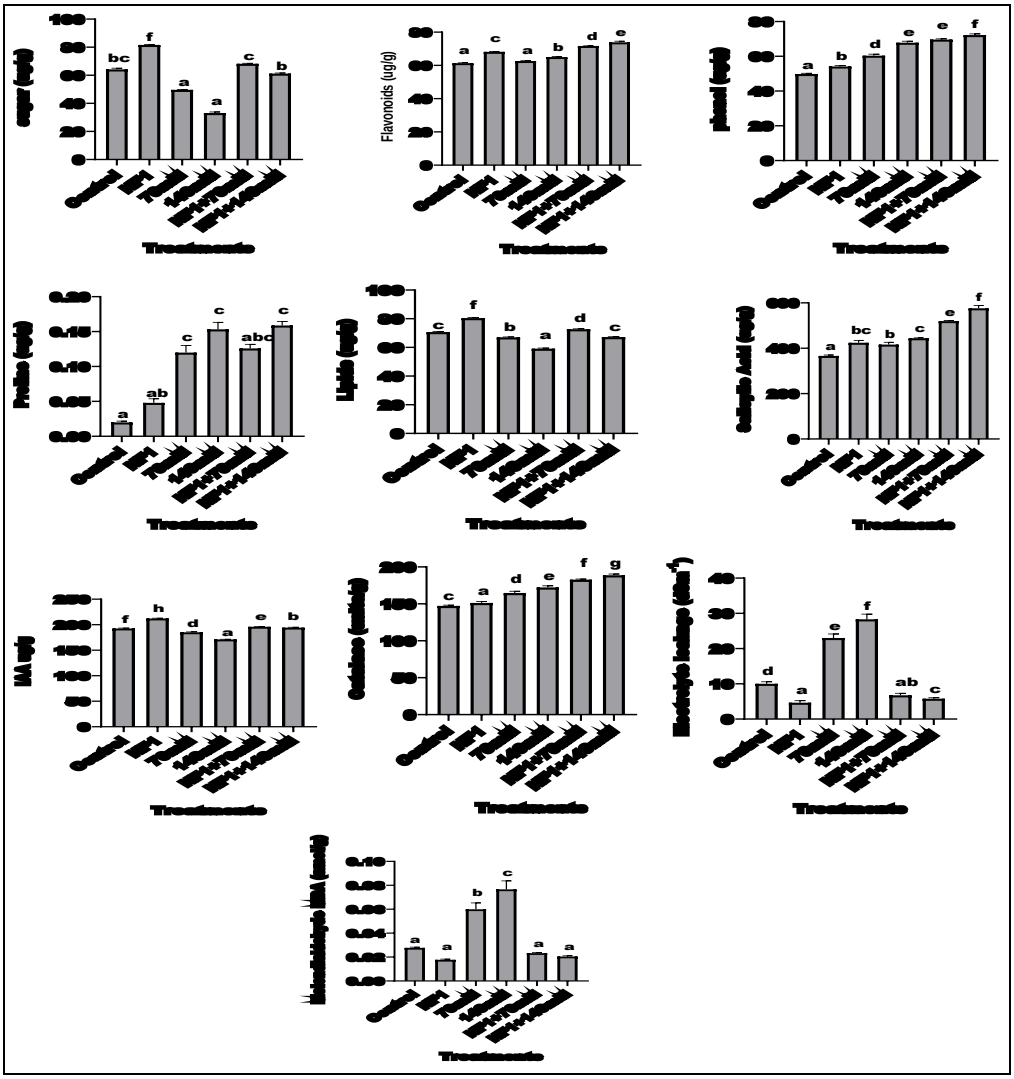

Figure 5. Effect of endophytic fungi (NF1) on total sugars, flavonoids, phenol, proline, lipids, SA, IAA, catalase, electrolyte leakage, and MDA of maize under salinity stress. The data are presented as mean ± SE (n=3). The superscripted lowercase letters in each bar denote statistical differences between treatments determined by one-way ANOVA and post hoc DMRT test at P≤0.05.

Auxin plays a central role in plant stress responses, but severe salt stress reduces its levels in rice by downregulating auxin biosynthetic genes due to oxidative stress and Na^+/Cl^- toxicity (Du et al., 2013). Concurrently, abscisic acid accumulates, antagonizing auxin and redirecting energy from growth to stress adaptation (Fahad et al., 2015). IAA is critical for regulating Na^+ transport, mitigating salt-induced damage, and enhancing nutrient uptake. Salt-exposed wheat accumulates salicylic acid, which chelates excess ions and scavenges ROS, enhancing stress tolerance (Hoque et al., 2020). Endophyte-associated seedlings maintain adequate SA levels, unlike non-endophyte plants.

Salt stress also elevates flavonoids, phenols, and proline, acting as antioxidants and osmolytes to preserve cell integrity and turgor (Bagheri et al., 2013; Yang et al., 2026). Maize treated with the endophytic strain NF1 showed increased accumulation of these metabolites under normal and saline conditions. NaCl stress reduced sugar and lipid content due to stomatal closure, decreased photosynthesis, and ROS-induced lipid peroxidation, whereas endophyte inoculation restored sugar and lipid levels, stabilizing membranes and improving stress resilience (Zhang et al., 2019). Catalase activity, indicative of ROS detoxification, increased under salt stress and was further enhanced by endophyte association, supporting antioxidant defense (Ali et al., 2022a, b). Fungal colonization of maize roots decreased under salt stress, likely due to altered root biochemistry and direct inhibition of hyphal growth (Navarro et al., 2014). MDA levels and electrolyte leakage, markers of oxidative stress and membrane damage, rose under salinity but were mitigated by endophyte treatment through improved ionic balance and metabolic adjustments (Khushdil et al., 2019).

Root colonization of *Zea mays* L.

Root colonization of endophytic fungi in *Zea mays* L. was observed microscopically using lactophenol (cotton blue) following exposure to NaCl stress (Figure 6). The results indicate that endophytic fungi effectively colonize the root tissues of *Zea mays* L. seedlings. Additionally, the presence of NaCl stress reduces the number of colonized hyphae.

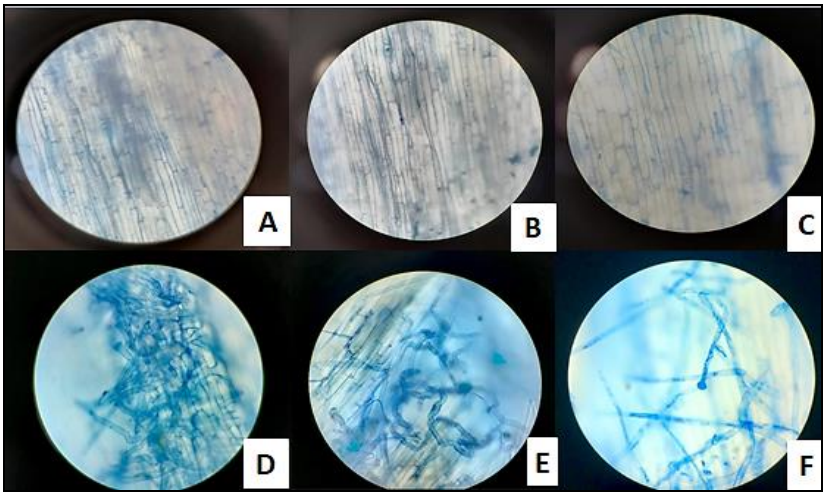

Figure 6. Effect of NaCl stress on *Zea mays* L. root colonization of endophytic fungi NF1
A) Control; **B)** 70 mM NaCl; **C)** 140 mM NaCl; **D)** NF1 strain; **E)** 70 mM+NF1 strain; **F)** 140 mM+NF1 strain

DAB staining (accumulation of ROS in leaves of maize seedlings)

To determine the existence of reactive oxygen species in the leaves of maize seedlings, a DAB staining method was used. An examination of the leaves under a microscope revealed the presence of ROS, which appeared as a dark stain on the surface of the leaf. Elevated levels of reactive oxygen species were observed in the host plant under NaCl stress. Microbial species play a crucial role in protecting the host plant and

decreasing the production of ROS. Exposed to NaCl stress at concentrations of 70 mM and 140 mM, the maize seedlings exhibited increased levels of reactive oxygen species. The seedlings exposed to a stress level of 140 mM exhibited a higher accumulation of ROS in comparison to those exposed to a stress level of 70 mM. In contrast, the seedlings showed a decrease in the accumulation of ROS when their exposure to NF1 endophytic fungus treatment was observed, even when subjected to NaCl stress at levels of 70 mM and 140 mM (Figure 7). In summary, the DAB staining experiments indicate that *A. terreus* NF1 is capable of ameliorating the oxidative stress induced by salt by inhibiting the production of reactive oxygen species as well as enhancing the effectiveness of the antioxidant defense system. The outcomes show that *A. terreus* NF1 has great potential to be used as an eco-friendly biological inoculum for growing maize in saline soils (Chauhan et al. 2024).

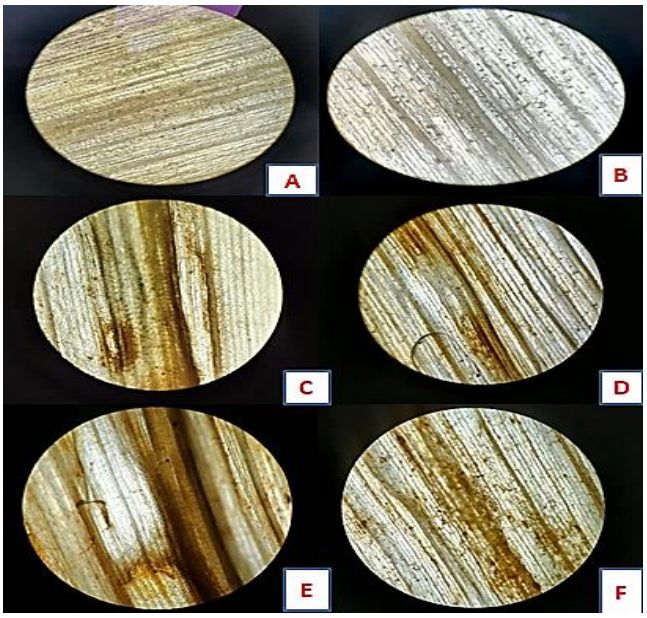

Figure 7. **A)** Control (without any treatment); **B)** NF1 strain; **C)** 70 mM NaCl; **D)** 70 mM+NF1 strain; **E)** 140 mM NaCl; **F)** 140 mM+ NF1 strain

CONCLUSIONS

Research indicates that endophytic fungi can protect plants from salinity's harmful effects on growth. These microorganisms enhance host antioxidant defense systems and contribute to increased biomass by producing phytohormones and secondary metabolites. Salinity significantly impacts crop yield, so utilizing these microorganisms offers new opportunities to improve crop production and reduce reliance on chemical fertilizers.

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