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Bio-amelioration of saline soil using *Aeluropus littoralis*, arbuscular mycorrhizal fungus (AMF), and salt-resistant PGPB

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Abstract

This study aimed to evaluate the capability of the halophyte *A. littoralis* in symbiosis with *Rhizophagus intraradices* and *Nocardia Halotolerans* an indigenous bacterium of saline soils- on phytoextraction of Na under saline conditions. Salinity treatments included 0 (S0), 100 mM NaCl (S1), 200 mM NaCl (S2), 100 mM NaCl+50mM K_2SO_4 (S3), 200 mM NaCl+50mM K_2SO_4 (S4) levels. Plant fresh and dry weight, chlorophyll content decreased as salinity increased up to S2 level and increased thereafter. Plant root colonization in the inoculation and co-inoculation of AMF+SR-PGPB were similar. Compared to the S0 treatment, root colonization in the AMF group decreased by 23.5%, 32.6%, 13.5%, and 26.7% under S1, S2, S3, and S4 treatments, respectively. In the Bacteria+AMF group, the reduction was smaller, with decreases of 2.8%, 3.4%, and an increase of 6.8% and 1.4% under S1, S2, S3, and S4 treatments, respectively. These results indicate that co-inoculation with PGPB mitigated the negative effects of salinity on root colonization. The root and soil glomalin contents increased as salinity increased. Root glomalin in plants inoculated by AMF+SR-PGPB was more than in a single inoculation of AMF under salt stress. This study highlights the potential application of salt-tolerant bacteria and AMF as effective strategies for enhancing plant growth and productivity in saline environments, contributing to sustainable agricultural practices in affected regions.

Keywords: Halophyte, mycorrhizal symbiosis, plant growth promoting traits, phytoremediation, salinity stress.

INTRODUCTION

Soil salinization is a growing global concern, with estimates suggesting that by 2050, more than 50% of arable land will be affected, leading to a significant decline in crop production (Talbi Zribi *et al*., 2020). Soils affected by salinity in Iran constitute about 55.6 million hectares, which is 34% of the country's total land. Saline soil is mainly affected by Na, Ca and Mg chloride and sulfate salts, but bicarbonates are present in low amounts (Barhoumi, 2018; Barzegargolchini *et al*., 2017). Salinity stress limits water availability to plant roots through osmotic stress and, over time, causes

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ionic toxicity and nutrient imbalances, leading to growth reduction (Animasaun *et al*., 2020). With the global population expected to reach 9 billion by 2050, agricultural production must increase by approximately 44 million tons annually. However, challenges such as climate change, global warming, excessive fertilizer use, inadequate drainage, and declining water quality make addressing salinity stress essential for sustainable crop production (Fakhrfeshani *et al*., 2015).

Halophytes are plants that grow in soils with a high concentration of salts, especially NaCl, and their adaptation mechanism includes salt tolerance and/or salt avoidance (Animasaun *et al*., 2020). *A. littoralis* plant is a monocotyledonous and perennial halophyte from the Poaceae family with a C4 photosynthetic system, which is widely distributed in coastal areas and saline soils with a high water table in Iran (Barhoumi, 2018). It is a critical economic plant, because is important as fodder and biological improvement and regeneration of barren saline soils (Barzegargolchini *et al*., 2017).

Salt-resistant plant growth-promoting bacteria (PGPB) cause tolerance to stress and improve plant growth directly through mechanisms such as N fixation, soil P dissolution and production of phytohormones, and indirectly by siderophores, antibiotics, ACC-deaminase enzyme production, change in selective absorption of sodium, potassium, and calcium to keep K:Na ratio at a higher level, cellular regulation of various antioxidant enzymes levels, induction of heat shock proteins, osmotic protectors and proline accumulation (Qin *et al*., 2018; Al- Garni *et al*., 2019; Yasmin *et al*., 2020; Yanez-Yazlle *et al*., 2021). Yanez-Yazlle *et al* (2021) investigated the PGP properties of three salt-resistant bacteria and their effect on *Salvia hispanica* L. and *Chenopodium quinoa* Wild growth and development under saline conditions and reported that all three isolates have growth-promoting properties such as P solubilization, siderophore and auxin production. The isolate of *bacillus* sp. HX11 has all three growth-stimulating properties and compared to other isolates, improves the salinity tolerance of the plant. It has been reported that AM fungi exist in highly saline soils and could colonize several halophyte species (Hajiboland *et al*., 2015; Yang *et al*., 2019), however, the effect of AMF symbiosis with halophyte plants on their resistance to salinity has been rarely investigated. AM fungi have a beneficial symbiosis with most plants and play a vital role in plant growth under different conditions, which is performed by modifying the root system, increasing the mobility and nutrient uptake, enzymatic and non-enzymatic antioxidant defence system induction, and phytohormone synthesis (Hashem *et al*., 2016; Yang *et al*., 2019). It is reported that plant growth and salinity tolerance are positively affected by PGPBs and AM fungi. These microorganisms act as bio-amelioration by regulating hormonal and nutritional balance and

systematic resistance reduction to stress (Solórzano-Acosta *et al*., 2023; Hashem *et al*., 2016; Moreira *et al*., 2020).

Although extensive research has been conducted on strategies to mitigate soil salinity, limited studies have explored the integrated effects of halophytic plants, AMF, and salt-resistant-PGPB on soil amelioration. Previous research has primarily focused on the individual roles of AMF or PGPB in enhancing plant tolerance to salinity; however, the synergistic interactions between these biological agents, particularly in association with halophytes such as *A. littoralis*, remain insufficiently understood. Furthermore, the extent to which these biological components collectively influence soil physicochemical properties, nutrient dynamics, and plant resilience under saline conditions has not been comprehensively examined. Addressing this research gap, the present study investigates the combined impact of *A. littoralis*, AMF, and salt-resistant PGPB on the bio-amelioration of saline soils, contributing to the development of sustainable and biologically driven soil management strategies.

MATERIAL AND METHODS

Strain details

Nocardia Halotolerans sp. Nov was prepared by the National Center of Genetic and Biological Resources of Iran with the accession code of IBRC-M 10490. This actinomycete bacterium is native to the saline soil around Inche-Broun Golestan province, Iran (Moshtaghi *et al*., 2015). The *Rhizophagus intraradices* fungus (syn. *Glomus intraradices*) was obtained from Turan biotechnology knowledge-based Co. Iran.

Salt resistance test

The bacterium was cultured on nutrient agar medium supplemented with NaCl at concentrations of 0, 2.5, 5, 7.5, 10, and 12% (W/V). After 72 hours of incubation at 30 °C, bacterial growth was assessed using a visual growth observation method to determine the tolerance of the strain to different salinity levels.

The production of Indole 3-acetic acid (IAA)

The bacterium was cultured in nutrient broth (NB) medium supplemented with L-tryptophan (50 mg/L) and NaCl (5% W/V) at 30 °C for 72 hours. After incubation, bacterial cells were separated by centrifugation at 10,000 rpm for 10 minutes. Then, 1 mL of the supernatant was mixed with 4

ml of Salkowski's reagent, which was prepared by mixing 150 mL of concentrated sulfuric acid, 250 mL of distilled water, and 7.5 mL of 0.5 M $FeCl_3 \cdot 6H_2O$ solution. The mixture was allowed to react for 20 minutes at room temperature, and the auxin production potential was quantified by measuring absorbance at 535 nm using a spectrophotometer (Patten and Glick, 2002).

Solubility of inorganic phosphorus

The strain was cultured in Pikovsky's solid medium (PKV) containing 5% NaCl (%W/V) at 30 ˚C for 72 h. After centrifuging the medium culture, 1 ml of the supernatant solution was mixed with 3 ml of distilled water and 1 ml of molybdate-vanadate reagent, after 20 minutes, the absorbance of the mixture was read using a spectrophotometer at 430 nm (Jeon *et al*., 2003).

Siderophore production

This trait was measured using a Chrome-Azurol-S agar plate (kept for 72 hours at 30 ˚C) based on the calculation of the orange halo diameter to the colony to diameter ratio (Alexander and Zuberer, 1991).

Potassium release potential

The amount of potassium released was assayed using Aleksandrov medium containing white mica (muscovite) and 5% NaCl (W/V) at 30 ˚C for 72 h by a flame photometer (Meena *et al*., 2015).

The ability of ACC-deaminase enzyme production

This part was estimated by the modified method of Penrose and Glick (2004) in a DF culture medium by determining the released α-ketobutyrate concentration.

Greenhouse cultivation

A soil sample was randomly collected from 0 to 30 cm soil depth with the longitude 28˚54́ 23˝and latitude 36˚ 42́ 23˝located in Ziarat village, Gorgan, Iran. Soil samples after air drying were mixed with the sand at the ratio of 3 to 1 (soil: sand). Physiochemical properties of saturated soil paste extract are provided in Table 1.

Table 1. Physiochemical properties of soil.

Properties	Unit	Value
Soil texture	-	Sandy loam
pH	-	7.70
EC	dS/m	1.81
Saturation point	%	34
Total neutralizing value (TNV)	%	8.90
Organic carbon	%	1.30
Total nitrogen	%	0.1
Available P	mg/kg	22.72
Available K	mg/kg	805

Culture media was sterilized by autoclaving at 121 ˚C after passing through a 4 mm sieve. Plastic pots (20 cm×17 cm) were prepared and after disinfection with 70% ethanol were filled with 2 kg soil. To avoid salt build-up of salt, three holes were made in the bottom of each pot (1 cm diameter), gravels were placed to 3 cm height in the bottom of the pot.

A. littoralis seeds were collected from its natural habitats in the Agh Qala region, Golestan Province, Iran. For surface sterilization of the seeds, they were immersed in 96% ethanol for 5-10 seconds. Then, the seeds were drenched in distilled water and disinfected for 10 minutes in a 0.5% NaClO solution. The seeds were then drenched again in distilled water. 30 seeds were sown in each pot. Upon emergence, the plants were thinned so that each pot contained 20 seedlings. The plants were grown in a greenhouse at a temperature of 20-25 ˚C and under 14/10 h (light/dark), and 70-80% relative humidity conditions.

Microbial inoculation

Microbial treatments were inoculated at the same time as the seeds were planted. For this, *N. halotolerans* was inoculated with a population density of 10^8 CFU/mL per seed. Also, 50 g of *R. intraradices* (~150 spores per 100 g of inoculum) were uniformly spread as one cm thin layer below the seeds. To equalize the treatments, 50 g of three times autoclaved inoculum was added to the non-mycorrhizal treatments.

Applying different levels of salinity

After planting, the plants were irrigated for 4 weeks with Hoagland solution (Millner and Kit, 1992) with half the concentration of phosphorus (to stimulate symbiosis). After 4 weeks, salinity treatments at 0 (S0), 100 mM NaCl (S1), 200 mM NaCl (S2), 100 mM NaCl+50mM K_2SO_4 (S3), 200 mM NaCl+50 mM K_2SO_4 (S4) were applied as 2.5 times the pore volume of the soil by

Hoagland's solution (Kutilek and Nielsen, 1994). To avoid osmotic stress, the treatments were administered slowly over two weeks (Dashtebani *et al*., 2014). Then, the plants were treated with different concentrations of salinity for 4 weeks. The average soil EC in salinity treatments was 4, 14.2, 22.13, 17.96 and 28.1 dS/m, respectively. In the last 4 weeks of the growth period, pots were irrigated with tap water by maintaining a humidity of 70-80% FC.

Cell membrane stability

The membrane stability index was evaluated by measuring the leakage of leaf electrolytes (Shiferaw and Baker 1996). Electrolyte leakage was calculated from the following equation:

$$\text{EL}\% = [1 - \left(\frac{\text{EC1}}{\text{EC2}}\right)] \times 100$$

Where, EC1 is the initial electrical conductivity of the medium and EC2 is final EC.

Photosynthetic pigments

Chlorophyll a and b content in the plant fresh leaves were measured using 80% acetone, according to the method of Arnon (1967), and were obtained by the formula presented below in terms of mg/g of fresh weight:

Chl. a (mg/g FW)= $[19.3\ (A663) - 0.86\ (A645)] \times V/100W$

Chl. b (mg /g FW) = $[19.3(A645) - \ 3.6\ (A663)] \times \ V/100W$

Where, A is the absorbance at a specific wavelength (nm), V is the volume of chlorophyll extract 80% acetone and W is leaf fresh weight (g).

Root colonization

Root staining was performed using the modified method of Kormanik and McGraw (1982). The staining solution consisted of a 1:1:1 ratio of distilled water, glycerin, and lactic acid, along with 0.05% (W/V) trypan blue. The stained roots were first cut into 1.0 cm pieces, then 30 pieces were randomly placed on a petri dish with a 1.0 × 1.0 cm grid. Using the grid line intersection method, the number of colonized intersecting lines was counted under a binocular microscope (30X), and the root colonization percentage was determined.

Total glomalin assay

To extract of total glomalin, the soil and root samples were autoclaved by 50 mM sodium citrate buffer (pH=8) for 60 minutes at 121˚C in three continuous cycles. Supernatant from the extraction were combined after centrifugation for 10 min at 10000×g (Rosier *et al*., 2006). The Bradford-reactive total glomalin was measured via Bradford's total protein assay method (Wright *et al*., 1996; Bradford, 1976). A standard curve was drawn by plotting absorbance against 0-10 µg concentrations of bovine serum albumin.

Statistical analysis

The data was analyzed using analysis of variance (ANOVA) for each variable. Three replicates were used as a factorial in a completely randomized design (CRD). The treatment means were compared with Duncan's test at a $p<0.05$ probability level. All analyzes were performed with the SAS software.

RESULTS AND DISCUSSION

Growth-promoting properties of salt-resistant bacteria

The salinity resistance of *N. halotolerans* was up to 10% NaCl (W/V), which was considered as a measure of salinity tolerance on NA medium after 72 h. Moshtagi *et al* (2015) reported that this strain tolerated NaCl concentration up to 12.5% (W/V) by growing on ISP4 solid medium. Zhou *et al* (2017) collected 23 isolates of halophyte bacteria from the rhizosphere of plants belonging to the Chenopodiaceae family, which were able to tolerate salt in the range of 0.34 to 1.71 M NaCl.

This study investigated the bacterial PGP properties at 5% NaCl concentration. However, PGP properties can be different at various salinity levels. Some studies reported that salt-tolerant bacteria require moderate salinity levels in the growth medium to maintain PGP properties (Zhou *et al*., 2017). IAA production ability, inorganic phosphate solubility potential, siderophore production (halo to colony diameter ratio), ACC-deaminase enzyme activity and K-release ability of N, halotolerans bacteria in the presence of 5% NaCl were 5.8 µg/ml, 11.7 µg/ml, 1.4, 6 µmol α-ketobutyrate mg protein^{-1} h^{-1} and 8.05 µg/ml, respectively. IAA production potential is an important PGP trait for bacteria. This phytohormone extends the plant root system and causes better absorption of nutrients (Zhou *et al*., 2017). A low concentration of IAA stimulates germination and

root growth, while a high concentration is inhibitory (Yanez-Yazlle *et al*., 2021). According to Qin *et al* (2018), among 15 isolates belonging to the *Glutamicibacter* genus isolated from the root, leaf and rhizosphere of the *Limonium sinense* a coastal halophyte, six isolates had the ability to IAA production.

Zhou *et al* (2017) reported that the highest P solubilizing ability of bacteria was at a salt level of 2.5%, which decreased with increasing salinity levels. Meanwhile, Rojas-Tapias *et al* (2012) showed that by increasing the NaCl concentration in medium culture from 0 to 5.85 g/L, the phosphate-solubilizing ability of *Azotobacter* sp. C9 increased. The availability of P is reduced in saline soils, which is due to the increase in the sorption capacity of soil particles and the decrease in the solubility of elements under salt stress (Moreira *et al*., 2020; Dey *et al*., 2021). Salt-tolerant phosphate dissolving bacteria is a unique approach to increasing P availability in salt-affected soils.

Plant growth-promoting bacteria overcome Fe deficiency by producing siderophore, which often occurs in salt-affected soils by chelating and increasing plant Fe availability (Moreira *et al*., 2020; Dey *et al*., 2021). Yanez-Yazlle *et al* (2021) reported that the highest siderophore production ability was in *Halomonas* sp. SFS which the ratio of halo to colony diameter with 1mol/L of NaCl compared to without salt was 3 and 2.93, respectively. Siderophore production potential by salt-tolerant bacteria has also been observed by other researchers (Zhou *et al*., 2017; Yasmin *et al*., 2020; Moreira *et al*., 2020).

PGP bacteria convert 1-aminocyclopropane-1-carboxylate (i.e. ethylene precursor) into α-ketobutyrate and ammonia by producing the ACC-deaminase enzyme, which reduces ethylene concentration and its negative effect under salinity stress conditions (Zhou *et al*., 2017; Yasmin *et al*., 2020; Solorzano-Acosta *et al*., 2023). It is reported that all halophyte bacterial isolates had the ACC-deaminase enzyme production ability. Also, the synthesis of ACC-Oxidase which is responsible for the synthesis of ethylene from ACC was significantly reduced in plants inoculated by *G. halophytocola* bacterium compared to non-inoculated plants. This reduction could be due to bacterial function in ACC degradation (Qin *et al*., 2018). Potassium-solubilizing bacteria dissolves K-containing minerals by releasing organic acids (Feng *et al*., 2019). Ashfag *et al* (2020) reported that all the isolated bacteria from the rice rhizosphere were able to release K at 7% NaCl level. However, this ability decreased significantly by increasing salt concentration. They stated that the reason for the improvement of plant growth in paddy soil was the ability to inhibit Na absorption and improve K uptake under salt stress.

Fresh and dry weight of the plants

As salinity was increased up to the S2 level, plant fresh and dry weights decreased, which can be due to the increase in Na uptake as well as the decrease in the uptake of essential elements (such as K and P), the synthesis of chlorophyll and photosynthesis (Ansari *et al*., 2019). However, these traits increased at the salinity levels of S3 and S4 compared to the levels of S1 and S2 (**Fig.** 1a, **Fig.** 1b), which can improve plant productivity due to the more K availability (Ashfag *et al*., 2020). The results showed the positive effect of microbial inoculation on the fresh and dry plant weights, so that the highest values were in the co-inoculation treatment of AMF+PGPB under no salinity condition, which is following the results obtained by Hashem *et al* (2016) regarding the positive effect of AMF+PGPB co-inoculation on plant growth and salinity tolerance. Growth-promoting bacteria increase nutrient uptake and plant tolerant by different mechanisms such as phytohormone production, increasing the release of nutrients, especially P and K, ACC-deaminase production, dissolving the insoluble compounds (Al-Garni *et al*., 2019; Suarez *et al*., 2015). Bacteria IAA hormone along with plant IAA can increase water and nutrient uptake by modifying the root systems and/or generating hairy and lateral roots (Al-Garni *et al*., 2019). Besides, bacterial ACC-deaminase enzyme can reduce ethylene synthesis and improve plant tolerance to salinity (Yasmin *et al*., 2020; Al-Garni *et al*., 2019; Ansari *et al*., 2019). AMF can increase the photosynthetic products and plant biomass by increasing the bioavailability of macro elements, especially P and microelements such as Zn and Cu (Begum *et al*., 2019).

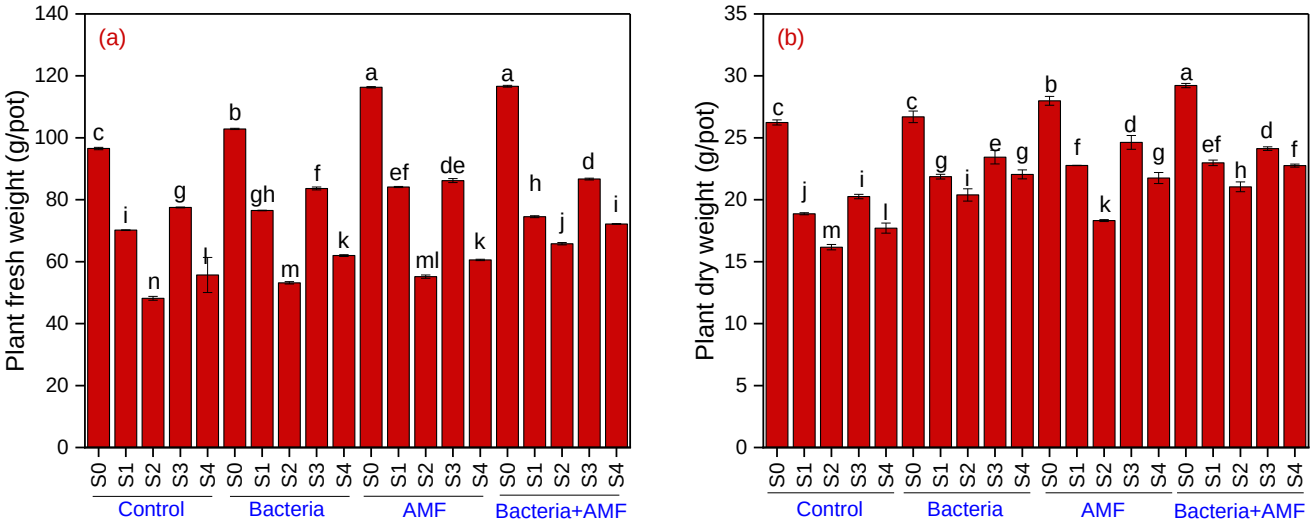

Fig. 1. Fresh (a) and dry (b) weights of *A. littoralis* influenced by microbial inoculation and different levels of salinity.

Chlorophyll a and b contents

Chlorophyll a and b contents decreased with increasing salinity, but in salinity treatments containing K_2SO_4, these pigments were less affected by salinity (**Fig.** 2a,b). Potassium sulfate (K_2SO_4) mitigates the effects of salinity on chlorophyll content by enhancing plant tolerance to salt stress through several mechanisms. Potassium (K^+) plays a crucial role in maintaining osmotic balance, regulating stomatal opening, and activating enzymes involved in photosynthesis (Cakmak, 2005; Britto and Kronzucker, 2008; Praveen and Singh, 2024). Under saline conditions, high levels of sodium (Na^+) and chloride (Cl^-) ions disrupt nutrient uptake and enzyme activities, impairing chlorophyll synthesis and photosynthetic efficiency (Debouba *et al*., 2006; Flowers *et al*., 2015; Gengmao *et al*., 2015). Potassium from K_2SO_4 helps counteract these effects by promoting selective ion uptake, reducing sodium accumulation in plant tissues, and maintaining chlorophyll stability (Akram *et al*., 2009; Wasaya *et al*., 2021). Additionally, sulfate (SO_4^{2-}) contributes to sulfur nutrition, which is essential for synthesizing amino acids and proteins involved in chlorophyll production (Adak and Sengupta, 2023; Oksana *et al*., 2024). Together, these effects help maintain chlorophyll content and photosynthetic capacity under salinity stress. Wei *et al* (2016) stated that potassium use increased the chlorophyll content in *Sophora alopecuroides* under salt stress. The reduction of chlorophyll content can be due to the inhibition of enzymes involved in the synthesis of these pigments. Increased activity of the chlorophyllase enzyme, which decomposes chlorophyll in plants under salt stress is another possible reason for the reduction of

photosynthetic pigments (Al-Garni *et al*., 2019). Also, inoculation with PGPB, AMF, and AMF+PGPB increased chlorophyll a content by 34.2, 25.2, and 28.1%, respectively, and chlorophyll b content by 44.1, 50.9, and 25.1%, respectively, compared to the control (without inoculation). Therefore, an increase in chlorophyll content can lead to an increase in photosynthesis rate that leads to improved growth of the plant under salinity stress. These results follow those observed by Al-Garni *et al* (2019). Increased content of chlorophyll in inoculated plants can be due to more Fe, Mg, N uptake, and less synthesis of stress-related ethylene (Ansari *et al*., 2019). Elhindi *et al* (2017) reported that mycorrhizal inoculation in *Ocimum basilicum* L. improves photosynthesis rate, gas exchange, water use efficiency, and content of chlorophyll under salt stress. More chlorophyll content in mycorrhizal plants in comparison to mycorrhizal-free ones means an increase in the photosynthesis rate and carbon fixation, which can be used to maintain the symbiotic relationship between AM and plants (Elhindi *et al*., 2017).

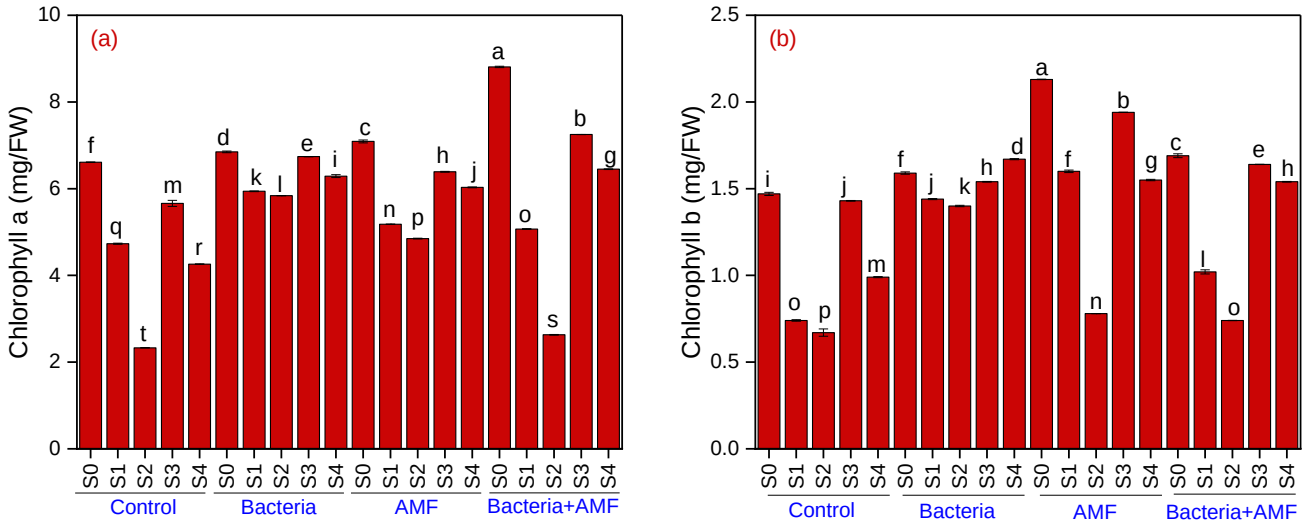

Fig. 2. (a) Chlorophyll a and (b) Chlorophyll b content of *A. littoralis* influenced by microbial inoculation and different levels of salinity.

Electrolyte leakage

Salt stress causes lipid oxidation in the cell membrane and increases the permeability of the membrane, and the plant wilts due to the leakage of cell electrolytes (Filek *et al*., 2012). In general, with increasing salinity level up to S2 level, electrolyte leakage increased, and in the S3 and S4 levels, this trait significantly decreased compared to the S1 and S2 treatments. Also, microbial inoculation reduced electrolyte leakage compared to the control. The greatest membrane stability was related to single bacterial treatment, because it had less electrolyte leakage than single AMF

and co-inoculation of AMF+PGPB treatments (10.57 and 14.2%, respectively) (**Fig**. 3). Mycorrhizal plants reduce the damage inflicted by free radicals through reduced lipid peroxidation in membranes under saline conditions (Begum *et al*., 2019).

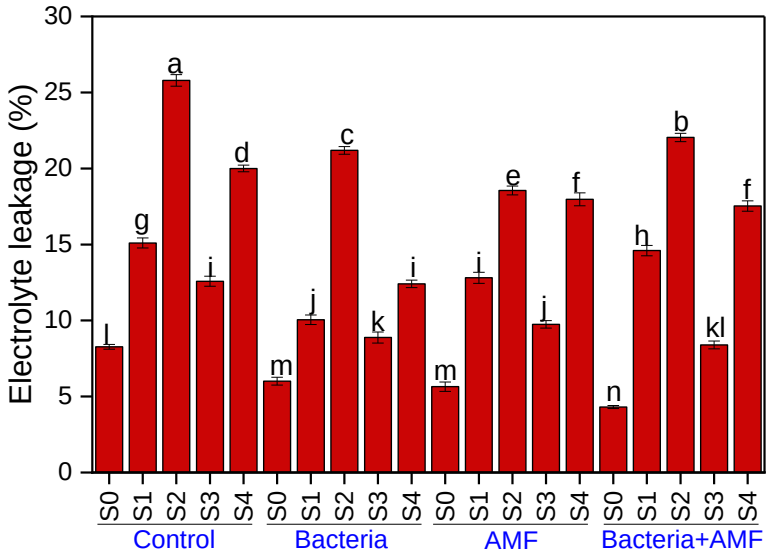

Fig. 3. Electrolyte leakage of *littoralis* influenced by microbial inoculation and different levels of salinity.

Root colonization

The highest root colonization (57.45%) was related to the AMF treatment at the S1 salinity level, which was significantly higher than other treatments. The AMF treatment had a 32.7% enhancement compared with AMF treatments at S2 salinity level (with the lowest root colonization, 38.66%) (**Fig**. 4). Under salt stress, the reason for increased root colonization in co-inoculation of AMF+PGPB compared to single AMF treatment can be due to metabolites production which improves root colonization by stimulating the hyphal growth and increasing the cell permeability (Moreira *et al*., 2020). Root colonization reduction with increasing salinity up to S2 level could be due to the inhibition of hyphal growth and/or its development after initial colonization under salt conditions (Hajiboland *et al*., 2015). According to Elhindi *et al* (2017), mycorrhizal colonization of Sweet basil (*Osmium basilicum*) by *Glomus deserticola* decreased with increasing salinity up to 10 $ds.m^{-1}$. Hashemi *et al* (2016) reported that with increasing NaCl concentration, spore density, hyphal, vesicle and arbuscular abundancy decreased by 24.8, 63.6, 20.7, and 60.4%, compared to the salinity control treatment, respectively. They found that co-inoculation of *Bacillus subtilis* with AMF improved mycorrhizal root colonization and abundance of fungal organs under salt stress.

Following our results, there are other reports on the reduction of salinity stress effect in symbiosis of AMF with halophyte plants such *Punccinellia tenuiflora* and *A. littoralis* (Wang *et al*., 2019; Hajiboland *et al*., 2015; Diao *et al*., 2021).

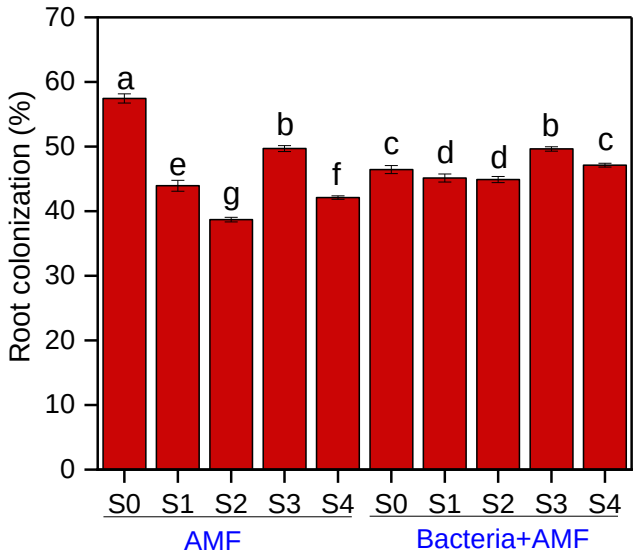

Fig. 4. Root colonization of *A. littoralis* influenced by microbial inoculation and different levels of salinity.

Soil and root glomalin

The root glomalin concentration increased with increasing salinity level, so that the highest amount in the inoculated treatments was at the S4 level (**Fig**. 5a). The results showed that the glomalin production as a heat shock glycoprotein increased with increasing salinity, and glomalin production is probably a defense mechanism of the fungus to protect the host plant (Garcia *et al*., 2019). The co-inoculation of AMF+PGPB increased glomalin production under salt stress. Gacia *et al* (2019) reported that the glomalin production in the co-inoculation of rhizobium bacteria with AM fungi was higher than in single inoculation of AM fungi at all salinity levels.

Soil glomalin content increased with increasing salinity, but the effect of microbial inoculation on this trait was not significant. The greatest amount of glomalin (19.81 µg/g) was related to the S2 level, which was not significantly different from the S4 salinity treatment. Also, these treatments had an increase of 6.4 and 4.9%, respectively, compared to the control salinity level (with the lowest value, 18.55 µg/g) (**Fig**. 5b). Glomalin, in different biotic stresses, increases the soil water holding capacity, which is due to its 30-40% carbon content (Begum *et al*., 2019; Aalipour *et al*.,

2021). Aggregate stability, nutrient cycle, enhancement of soil organic and nitrogen content, and improvement of water-soil-plant relationship are other benefits of soil glomalin (Garcia *et al*., 2019).

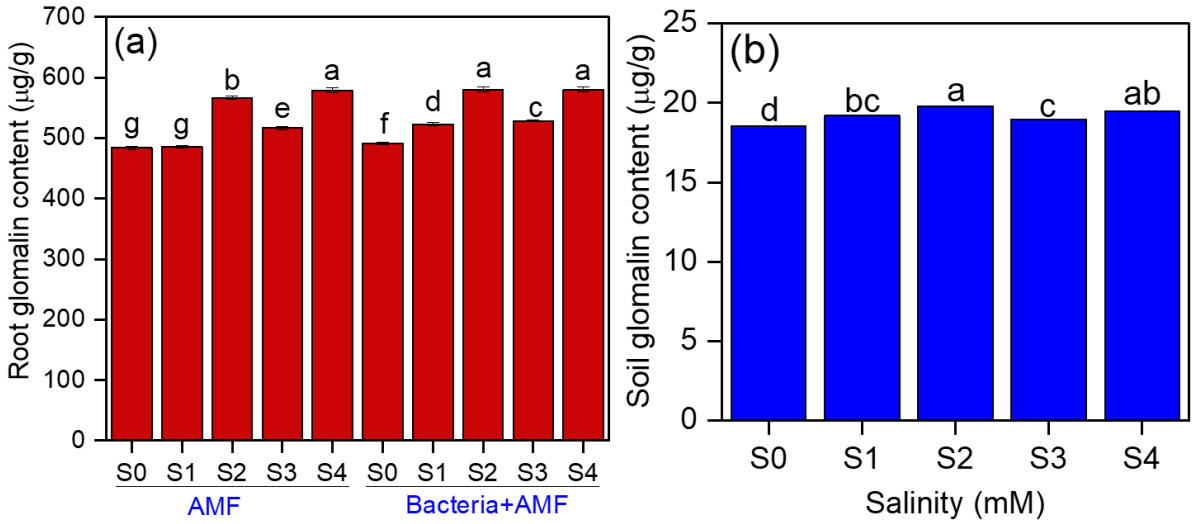

Fig. 5. Root (a) and soil (b) glomalin of *A. littoralis* influenced by microbial inoculation and different levels of salinity.

Conclusions

The ability *of N. halotolerans* to survive in high salinity and its beneficial plant growth-promoting (PGP) traits, such as IAA production, phosphate solubilization, and potassium release, offer promising insights for improving plant productivity in saline environments. Furthermore, the positive effects of microbial inoculation, particularly the co-inoculation of AMF and PGPB, were demonstrated through increased fresh and dry plant weights, enhanced chlorophyll content, and reduced electrolyte leakage. The study confirmed the role of ACC-deaminase and siderophore production in reducing ethylene synthesis and enhancing iron availability, further promoting plant resilience. Root colonization and glomalin production were also positively influenced by microbial inoculation, providing additional evidence of the symbiotic relationship's contribution to plant health under salt stress. In comparison to the S0 treatment, root colonization in the AMF group exhibited a significant decline, with reductions of 23.5%, 32.6%, 13.5%, and 26.7% under S1, S2, S3, and S4 treatments, respectively. However, in the bacteria+AMF group, the extent of reduction was notably smaller, with decreases of only 2.8% and 3.4% under S1 and S2 treatments, while an increase of 6.8% and 1.4% was observed under S3 and S4 treatments, respectively. Overall, the

findings underscore the potential of using salt-tolerant microbes to alleviate salinity stress in plants, thereby improving crop performance in salt-affected soils.

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