

## Bio-Amelioration of Saline Soil Using *Aeluropus littoralis*, Arbuscular Mycorrhizal Fungus (AMF) and Salt-Resistant PGPB

Masoumeh Zarei<sup>1</sup>, Elham Malekzadeh<sup>1\*</sup>, and Alireza Movahedi Naeini<sup>1</sup>

### 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 phyto-extraction of Na under saline conditions. Salinity treatments included 0 (S0), 100 mM NaCl (S1), 200 mM NaCl (S2), 100 mM NaCl+50mM K<sub>2</sub>SO<sub>4</sub> (S3), and 200 mM NaCl+50mM K<sub>2</sub>SO<sub>4</sub> (S4) levels. Plant fresh and dry weight and 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 (Arbuscular Mycorrhizal Fungi (AMF) and Salt-Resistant Plant Growth-Promoting Bacteria) 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 the 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 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).

<sup>1</sup> Department of Soil Science, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Islamic Republic of Iran.

\*Corresponding author, e-mail: emalekzadeh@gau.ac.ir



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 (Barhouri, 2018). It is a critical economic plant, because it 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 had 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 defense 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.

## MATERIALS 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.

### Production of Indole 3-Acetic Acid (IAA)

The bacterium was cultured in Nutrient Broth (NB) medium supplemented with L-tryptophan (50 mg L<sup>-1</sup>) and NaCl [5% w/v (weight/volume)] 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<sub>3</sub>·6H<sub>2</sub>O 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 hours. 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 hours 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 (2001) in a DF culture medium by determining the released  $\alpha$ -ketobutyrate concentration.

### Greenhouse Cultivation

A soil sample was randomly collected from 0 to 30 cm soil depth, at a location with the longitude 28° 54' 23" and latitude 36° 42' 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.

Culture media was sterilized by autoclaving at 121°C after passing through a 4 mm sieve. Plastic pots (20×17 cm) were prepared and, after disinfection with 70% ethanol, were filled with 2 kg soil. To avoid build-up of salt, three holes were made in

**Table 1.** Physiochemical properties of soil.

Properties	Unit	Value
Soil texture	-	Sandy loam
pH	-	7.70
EC	dS m <sup>-1</sup>	1.81
Saturation point	%	34
Total Neutralizing Value (TNV)	%	8.90
Organic carbon	%	1.30
Total nitrogen	%	0.1
Available P	mg kg <sup>-1</sup>	22.72
Available K	mg kg <sup>-1</sup>	805

the bottom of each pot (1 cm diameter) and 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. Thirty 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 hours (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<sup>8</sup> CFU mL<sup>-1</sup> per seed. Also, 50 g of *R. intraradices* (~150 spores per 100 g of inoculum) were uniformly spread as 1 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 Kitt, 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<sub>2</sub>SO<sub>4</sub> (S3), 200 mM NaCl+50 mM K<sub>2</sub>SO<sub>4</sub> (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<sup>-1</sup>, respectively. In the last 4 weeks of the growth period, the pots were irrigated with tap water while maintaining the soil moisture content at 70–80% of Field Capacity (FC).

### Cell Membrane Stability

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

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

Where, EC1 is the initial Electrical Conductivity of the medium and EC2 is the 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<sup>-1</sup> of Fresh Weight (FW):

$$\text{Chl. a (mg g}^{-1}\text{ FW)} = [19.3 (A663) - 0.86 (A645)] \times V/100W$$

$$\text{Chl. b (mg g}^{-1}\text{ FW)} = [19.3(A645) - 3.6 (A663)] \times V/100W$$

Where, A is the Absorbance at a specific wavelength (nm), V is the Volume of 80% acetone chlorophyll extract 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 the 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 minutes at 10,000×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 were 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 analyses 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. Moshtaghi *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 1.71M 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, halo-tolerant bacteria in the presence of 5% NaCl were 5.8 µg mL<sup>-1</sup>, 11.7 µg mL<sup>-1</sup>, 1.4, 6 µmol α-ketobutyrate mg protein<sup>-1</sup> h<sup>-1</sup> and 8.05 µg mL<sup>-1</sup>, 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 produce IAA.

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<sup>-1</sup>, 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.

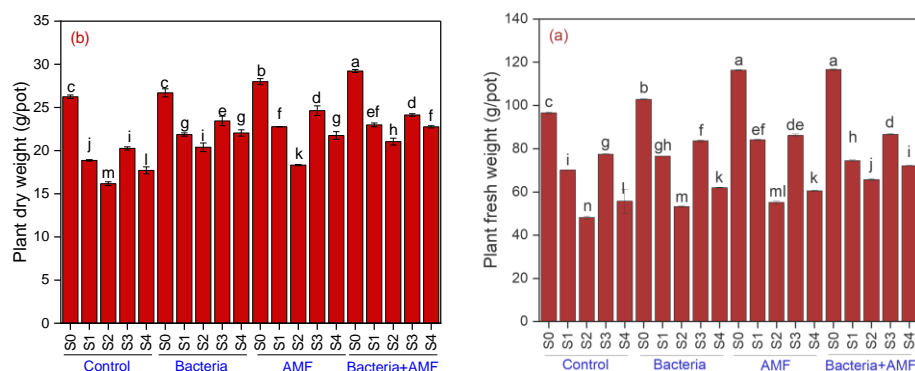
Plant growth-promoting bacteria overcome Fe deficiency by producing siderophore, which often occurs in salt-affected soils by chelating and increasing plant Fe availability (Moreira *et al.*, 2020; Dey *et al.*, 2021). Yanez-Yazlle *et al.* (2021) reported that *Halomonas* sp. SFS showed the highest siderophore production ability, with halo-to-colony diameter ratios of 3.00 and 2.93 under 1 mol L<sup>-1</sup> NaCl and salt-free conditions, 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  $\alpha$ -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). Ashfaq *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. This 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 (Figures 1-a and -b), which can improve plant productivity due to the more K availability (Ashfaq *et al.*, 2020). The results showed a positive effect of microbial inoculation on fresh and dry plant weights. The highest values were observed in the co-inoculation treatment of AMF+PGPB under non-saline conditions. These findings are consistent with the results of Hashem *et al.* (2016), who reported 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;



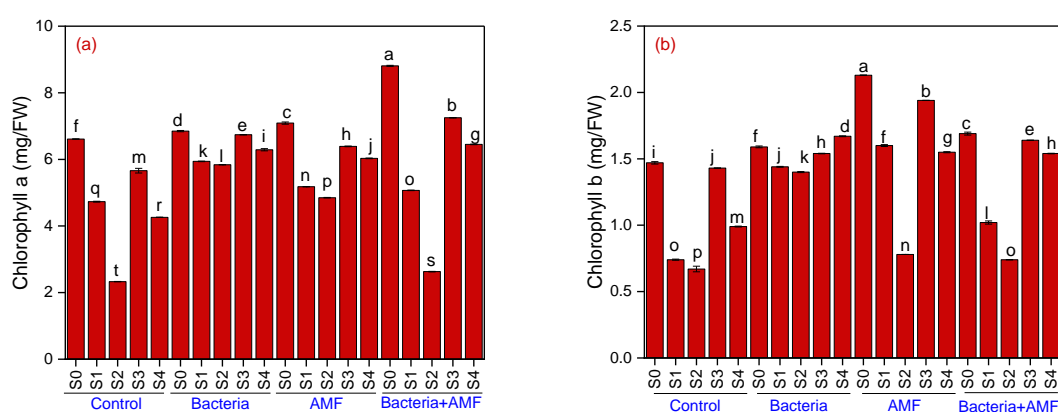
**Figure 1.** Fresh (a) and dry (b) weights of *A. littoralis* influenced by microbial inoculation and different levels of salinity (S0= 0 mM NaCl, S1= 100 mM NaCl, S2= 200 mM NaCl, S3= 100 mM NaCl+50 mM K<sub>2</sub>SO<sub>4</sub>, S4= 200 mM NaCl+50 mM K<sub>2</sub>SO<sub>4</sub>). Different lowercase letters above the bars indicate statistically significant differences among treatments at P< 0.05, based on Duncan's multiple range test.

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).

### Chlorophyll a and b Contents

Chlorophyll a and b contents decreased with increasing salinity, but in salinity treatments containing K<sub>2</sub>SO<sub>4</sub>, these pigments were less affected by salinity (Figures 2-a and -b). Potassium sulfate (K<sub>2</sub>SO<sub>4</sub>) mitigates the effects of salinity on chlorophyll content by enhancing plant tolerance to salt stress through several mechanisms. K<sup>+</sup> plays a



**Figure 2.** (a) Chlorophyll a and (b) Chlorophyll b content of *A. littoralis* influenced by microbial inoculation and different levels of salinity (S0= 0 mM NaCl, S1= 100 mM NaCl, S2= 200 mM NaCl, S3= 100 mM NaCl+50 mM K<sub>2</sub>SO<sub>4</sub>, S4= 200 mM NaCl+50 mM K<sub>2</sub>SO<sub>4</sub>). Different lowercase letters above the bars indicate statistically significant differences among treatments at P< 0.05, based on Duncan's multiple range test.



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 and chloride 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 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,  $\text{SO}_4^{2-}$  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, and N uptake and less synthesis of stress-related ethylene (Ansari *et al.*, 2019). Elhindi *et al.* (2017) reported that mycorrhizal inoculation in *Ocimum*

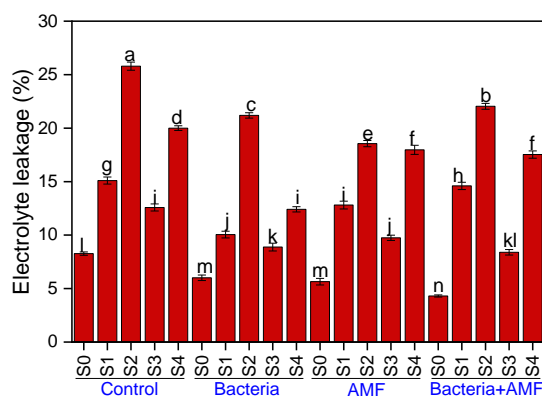
*basilicum* L. improved photosynthesis rate, gas exchange, water use efficiency, and content of chlorophyll under salt stress. More chlorophyll content in mycorrhizal plants in comparison to mycorrhizal-free ones means an increase in the photosynthesis rate and carbon fixation, which can be used to maintain the symbiotic relationship between AM and plants (Elhindi *et al.*, 2017).

### Electrolyte Leakage

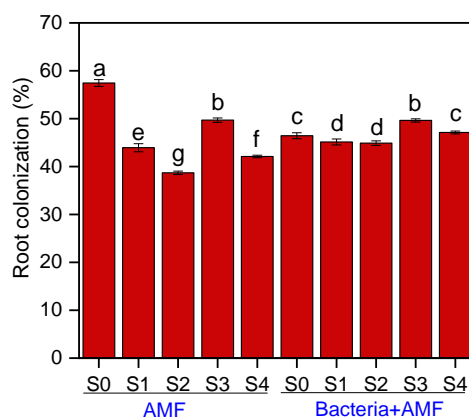
Salt stress causes lipid oxidation in the cell membrane and increases the permeability of the membrane, and the plant wilts due to the leakage of cell electrolytes (Filek *et al.*, 2012). In general, with increasing salinity level up to S2 level, electrolyte leakage increased, and in the S3 and S4 levels, 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) (Figure 3). Mycorrhizal plants reduce the damage inflicted by free radicals through reduced lipid peroxidation in membranes under saline conditions (Begum *et al.*, 2019).

### Root Colonization

The highest root colonization (57.45%) was related to the AMF treatment at the S1, which was significantly higher than the other treatments. The AMF treatment had a 32.7% enhancement compared with AMF treatments at S2 (with the lowest root colonization, 38.66%) (Figure 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



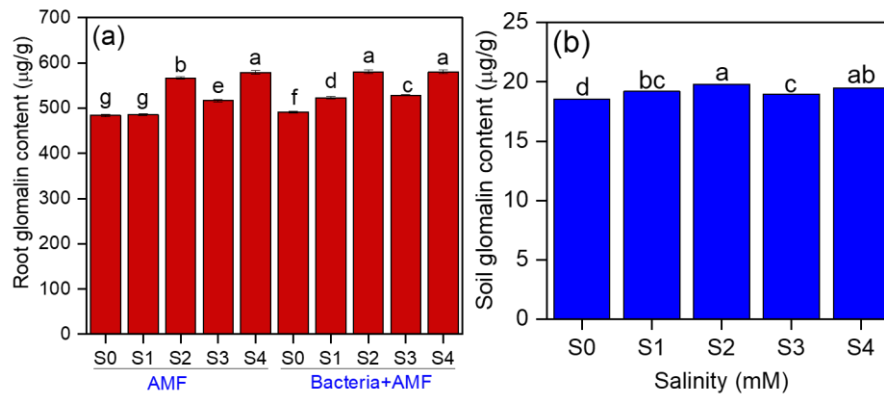
**Figure 3.** Electrolyte leakage of *littoralis* influenced by microbial inoculation and different levels of salinity (S0= 0 mM NaCl, S1= 100 mM NaCl, S2= 200 mM NaCl, S3= 100 mM NaCl+50 mM K<sub>2</sub>SO<sub>4</sub>, S4= 200 mM NaCl+50 mM K<sub>2</sub>SO<sub>4</sub>). Different lowercase letters above the bars indicate statistically significant differences among treatments at P< 0.05, based on Duncan's multiple range test.



**Figure 4.** Root colonization of *A. littoralis* influenced by microbial inoculation and different levels of salinity (S0= 0 mM NaCl, S1= 100 mM NaCl, S2= 200 mM NaCl, S3= 100 mM NaCl+50 mM K<sub>2</sub>SO<sub>4</sub>, S4= 200 mM NaCl+50 mM K<sub>2</sub>SO<sub>4</sub>). Different lowercase letters above the bars indicate statistically significant differences among treatments at P< 0.05, based on Duncan's multiple range test.

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<sup>-1</sup>. Hashem *et al.* (2016) reported that with increasing NaCl concentration, spore

density, hyphal, vesicle and arbuscular abundance 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 *Puccinellia tenuiflora* and *A. littoralis* (Wang *et al.*, 2019; Hajiboland *et al.*, 2015; Diao *et al.*, 2021).



**Figure 5.** Root (a) and soil (b) glomalin of *A. littoralis* influenced by microbial inoculation and different levels of salinity (S0= 0 mM NaCl, S1= 100 mM NaCl, S2= 200 mM NaCl, S3= 100 mM NaCl+50 mM K<sub>2</sub>SO<sub>4</sub>, S4= 200 mM NaCl+50 mM K<sub>2</sub>SO<sub>4</sub>). Different lowercase letters above the bars indicate statistically significant differences among treatments at  $P < 0.05$ , based on Duncan's multiple range test.

### 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 (Figure 5-a). 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. Garcia *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 ( $19.81 \mu\text{g g}^{-1}$ ) was related to the S2 level, which was not significantly different from S4. Also, these treatments had an increase of 6.4 and 4.9%, respectively, compared to the control salinity level (with the lowest value,  $18.55 \mu\text{g g}^{-1}$ ) (Figure 5-b). 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).

### 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.

## REFERENCES

1. Aalipour, H., Nikbakht, A., Etemadi, N. and MacDonald, J. E. 2021. Co-Inoculation of *Arizona cypress* with Mycorrhizae and Rhizobacteria Affects Biomass, Nutrient Status, Water-Use Efficiency, and Glomalin-Related Soil Protein Concentration. *Urban For. Urban Green.*, **60**: 1-9.
2. Adak, E. and Sengupta, S. 2023. Role of Polyhalite in Soil-Plant Nutrition Studies. *Int. J. Agric. Nutr.*, **6(2)**: 32-34.
3. Akram, M. S., Ashraf, M. and Akram, N. A. 2009. Effectiveness of Potassium Sulfate in Mitigating Salt-Induced Adverse Effects on Different Physio-Biochemical Attributes in Sunflower (*Helianthus annuus* L.). *Flora: Morphol. Distrib. Funct. Ecol. Plants*, **204(6)**: 471-483.
4. Alexander, D. B. and Zuberger, D. A. 1991. Use of Chrome Azurol S Reagent to Evaluate Siderophore Production by Rhizobacteria. *Biol. Fertil. Soils*, **12(1)**: 39-45.
5. Al-Garni, S. M., Khan, M. M. and Bahieldin, A. 2019. Plant Growth-Promoting Bacteria and Silicon Fertilizer Enhance Plant Growth and Salinity Tolerance in *Coriandrum sativum*. *J. Plant Interact.*, **14**: 386-396.
6. Animasaun, D. A., Oyedeji, S., Joseph, G. G., Adedibu, P. A. and Krishnamurthy, R. 2020. Sodium Chloride Stress Induced Differential Growth, Biomass Yield, and Phytochemical Composition Responses in the Halophytic Grass *Aeluropus lagopoides* L. *West Afr. J. Appl. Ecol.*, **28(2)**: 31-40.
7. Ansari, M., Shekari, F., Mohammadi, M. H., Juhos, K., Végvári, G. and Biró, B. 2019. Salt-Tolerant Plant Growth-Promoting Bacteria Enhanced Salinity Tolerance of Salt-Tolerant Alfalfa (*Medicago sativa* L.) Cultivars at High Salinity. *Acta Physiol. Plant*, **41**: 195.
8. Arnon, A. N. 1967. Method of Extraction of Chlorophyll in the Plants. *Agron. J.*, **23**: 112-121.
9. Ashfaq, M., Hassan, H. M., Ghazali, A. H. and Ahmad, M. 2020. Halotolerant Potassium Solubilizing Plant Growth Promoting Rhizobacteria May Improve Potassium Availability under Saline Conditions. *Environ. Monit. Assess.*, **192**: 697.
10. Barhoumi, Z. 2018. Physiological Response of the Facultative Halophyte, *Aeluropus littoralis*, to Different Salt Types and Levels. *Plant Biosyst.*, **153(2)**: 298-305.
11. Barzegargolchini, B., Movafeghi, A., Dehestani, A. and Mehrabanjoubani, P. 2017. Morphological and Anatomical Changes in Stems of *Aeluropus littoralis* under Salt Stress. *J. Plant Mol. Breed.*, **5(1)**: 40-48.
12. Begum, N., Qin, C., Ahanger, MA., Raza, S, Khan, MI., Ashraf, M., Ahmed, N. and Zhang, L. 2019. Role of Arbuscular Mycorrhizal Fungi in Plant Growth Regulation: Implications in Abiotic Stress Tolerance. *Front. Plant Sci.*, **10**: 1068.
13. Bradford, M. M. 1976. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem.*, **72**: 248-254.
14. Britto, D. T. and Kronzucker, H. J. 2008. Cellular Mechanisms of Potassium Transport in Plants. *Physiol. Plant.*, **133(4)**: 637-650.
15. Cakmak, I. 2005. The Role of Potassium in Alleviating Detrimental Effects of Abiotic



- Stresses in Plants. *J. Plant Nutr. Soil Sci.*, **168**(4): 521-530.
16. Dashtebani, F., Hajiboland, R. and Aliasghar zad, N. 2014. Characterization of Salt-Tolerance Mechanisms in Mycorrhizal (*Claroideoglossum etunicatum*) Halophytic Grass, *Puccinellia distans*. *Acta Physiol. Plant.*, **36**: 1713–1726.
  17. Debouba, M., Gouia, H., Suzuki, A. and Ghorbel, M. H. 2006. NaCl Stress Effects on Enzymes Involved in Nitrogen Assimilation Pathway in Tomato "*Lycopersicon esculentum*" Seedlings. *J. Plant Physiol.*, **163**(12): 1247-1258.
  18. Dey G, Banerjee, P., Sharma, R. K., Maity, J. P., Etesami, H., Shaw, A. K., Huang, Y. H., Huang, H. B. and Chen, C. Y. 2021. Management of Phosphorus in Salinity-Stressed Agriculture for Sustainable Crop Production by Salt-Tolerant Phosphate-Solubilizing Bacteria—A Review. *Agronomy*, **11**(8): 1552.
  19. Diao, F., Dang, Z., Xu, J., Ding, S., Hao, B., Zhang, Z., Zhang, J., Wang, L. and Guo, W. 2021. Effect of Arbuscular Mycorrhizal Symbiosis on Onion Homeostasis and Salt Tolerance-Related Gene Expression in Halophyte *Suaeda salsa* under Salt Treatments. *Microbiol. Res.*, **24**: 126688.
  20. Elhindi, K. M., El-Din, A. S. and Elgorban, A. M. 2017. The Impact of Arbuscular Mycorrhizal Fungi in Mitigating Salt-Induced Adverse Effects in Sweet Basil (*Ocimum basilicum* L.). *Saud. J. Biol. Sci.*, **24**: 170-179.
  21. Fakhreshani, M., Shahriari-Ahmadi, F., Niazi, A., Moshtaghi, N. and Zare-Mehrjerdi, M. 2015. The Effect of Salinity Stress on Na<sup>+</sup>, K<sup>+</sup> Concentration, Na<sup>+</sup>/K<sup>+</sup> Ratio, Electrolyte Leakage and HKT Expression Profile in Roots of *Aeluropus litoralis*. *J. Plant Mol. Breed.*, **3**(2): 1-10.
  22. Feng, K., Cai, Z., Ding, T., Yan, H., Liu, X. and Zhang, Z. 2019. Effects of Potassium-Solubilizing and Photosynthetic Bacteria on Tolerance to Salt Stress in Maize. *J. Appl. Microbiol.*, **126**(5): 1530-1540.
  23. Filek, M., Walas, S., Mrowiec, H., Rudolphy-Skórska, E., Sieprawska, A. and Biesaga-Kościelniak, J. 2012. Membrane Permeability and Micro- and Macroelement Accumulation in Spring Wheat Cultivars during the Short-Term Effect of Salinity- and PEG-Induced Water Stress. *Acta Physiol. Plant.*, **34**: 985-995.
  24. Flowers, T. J., Munns, R. and Colmer, T. D. 2015. Sodium Chloride Toxicity and the Cellular Basis of Salt Tolerance in Halophytes. *Annal. Bot.*, **115**(3): 419-431.
  25. Garcia, CL., Dattamudi, S., Chanda, S. and Jayachandran, K. 2019. Effect of Salinity Stress and Microbial Inoculations on Glomalin Production and Plant Growth Parameters of Snap Bean (*Phaseolus vulgaris*). *Agronomy*, **9**(9): 545.
  26. Gengmao, Z., Yu, H., Xing, S., Shihui, L., Quanmei, S. and Changhai, W. 2015. Salinity Stress Increases Secondary Metabolites and Enzyme Activity in Safflower. *Ind. Crops Prod.*, **64**: 175-181.
  27. Hajiboland, R., Dashtebani, F. and Aliasghar zad, N. 2015. Physiological Responses of Halophytic C<sub>4</sub> Grass *Aeluropus litoralis* to Salinity and Arbuscular Mycorrhizal Fungi Colonization. *Photosynthetica*, **53**: 572-584.
  28. Hashem, A., Abd\_Allah, E. F., Alqarawi, A. A., Al-Huqail, A. A., Wirth, S. and Egamberdieva, D. 2016. The Interaction between Arbuscular Mycorrhizal Fungi and Endophytic Bacteria Enhances Plant Growth of *Acacia gerrardii* under Salt Stress. *Front. Microbiol.*, **7**: 1089.
  29. Jeon, J. S., Lee, S. S., Kim, H. Y., Ahn, T. S. and Song, H. G. 2003. Plant Growth Promoting in Soil by Some Inoculated Microorganism. *J. Microbiol.*, **41**(4): 271-276.
  30. Kormanik, P. P. and McGraw A. C. 1982. Quantification of Vesicular-Arbuscular Mycorrhizae in Plant Roots. In: "*Methods and Principles of Mycorrhizal Research*", (Ed.): Schenck, N. C. American Phytopathological Society, St. Paul, PP. 37-45.
  31. Kutilek, M. and Nielsen, D. R. 1994. *Soil Hydrology*. Catena Verlag, Cremlingen.
  32. Meena, V. S., Maurya, B. R., Verma, J. P., Aeron, A., Kumar, A., Kim, K. and Bajpai, V. K. 2015. Potassium Solubilizing

- Rhizobacteria (KSR): Isolation, Identification, and K-Release Dynamics from Waste Mica. *Ecol. Eng.*, **81**: 340-347.
33. Millner, P. D. and Kitt, D. G. 1992. The Beltsville Method for Soilless Production of Vesicular Arbuscular Mycorrhizal Fungi. *Mycorrhiza*, **2**: 9-15.
34. Moreira, H., Pereira, S. I., Vega, A., Castro, P. M. and Marques, A. P. 2020. Synergistic Effects of Arbuscular Mycorrhizal Fungi and Plant Growth-Promoting Bacteria Benefit Maize Growth under Increasing Soil Salinity. *J. Environ. Manag.*, **257**: 109982.
35. Moshtaghi Nikou, M., Ramezani, M., Ali Amoozegar, M., Rasooli, M., Harirchi, S., Shahzadeh Fazeli, S. A., Schumann, P., Spröer, C. and Ventosa, A. 2015. *Nocardia halotolerans* sp. nov., a Halotolerant Actinomycete Isolated from Saline Soil. *Int. J. Syst. Evol. Microbiol.*, **65**(9):3148-3154.
36. Oksana, O., Hermansah, H., Agustian, A., Syafrimen, S. and Yasin, S. 2024. Soil Sulfur Dynamics and Their Role in Plant Growth and Development. *J. Agron. Tanaman Tropika (JUATIKA)*, **6**(3): 850-868.
37. Patten, C. L. and Glick, B. R. 2002. Role of *Pseudomonas putida* Indoleacetic Acid in Development of the Host Plant Root System. *Appl. Environ. Microbiol.*, **68**(8): 3795-3801.
38. Penrose, D. M. and Glick, B. R. 2001. Levels of ACC and Related Compounds in Exudate and Extracts of Canola Seeds Treated with ACC Deaminase-Containing Plant Growth-Promoting Bacteria. *Can. J. Microbiol.*, **47**(4): 368-372.
39. Praveen, A. and Singh, S. 2024. The Role of Potassium under Salinity Stress in Crop Plants. *Cereal Res. Commun.*, **52**(2): 315-322.
40. Qin, S., Feng, W. W., Zhang, Y. J., Wang, T. T., Xiong, Y. W. and Xing, K. 2018. Diversity of Bacterial Microbiota of Coastal Halophyte *Limonium sinense* and Amelioration of Salinity Stress Damage by Symbiotic Plant Growth-Promoting Actinobacterium *Glutamicibacter halophytocola* KLBMP 5180. *Appl. Environ. Microbiol.*, **84**: e01533-18.
41. Rojas-Tapias, D., Moreno-Galván, A., Pardo-Díaz, S., Obando, M., Rivera, D. and Bonilla, R. 2012. Effect of Inoculation with Plant Growth-Promoting Bacteria (PGPB) on Amelioration of Saline Stress in Maize (*Zea mays*). *Appl. Soil Ecol.*, **61**: 264-272.
42. Rosier, C. L., Hoyer, A. T. and Rillig, M. C. 2006. Glomalin-Related Soil Protein: Assessment of Current Detection and Quantification Tools. *Soil. Biol. Biochem.*, **38**: 2205-2211.
43. Shiferaw, B. and Baker, D. A. 1996. An Evaluation of Drought Screening Techniques for *Eragrostis tef*. *Trop. Sci.*, **36**: 74-85.
44. Solórzano-Acosta, R., Toro, M. and Zúñiga-Dávila, D. 2023. Plant Growth Promoting Bacteria and Arbuscular Mycorrhizae Improve the Growth of *Persea americana* var. Zutano under Salt Stress Conditions. *J. Fungi.*, **9**: 233.
45. Suarez, C., Cardinale, M., Ratering, S., Steffens, D., Jung, S., Montoya, A. M., Geissler-Plaum, R. and Schnell, S. 2015. Plant Growth-Promoting Effects of *Hartmannibacter diazotrophicus* on Summer Barley (*Hordeum vulgare* L.) under Salt Stress. *Appl. Soil Ecol.*, **95**: 23-30.
46. Talbi Zribi, O., Mbarki, S., Metoui, O., Trabelsi, N., Zribi, F., Ksouri, R. and Abdelly, C. 2020. Salinity and Phosphorus Availability Differentially Affect Plant Growth, Leaf Morphology, Water Relations, Solutes Accumulation and Antioxidant Capacity in *Aeluropus littoralis*. *Plant Biosyst.*, **155**(4): 935-943.
47. Wang, H., An, T., Huang, D., Liu, R., Xu, B., Zhang, S., Deng, X., Siddique, K. H. and Chen, Y. 2021. Arbuscular Mycorrhizal Symbiosis Alleviating Salt Stress in Maize is Associated with a Decline in Root-to-Leaf Gradient of Na<sup>+</sup>/K<sup>+</sup> Ratio. *BMC Plant Biol.*, **21**: 457.
48. Wang, Y., Lin J., Huang, S., Zhang, L., Zhao, W. and Yang, C. 2019. Isobaric Tags for Relative and Absolute Quantification-Based Proteomic Analysis of *Puccinellia tenuiflora* Inoculated with Arbuscular Mycorrhizal Fungi Reveal Stress Response



- Mechanisms in Alkali-Degraded Soil. *Land Degrad. Dev.*, **30**: 1584-1598.
49. Wasaya, A., Affan, M., Ahmad Yasir, T., Mubeen, K., Rehman, H. U., Ali, M., Nawaz, F., Galal, A., Iqbal, M. A., Islam, M. S., El-Sharnouby, M., Rahman, M. H. u. and EL Sabagh, A. 2021. Foliar Potassium Sulfate Application Improved Photosynthetic Characteristics, Water Relations and Seedling Growth of Drought-Stressed Maize. *Atmosphere*, **12(6)**: 663.
50. Wei, D. D., Cheng, D., Liu, W. B., Liu, T., Yang, X. H. and Zheng, Y. H. 2016. Adequate Potassium Application Enhances Salt Tolerance of Moderate-Halophyte *Sophora alopecuroides*. *Plant Soil Environ.* **61**: 364-370.
51. Wright, S. F., Franke-Snyder, M., Morton, J. B. and Upadhyaya, A. 1996. Time-Course Study and Partial Characterization of a Protein on Hyphae of Arbuscular Mycorrhizal Fungi during Active Colonization of Roots. *Plant Soil.*, **181**: 193-203.
52. Yañez-Yazlle, M. F., Romano-Armada, N., Acreche, M. M., Rajal, V. B. and Irazusta, V. P. 2021. Halotolerant Bacteria Isolated from Extreme Environments Induce Seed Germination and Growth of Chia (*Salvia hispanica* L.) and Quinoa (*Chenopodium quinoa* Willd.) under Saline Stress. *Ecotoxicol. Environ. Saf.*, **218**: 112273.
53. Yang, C. X., Zhao, W. N. and Wang, Y. D. 2019. Isolation and Identification of Three Dominant Arbuscular Mycorrhizal Fungi in Rhizosphere of *Puccinellia tenuiflora* from Saline-Alkaline Grassland of Songnen Plain. *Sydowia.*, **71**: 247-253.
54. Yasmin, H., Naeem, S., Bakhtawar, M., Jabeen, Z., Nosheen, A., Naz, R., Keyani, R., Mumtaz, S. and Hassan, M. N. 2020. Halotolerant Rhizobacteria *Pseudomonas pseudoalcaligenes* and *Bacillus subtilis* Mediate Systemic Tolerance in Hydroponically Grown Soybean (*Glycine max* L.) against Salinity Stress. *PLoS ONE*, **15(4)**: e0231348.
55. Zhou, N., Zhao, S. and Tian, C. Y. 2017. Effect of Halotolerant Rhizobacteria Isolated from Halophytes on the Growth of Sugar Beet (*Beta vulgaris* L.) under Salt Stress. *FEMS Microbiol. Lett.*, **364(11)**: fnx091.

## اصلاح زیستی خاک شور با استفاده از *Aeluropus littoralis*، قارچ میکوریز آربوسکولار (AMF) و باکتری‌های محرک رشد (PGPB) مقاوم به شوری

معصومه زارعی، الهام ملک زاده، و علیرضا موحدی نایینی

### چکیده

این مطالعه با هدف ارزیابی توانایی گیاه شورپسند *A. littoralis* در همزیستی با *Rhizophagus intraradices* و *Nocardia halotolerans*، به عنوان یک باکتری بومی خاک‌های شور، در استخراج گیاهی سدیم (Na) در شرایط شوری انجام شد. تیمارهای شوری شامل سطوح 0 (S0)، 100 میلی‌مولار (S1) NaCl، 200 میلی‌مولار (S2) NaCl، 100 میلی‌مولار + 50 میلی‌مولار NaCl (S3) و 200 میلی‌مولار (S3)  $K_2SO_4$  + 50 میلی‌مولار  $K_2SO_4$  (S3) بودند. وزن تر و خشک گیاه و همچنین محتوای کلروفیل با افزایش شوری تا سطح S2 کاهش و پس از آن افزایش یافت. کلونیزاسیون ریشه گیاه در تیمارهای تلقیح منفرد و همزمان (قارچ‌های میکوریز آربوسکولار و باکتری‌های محرک رشد گیاه

مقاوم به شوری) AMF+SR-PGPB مشابه بود. در مقایسه با تیمار S0، کلونیزاسیون ریشه در تیمار AMF به ترتیب در سطوح شوری S1، S2، S3 و S4 به میزان 23.5، 32.6، 13.5 و 26.7 درصد کاهش یافت. در تیمار Bacteria + AMF این کاهش کمتر بود، به طوری که در سطوح شوری S1 و S2 به ترتیب 2.8 و 3.4 درصد کاهش، و در سطوح شوری S3 و S4 به ترتیب 6.8 و 1.4 درصد افزایش مشاهده شد. این نتایج نشان می‌دهد که تلقیح همزمان با باکتری‌های محرک رشد گیاه (PGPB) مقاوم به شوری اثرات منفی شوری بر کلونیزاسیون ریشه را کاهش داده است. مقدار گلومالین ریشه و خاک با افزایش شوری افزایش یافت. گلومالین ریشه در گیاهان تلقیح شده با AMF + SR-PGPB در شرایط تنش شوری بیشتر از تلقیح منفرد AMF بود. این مطالعه کاربرد بالقوه باکتری‌های مقاوم به شوری و قارچ‌های میکوریز آربوسکولار را به عنوان راهبردهایی مؤثر برای بهبود رشد و بهره‌وری گیاه در محیط‌های شور نشان می‌دهد؛ امری که می‌تواند به توسعه کشاورزی پایدار در مناطق آسیب‌دیده از شوری کمک کند.