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Research Article

Effect of Mycorrhiza Applications on Some Physiological and Macro-Micro Element Contents in Soybean (*Glycine max* L.) Grown under Salt Stress Condition

Erol ORAL^{*1}, Rüveyde TUNÇTÜRK², Murat TUNÇTÜRK³, Lütfi NOHUTÇU⁴, Ezelhan ŞELEM⁵

^{1,2,3,4}Van Yuzuncu Yil University, Faculty of Agriculture, Field Crops Department, Van, Türkiye

⁵Van Yuzuncu Yil University, Muradiye Vocational School, Department of Landscape and Ornamental Plants, Türkiye

¹<https://orcid.org/0000-0001-9413-1092>, ²<https://orcid.org/0000-0002-3759-8232>, ³<https://orcid.org/0000-0002-7995-0599>

⁴<https://orcid.org/0000-0003-2250-2645>, ⁵<https://orcid.org/0000-0003-4227-5013>

*Corresponding author e-mail: eroloral@yyu.edu.tr

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Abstract: This study was carried out to determine the effects of mycorrhizal applications on some biochemical and macro/micro nutrient characteristics of soybean (*Glycine max* L.) under salt stress (0, 50, 100, 150, 200 mM NaCl). In the research investigated chlorophyll a (17.30-22.61 $\mu\text{g g}^{-1}$ TA), chlorophyll b (3.05-5.78 $\mu\text{g g}^{-1}$ TA), total chlorophyll (20.46-27.72 $\mu\text{g g}^{-1}$ TA), carotenoids (3.57-4.72 $\mu\text{g g}^{-1}$ TA), proline (0.43-1.81 $\mu\text{g g}^{-1}$ TA), malondialdehyde (MDA) (13.1-18.3 nmol g^{-1}), and several macro- and micro-elements, including Ca (9.43-12.8 g kg^{-1}), K (9.97-11.8 g kg^{-1}), Na (0.94-3.52 g kg^{-1}), P (1.49-2.44 g kg^{-1}), Mg (3.03-3.46 g kg^{-1}), Zn (3.71-7.63 g kg^{-1}), K/Na ratio (3.32-7.17%), Mn (23.6-56.5 g kg^{-1}), Mo (0.81-1.26 g kg^{-1}), Cu (0.76-1.78 g kg^{-1}), As (2.17-5.26 g kg^{-1}), Ni (0.99-1.97 g kg^{-1}), Pb (0.07-0.12 g kg^{-1}), Cd (0.06-0.13 g kg^{-1}), Co (0.06-0.08 g kg^{-1}) and Cr (0.78-1.48 g kg^{-1}). As a result of the study; a decrease or an initial increase followed by a decrease was observed in chlorophyll a and b, total chlorophyll, carotenoids, P, Zn, K/Na, Ca/Na, Mn, Mo, Cu, As, Ni, Pb, Cd, Co and Cr contents in mycorrhiza -treated plants under salt stress. The levels of Ca, K and Na increased, while Mg levels remained statistically insignificant, following a fluctuating pattern. Additionally, boron applications were found to increase the nitrogen balance index, MDA, flavonol, anthocyanin, antioxidant and phenolic contents. Overall, the study demonstrated that mycorrhiza applications have a beneficial and regulatory effect on the biochemical composition and macro/micro-element levels in soybean under salt stress.

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1. Introduction

Since the dawn of human civilization, plants have been utilized for various purposes. Among these, the most notable include their use in disease treatment, nutrition, shelter, defense, and heating. Today, the utilization of plants is more systematic and conscious, extending to numerous fields such as food, cosmetics, medicine, industry, and agriculture (Göktaş and Gıdık, 2019). In particular, with the increasing emphasis on human health, many plant-based products have been incorporated into modern dietary habits. The demand for plant-based protein in the food industry has surged, prompting research

into alternative plant sources capable of replacing conventional animal-based proteins for both health and environmental reasons. Among these sources, soybean (*Glycine max* L. Merr.) stands out as an oilseed crop with an exceptionally high protein content (Qin et al., 2022; Çelebi and Şahar, 2023). Globally, soybean ranks second only to oil palm in vegetable oil production (FAOSTAT, 2024). As a highly versatile crop, soybean serves as a crucial industrial resource, providing raw materials for the manufacturing of approximately 400 different products in various industries. Its high-quality oil is widely used in liquid form for salad dressings and frying (Arıoğlu and Güllüoğlu, 2008). Additionally, soybean oil has diverse industrial applications, including the production of soap, paint, varnish, adhesives, and printing inks. Soybean meal, derived from oil extraction, is an indispensable source of animal feed due to its high protein content, which is rich in essential amino acids and offers significant nutritional value. Consequently, soybean protein is extensively utilized in human nutrition. Beyond its superior protein composition, soybean oil is also rich in essential fatty acids, such as Omega-3, which play a critical role in lipid metabolism in the human body. As a result, soybean-derived products are particularly beneficial in the diets of individuals with diabetes, atherosclerosis, and coronary heart disease (Özcan et al., 2015; Aktaş, 2021; Nas and Günal, 2024).

In recent years, the global soybean cultivation area has ranged between 129.0 million hectares and 139.7 million hectares. For the 2024/2025 period, projections estimate that this figure will rise to 145.8 million hectares. Brazil leads in soybean cultivation, with approximately 46 million hectares, followed by the United States with 33 million hectares and Argentina with 16 million hectares (USDA, 2024). In Türkiye, soybean farming was initially introduced in irrigated areas of the Mediterranean and Aegean regions as part of the second crop project implemented over the past two decades. However, today, production is concentrated mainly in the Çukurova region. According to 2023 data, soybean production in Türkiye covered 326 thousand hectares, yielding 137 thousand tons of product, with an average seed yield of 421 kg/da. Approximately 80–85% of soybean production takes place in Adana and Osmaniye provinces. However, in recent years, national production has declined to 50–60 thousand tons. To sustain and expand soybean production, it is essential to promote its widespread use as a second crop (Nazlıcan, 2017; TUIK, 2024).

Ensuring high yield and quality in soybean cultivation—critical for both domestic and international markets—requires understanding the impact of environmental factors on plant growth. Throughout their life cycle, plants may experience adverse conditions that hinder growth and reduce yield. These conditions, collectively known as stress factors, are classified into two categories:

1. Biotic stress factors: These include plants, fungi, nematodes, microorganisms, animals, and anthropogenic effects.

2. Abiotic stress factors: These encompass drought, salinity, nutrient deficiencies, radiation, atmospheric pollution, extreme temperatures, and variations in light intensity (Büyük et al., 2012). Among abiotic stress factors, salinity is particularly detrimental to plant growth. From seed germination onward, salinity disrupts plant development and significantly reduces yield. It is one of the most pressing environmental challenges in global agriculture, particularly in arid and semi-arid regions, where it affects approximately 20% of agricultural lands. If left unaddressed, this figure could rise to 50% within the next 20 years (Rengasamy, 2010; Hasanuzzaman et al., 2013). To combat salinity-related stress, various biological approaches have been explored. Among these, microbial applications have gained prominence due to their ease of application, long-term effectiveness, and ability to enhance salt tolerance. One of the most promising microbial solutions is mycorrhizal fungi. In their natural environment, plants interact with diverse microorganisms, some of which form symbiotic relationships that enhance plant growth and resilience. Mycorrhizal fungi, in particular, establish mutualistic associations with plant roots, facilitating the uptake of water and nutrients through their extensive hyphal networks. In return, they obtain carbohydrates that plants synthesize. Beyond nutrient acquisition, mycorrhizae enhance plant tolerance to both abiotic and biotic stress conditions (Smith and Read, 1997; Ruiz-Lozano, 2003; Carvalho et al., 2004). Research has demonstrated that mycorrhizal symbiosis significantly improves plant height, leaf area, and root and stem development under saline conditions (Ruiz-Lozano, 2003; Çekiç et al., 2012; Altunlu, 2019).

This study aims to evaluate the effects of Arbuscular Mycorrhizal Fungi (AMF) applications on selected physiological, biochemical, and macro- and micronutrient parameters in soybean (*Glycine max* L.) under saline conditions.

2. Material and Methods

The research was conducted in 2023 in a fully controlled plant growth chamber at the Department of Field Crops, Faculty of Agriculture, Van Yuzuncu Yil University. The soybean variety ‘Yeşilsoy’ used in the study was obtained from the Aegean Agricultural Research Institute, while AMF isolates were sourced from the culture collection of the Department of Plant Protection, Faculty of Agriculture, Urmia University, Iran. The experiment followed a completely randomized plot design with four replications. Five different salt concentrations (0, 50, 100, 150, and 200 mM NaCl), including the control, were applied to soybean plants at the young seedling stage. The AMF inoculum, containing *Glomus intraradices* at a density of 455 spores/g, was added to the pots at a 20% rate during seed sowing. To ensure seed sterilization, the seeds were first treated with 95% ethanol for 5 minutes, followed by 3% hydrogen peroxide for 5 minutes, and then rinsed six times with distilled water (Öğütçü et al., 2010). Soybean seeds (five per pot) were sown in 500 cc plastic cup pots filled with a growth medium comprising 40% sand, 40% soil, and 20% mycorrhizal inoculum. The pots were placed in a fully controlled climate chamber set to a 16/8-hour light/dark photoperiod, $250\pm 10 \mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity, 25 °C temperature, and 65% relative humidity. Soil moisture levels were monitored using a Field Scout digital moisture sensor, and irrigation was performed as needed using Hoagland nutrient solution. The pH of the Hoagland solution was maintained between 5.5 and 6.5 (Taiz and Zeiger, 2002) and stored under sterile conditions. The composition of the Hoagland nutrient solution used in the study is provided in Table 1.

Table 1. Hoagland nutrient content (Arnon, 1950)

Macronutrient Solution	(Gram)
MgSO ₄ .7H ₂ O (Magnesium sulphate heptahydrate)	246.5
Ca(NO ₃) ₂ .4H ₂ O (Calcium nitrate)	236.1
KH ₂ PO ₄ (Potassium dihydrogen phosphate)	136.1
KNO ₃ (Potassium nitrate)	101.1
Micronutrient Solution	(Gram)
H ₃ BO ₃ (Boric acid)	2.8
MnCl ₂ .4H ₂ O (Manganese chloride tetrahydrate)	1.8
ZnSO ₄ .5H ₂ O (Zinc sulphate)	0.2
CuSO ₄ .5H ₂ O (Copper sulphate)	0.1
NaMoO ₄ (Sodium molybdate)	0.025
Fe-EDTA (Iron Chelate)	15

When the plants had finished emerging, the thinning process was done so that there were three plants in each pot. When the plants reached a certain maturity (30±5 days), salt stress applications were started. In the study, where salt solutions with different osmotic pressures were given as irrigation water, pure water was given to the control applications. The plants were kept under controlled conditions in the climate chamber from seed emergence to harvest. When the severity of physiological problems increased in the experiment (45±5 days), they were harvested. Fresh plant samples were taken for some pigment analyses and other biochemical analyses and stored in a deep freezer at -20 °C. In the plant samples obtained, the following parameters were determined: malondialdehyde (nmol g⁻¹), proline (mmol g⁻¹ TA), chlorophyll a content (µg g⁻¹ TA), chlorophyll b content (µg g⁻¹ TA), total chlorophyll content (µg g⁻¹ TA), and total carotenoid content (µg g⁻¹ TA). In addition, phosphorus (P) was considered an important growth and nutrition criterion in the AMF × plant interaction in this study. Furthermore, the levels of Ca, Na, and K under salinity stress and their ratios to each other were determined. Then, macro- and micro-element analyses were performed on the samples. For the determination of macro- and micro-nutrient elements, after the samples were washed and dried, they were placed in paper bags, dried at 65 °C, and ground (Kacar and Inal, 2008). Then, 200 mg of ground samples were prepared according to the wet combustion method and analyzed at the Van YYU Science Application and Research Center. The amounts were determined using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) and an Atomic Absorption Spectrometer (AAS). For the determination of photosynthetic pigments, in the analyses performed according to Lichtenthaler and

Wellburn (1985), 0.2 g (200 mg) of fresh plant sample was extracted with 10 mL of 80% acetone and centrifuged at 4600 rpm for 15 minutes. The absorbance values of the aliquots taken after centrifugation were recorded at 662, 652, 645, and 470 nm using a spectrophotometer (PG T60 UV-VIS). Total chlorophyll, chlorophyll a, chlorophyll b, and carotenoid concentrations were calculated using the formulas determined by Lichtenthaler and Wellburn (1985), as shown below:

$$\begin{aligned} \text{Total chlorophyll } (\mu\text{g g}^{-1} \text{ TA}) &= (27.8 \times A_{652}) \\ \text{Chlorophyll a (Chl a; } \mu\text{g g}^{-1} \text{ TA)} &= (11.75 \times A_{662}) - (2.350 \times A_{645}) \\ \text{Chlorophyll b (Chl b; } \mu\text{g g}^{-1} \text{ TA)} &= (18.61 \times A_{645}) - (3.960 \times A_{662}) \\ \text{Carotenoid (Car; } \mu\text{g g}^{-1} \text{ TA)} &= [(100 \times A_{470}) - (2.270 \times \text{Chl a}) - (81.4 \times \text{Chl b})] / 227 \end{aligned}$$

where:

TA = Fresh weight

A662 = Absorbance reading at 662 nm

A652 = Absorbance reading at 652 nm

A645 = Absorbance reading at 645 nm

A470 = Absorbance reading at 470 nm For the determination of malondialdehyde (MDA), which indicates the level of lipid peroxidation, 0.5 g of leaf samples was homogenized with 10 mL of 0.1% trichloroacetic acid (TCA), and the homogenate was centrifuged at 15000 g for 5 minutes. One milliliter of the supernatant was taken, and 4 mL of 0.5% thiobarbituric acid (TBA) dissolved in 20% TCA was added. The mixture was incubated in a 95°C water bath for 30 minutes, then rapidly cooled in an ice bath and centrifuged at 10000 rpm for 10 minutes. The absorbance of the supernatant was measured at 532 and 600 nm, and the MDA content was calculated using the following equation (Heath and Packer, 1968; Sairam and Saxena, 2000):

$$\text{MDA } (\mu\text{mol g}^{-1}) = [6.45 \times (A_{532} - A_{600}) - (0.56 \times A_{450}) \times V_t / W], \text{ where } V_t = 0.0021; W = 0.2 \text{ g.} \quad (1)$$

For the determination of proline content, 0.5 g of fresh plant sample was digested with 3% sulfosalicylic acid and then filtered. Two milliliters of the filtered sample was taken, and 2 mL of acetic acid and 2 mL of ninhydrin reagent (containing ninhydrin, acetic acid, and orthophosphoric acid) were added. The samples were then incubated in a water bath at 100 °C for 1 hour, and the reaction was stopped by placing them on ice. Four milliliters of toluene was added to the cooled samples, and they were vortexed. The absorbance was recorded at 520 nm using a spectrophotometer, and calculations were performed using proline standards (Bates et al., 1973).

The data obtained from the research were subjected to variance analysis (ANOVA) according to the Randomized Complete Block Design (RCBD). Statistical calculations were performed using the COSTAT (Version 6.3) software package. Differences between means were determined according to Duncan's Multiple Comparison Test (Düzgüneş et al., 1987).

3. Results

In this study, the effects of AMF applications on chlorophyll a, chlorophyll b, and total chlorophyll were found to be insignificant, while the effects of salt applications and Salt × AMF interaction were found to be statistically significant (Table 2). As a result of salt treatments, the highest chlorophyll a value was 22.61 mg g⁻¹ with T1, the highest chlorophyll b value was 5.78 mg g⁻¹ with T0, and the highest total chlorophyll value was 27.72 mg g⁻¹ with T0 applications. The lowest chlorophyll a value was 17.30 mg g⁻¹ with T4, the lowest chlorophyll b value was 3.05 mg g⁻¹ with T3, and the lowest total chlorophyll value was 20.46 mg g⁻¹ with the T4 dose. As a result of the Salt × AMF application, the highest chlorophyll a value was 23.84 mg g⁻¹ with T1 × M0, and the lowest value was 17.18 mg g⁻¹ with T4 × M0 doses. In chlorophyll b values, the highest values were measured as 5.84 and 5.35 mg g⁻¹ in T2 × M0 and T0 × M1, respectively, while the lowest value was measured as 2.08 mg g⁻¹ in T4 × M0 applications. The highest total chlorophyll was determined to be 28.69 mg g⁻¹ in T0 × M1, and the lowest value was determined to be 19.26 mg g⁻¹ in T4 × M0 applications. In the study, the effect of Salt, AMF, and T × AMF interaction on carotenoid content was found to be significant at the 1% level. As a result of salt applications, the highest carotenoid value was obtained from T3

applications with 4.72 mg g^{-1} , while the lowest value was obtained from the T4 dose with 3.57 mg g^{-1} (Table 2). In terms of AMF applications, the highest value was determined to be 4.65 mg g^{-1} in M1 applications, which was 3.87 mg g^{-1} lower than the control group. The highest value in $T \times \text{AMF}$ interaction was determined to be 5.14 mg g^{-1} in $T0 \times M0$ applications. The lowest value in terms of this property was obtained from the $T4 \times M0$ application with 2.22 mg g^{-1} (Table 2).

Table 2. Effects of mycorrhiza applications on some physiological parameters in soybean under salt stress

Salt doses	Treatment				
	AMF Treatment	Chlorophyll a ($\text{mg g}^{-1} \text{ TA}$)	Chlorophyll b ($\text{mg g}^{-1} \text{ TA}$)	Total Chlorophyll ($\text{mg g}^{-1} \text{ TA}$)	Carotenoid ($\text{mg g}^{-1} \text{ TA}$)
T0	Control (M0)	20.54 b	2.21 d	22.75 b	5.14 a
	AMF (M1)	23.34 ab	5.35 a	28.69 a	4.29 b
T0 Ort.		21.94 B	5.78 A	27.72 A	4.66 A
T1 50 (T1)	Control (M0)	23.84 a	4.87 ab	23.76 ab	4.06 bc
	AMF (M1)	21.38 ab	2.70 cd	21.36 bc	4.75 ab
T1 Ort.		22.61 A	3.78 B	22.56 B	4.40 AB
T 100 (T2)	Control (M0)	17.35 c	5.84 a	23.42 a	3.58 c
	AMF (M1)	18.16 c	4.83 ab	23.18 ab	4.31 b
T2 Ort.		17.76 C	5.33 A	23.30 AB	3.94 B
T150 (T3)	Control (M0)	19.22 bc	3.60 c	22.73 b	4.47 ab
	AMF (M1)	17.27 c	2.50 d	21.36 bc	4.98 ab
T3 Ort.		18.24 BC	3.05 CD	22.04 B	4.72 A
T 200 (T4)	Control (M0)	17.18 d	2.08 e	19.26 c	2.22 d
	AMF (M1)	17.42 c	4.24 b	21.66 b	4.92 ab
T4 Ort.		17.30 D	3.16 C	20.46 C	3.57 B
AMF Treatment	Control (M0)	19.62	5.17	23.54	3.87 B
	AMF (M1)	19.52	5.16	22.01	4.65 A
Salt doses (T)		**	**	**	**
AMF		ns	ns	**	**
T x AMF		**	**	**	**
CV (%)		2.85	18.10	5.70	13.97
LSD (0.05)		1.18	3.52	2.89	1.32

*Significant at $P < 0.05$ level, **Significant at $P < 0.01$ level. ns: not significant.

While Salt and $T \times \text{AMF}$ interaction (1%) had significant effects on proline accumulation in soybean, AMF applications were found to have insignificant effects (Table 3). The highest proline accumulation was obtained from the T4 dose, with 1.81 mg g^{-1} , while the lowest value was obtained from control applications, with 0.43 mg g^{-1} . Proline content was determined to be between $0.88\text{--}1.01 \text{ mg g}^{-1}$ in AMF applications. In $T \times \text{AMF}$ interaction, the highest proline content was obtained from the $T4 \times M1$ interaction, with 2.17 mg g^{-1} . The lowest value was measured in the $T0 \times M0$ application, with 0.32 mg g^{-1} . Salt, AMF applications, and $T \times \text{AMF}$ interaction were found to be significant at the 1% level on MDA content. As a result of salt treatments, the highest MDA accumulation was obtained from T4 applications, with 18.3 nmol g^{-1} , while the lowest value was obtained from the T1 dose, with 13.1 nmol g^{-1} . As a result of AMF applications, MDA content (17.2 nmol g^{-1}) was obtained at a higher level in M0 (17.2 nmol g^{-1}) than in M1 (15.1 nmol g^{-1}). In $T \times \text{AMF}$ interaction, the highest MDA content was obtained as 22.1 nmol g^{-1} from the $T4 \times M0$ interaction, while 13.1 nmol g^{-1} was measured in $T1 \times M0$ and $T3 \times M0$ interactions (Table 3). The effect of salt treatments on Ca accumulation in soybean was found to be insignificant at the 1% level, while the effect of AMF and Salt \times AMF interactions was also found to be insignificant. As a result of salt treatments, the highest Ca accumulation was measured at 12.7 and 12.8 g kg^{-1} from T1 and T4 doses, while the lowest value was 9.43 g kg^{-1} in the control dose application (Table 3).

According to the results obtained at the end of the study, the effect of Salt and Salt \times AMF interactions on K content was found to be significant, while AMF was found to be insignificant ($P < 0.01$). According to salt treatments, the highest K content was measured in the T4 dose, with 11.8 g kg^{-1} , and it was in the same group as T1, T2, and T3. The lowest value was measured in the control dose,

with 9.97 g kg⁻¹. As a result of Salt × AMF applications, the highest K content was obtained at 13.9 g kg⁻¹ from T2 × M0, and the lowest value was obtained at 8.14 g kg⁻¹ from T0 × M0 doses (Table 3). According to Table 3, the effect of Salt and Salt × AMF interactions on Na content was found to be significant, while AMF was found to be insignificant. According to salt treatments, the highest Na content was measured in the T2 dose, with 3.70 g kg⁻¹, and it was in the same group as T4. The lowest value was measured in the control dose, with 0.94 g kg⁻¹. As a result of Salt × AMF applications, the highest Na content was obtained at 6.70 g kg⁻¹ from T2 × M0, and the lowest value was obtained at 0.59 g kg⁻¹ from T0 × M0 doses.

Table 3. Effect of mycorrhiza applications on some biochemical and elemental changes in soybean plants grown under salt stress conditions

Salt doses	Treatment					
	AMF Treatment	Proline (mg g ⁻¹ TA)	MDA (nmol g ⁻¹)	Ca (g kg ⁻¹)	K (g kg ⁻¹)	Na (g kg ⁻¹)
T0	Control (M0)	0.32 d	16.6 ab	7.26	8.14 d	0.59 d
	AMF (M1)	0.54 cd	13.9 c	11.6	11.8 ab	1.29 b
T0 Ort.		0.43 D	15.3 B	9.43 C	9.97 B	0.94 C
T1 50 (T1)	Control (M0)	0.90 bc	13.1 d	14.5	12.2 ab	2.26 ab
	AMF (M1)	1.13 bc	13.7 c	10.9	11.3 abc	0.82 c
T1 Ort.		1.02 AB	13.1 C	12.7 A	11.6 A	1.54 B
T 100 (T2)	Control (M0)	0.97 bc	16.4 ab	14.9	13.9 a	6.70 a
	AMF (M1)	0.43 c	17.6 ab	8.32	8.45 cd	0.70 c
T2 Ort.		0.70 B	15.1 B	11.6 B	11.2 A	3.70 A
T150 (T3)	Control (M0)	0.35 d	13.1 d	10.9	11.5 ab	0.87 c
	AMF (M1)	0.78 c	20.1 ab	10.1	10.6 bcd	2.29 ab
T3 Ort.		0.56 C	16.9 B	10.5 B	11.1 A	1.58 B
T 200 (T4)	Control (M0)	1.46 ab	22.1 a	10.1	11.2 abc	0.84 c
	AMF (M1)	2.17 a	14.6 b	15.5	12.1 ab	6.21 a
T4 Ort.		1.81 A	18.3 A	12.8 A	11.8 A	3.52 A
AMF Treatment	Control (M0)	0.88	17.2 A	11.5	11.4	2.25
	AMF (M1)	1.01	15.1 B	11.3	10.8	2.26
Salt doses (T)		**	**	**	**	**
AMF		ns	**	ns	ns	ns
T x AMF		**	**	ns	**	**
CV (%)		19.25	20.35	21.90	10.7	20.8
LSD (0.05)		0.87	7.31	5.90	2.67	4.53

*Significant at P<0.05 level, **Significant at P<0.01 level. ns: not significant.

In this study, it was determined that Salt, AMF applications, and T × AMF interaction were significant at the 1% level on P content. As a result of salt treatments, the highest P accumulation was measured as 2.44 g kg⁻¹ in control applications, while the lowest value was measured as 1.49 and 1.51 g kg⁻¹ in T3 and T4 doses. As a result of AMF applications, P content (1.57 g kg⁻¹) was obtained in M1 dose, which was higher than M0 (1.42 g kg⁻¹). In T × AMF interaction, the highest P content was obtained as 3.28 g kg⁻¹ from T0 × M0 interaction, while the lowest value was measured as 1.29 g kg⁻¹ in T4 × M0 application (Table 4). At the end of the research, the effect of Salt, AMF, and Salt × AMF applications on Mg content in soybean was found to be statistically insignificant. Mg content in soybean varied between 2.78–3.62 g kg⁻¹ (Table 4). Table 4 shows that salt, AMF applications, and T × AMF interaction on Zn content were significant at the 1% level. As a result of salt doses, the highest Zn accumulation was 7.63 g kg⁻¹ in T1 applications, while the lowest value was 3.71 g kg⁻¹ in T4 dose. As a result of AMF applications, Zn content (6.69 g kg⁻¹) was higher in M1 dose than in M0 (5.32 g kg⁻¹). In T × AMF interaction, the highest Zn content was 8.24 g kg⁻¹ in T1 × M1 interaction, while the lowest was measured in T4 × M0 application with 1.07 g kg⁻¹ (Table 4).

Table 4. Effect of mycorrhiza applications on some mineral changes in soybean plants grown under salt stress conditions

Salt doses	Treatment					
	AMF treatment	P (g kg ⁻¹)	Mg (g kg ⁻¹)	Zn (mg kg ⁻¹)	K/Na (%)	Ca/Na (%)
T0	Control (M0)	3.28 e	2.78	4.79 d	13.11 a	4.21 b
	AMF (M1)	1.61 bc	3.29	7.27 abc	10.26 ab	6.43 ab
T0 Ort.		2.44 A	3.03	6.03 BC	11.68 A	5.32 B
T1 50 (T1)	Control (M0)	1.87 ab	3.24	7.03 abc	9.60 ab	6.86 ab
	AMF (M1)	1.64 abc	3.26	8.24 a	14.20 a	5.84 b
T1 Ort.		1.75 A	3.25	7.63 A	11.90 A	6.35 B
T 100 (T2)	Control (M0)	2.09 a	3.42	7.89 ab	2.11 c	9.83 a
	AMF (M1)	1.41 c	2.85	5.75 cd	12.30 ab	4.51 b
T2 Ort.		1.76 A	3.13	6.82 B	7.21 C	7.17 A
T150 (T3)	Control (M0)	1.53 bc	3.37	5.85 cd	1.29 ab	4.71 b
	AMF (M1)	1.47 bc	2.78	5.88 cd	7.85 ab	4.23 b
T3 Ort.		1.49 B	3.07	5.86 C	10.58 AB	4.47 B
T 200 (T4)	Control (M0)	1.29 d	3.30	1.07 e	12.46 ab	3.28 c
	AMF (M1)	1.73 abc	3.62	6.35 bcd	2.97 b	3.37 c
T4 Ort.		1.51 B	3.46	3.71 D	8.21 B	3.32 C
AMF Treatment	Control (M0)	1.42 B	3.22	5.32 B	10.31	5.78
	AMF (M1)	1.57 A	3.16	6.69 A	9.52	4.88
Salt doses (T)		**	ns	**	**	**
AMF		**	ns	**	ns	ns
T x AMF		**	ns	**	*	**
CV (%)		13.80	11.22	13.10	21.20	21.40
LSD (0.05)		450.22	0.79	1.72	9.04	7.84

*Significant at P<0.05 level, **Significant at P<0.01 level. ns: not significant.

In this study, it was determined that AMF was insignificant on K/Na content, while Salt and T × AMF interaction were significant at the 1% and 5% levels. As a result of salt doses, the highest K/Na accumulation was 11.90% in T2 applications, and the lowest value was 7.21% in T2 dose. As a result of AMF applications, it was observed that K/Na content varied between 9.52–10.31%. In T × AMF interaction, the highest K/Na content was obtained as 14.20% in T1 × M1 interaction, while the lowest was measured as 2.11% g/kg in T2 × M0 application (Table 4). In Table 4, it was determined that Salt doses application and T × AMF interaction were significant at the 1% level on Ca/Na content. As a result of salt doses, the highest Ca/Na accumulation was 7.17% in T2 applications, and the lowest value was 3.32% in T4 dose. As a result of AMF applications, Ca/Na varied between 4.88–5.78%. In T × AMF interaction, the highest Ca/Na content was obtained from T2 × M0 interaction with 9.83%, while the lowest was measured in T4 × M0, M1 applications with 3.28 and 3.37% (Table 4). In this study, it was determined that Salt, AMF applications, and T × AMF interaction were significant at the 1% level on Mn content. As a result of salt doses, the highest Mn accumulation was 56.5 mg kg⁻¹ from T0 applications, and the lowest value was 23.6 mg kg⁻¹ from T4 dose. As a result of AMF applications, Mn content was measured as 44.1 mg/kg in M0 dose and 50.2 mg kg⁻¹ in M1 dose. In T × AMF interaction, the highest Mn content was obtained as 59.7 mg kg⁻¹ from T1 × M0 interaction, while the lowest value was measured as 0.03 mg kg⁻¹ in T4 × M0 application (Table 5).

AMF was determined to be insignificant on Mo content, while Salt and T × AMF interaction were significant at the 1% level. As a result of salt doses, the highest Mo accumulation was measured as 1.26 mg kg⁻¹ from T3 and T0 applications, while the lowest value was measured as 0.81 and 0.83 mg kg⁻¹ in T1 and T4 doses. As a result of AMF applications, it was observed that Mo content varied between 0.99–1.12 mg kg⁻¹. In T × AMF interaction, the highest Mo content was obtained as 1.64 mg kg⁻¹ from T3 × M0 interaction, while the lowest value was measured as 0.31 mg kg⁻¹ in T4 × M0 application (Table 5). As seen in Table 5, it was determined that AMF was insignificant on Cu content, while Salt and T × AMF interaction were significant at the 1% level. As a result of salt doses, the highest Cu accumulation was measured as 1.78 and 1.76 mg kg⁻¹ from T0 and T1 applications, while the lowest value was measured as 0.76 mg kg⁻¹ in T4 doses. As a result of AMF applications, Cu content was observed to vary between 1.42–1.48 mg kg⁻¹. In T × AMF interaction, the highest Cu content was

obtained as 1.99 mg kg⁻¹ from T0 × M1 interaction, while the lowest value was measured as 0.38 mg kg⁻¹ in T4 × M0 application (Table 5). At the end of the experiment, it was determined that Salt, AMF applications, and T × AMF interaction were significant at the 1% level on As content. As a result of salt doses, the highest Mn accumulation was obtained as 5.26 mg kg⁻¹ from T0 applications, while the lowest value was obtained as 2.17 mg kg⁻¹ in the T4 dose. T1, T2, T3, and T4, which had the lowest value in terms of As content, were statistically in the same group. As a result of AMF applications, the As content was measured as 2.37 mg kg⁻¹ at the lowest M1 dose and 3.68 mg kg⁻¹ at the highest M0 dose. In the T × AMF interaction, the highest As content was obtained as 8.75 mg kg⁻¹ from the T0 × M0 interaction, while the lowest value was measured as 1.86 mg kg⁻¹ in the T0 × M1 application (Table 5).

Table 5. Effect of mycorrhiza applications on some mineral changes in soybean plants grown under salt stress conditions

Treatment					
Salt doses	AMF Teratment	Mn (mg kg ⁻¹)	Mo (mg kg ⁻¹)	Cu (mg kg ⁻¹)	As (mg kg ⁻¹)
T0	Control (M0)	55.3 ab	1.27 ab	1.55 bcd	8.75 a
	AMF (M1)	57.7 ab	1.26 ab	1.99 a	1.86 c
T0 Ort.		56.5 A	1.26 A	1.78 A	5.26 A
T1 50 (T1)	Control (M0)	59.7 a	1.02 ab	1.88 ab	3.12 ab
	AMF (M1)	52.9 ab	0.60 bc	1.65 abc	2.43 b
T1 Ort.		56.3 A	0.81 B	1.76 A	2.78 B
T 100 (T2)	Control (M0)	51.2 ab	0.70 abc	1.67 abc	2.31 b
	AMF (M1)	49.1 ab	1.53 ab	1.27 cd	2.19 b
T2 Ort.		50.1 B	1.11 B	1.47 B	2.25 B
T150 (T3)	Control (M0)	54.1 ab	1.64 a	1.60 abc	2.12 b
	AMF (M1)	44.0 bc	0.87 abc	1.36 cde	2.65 b
T3 Ort.		49.1 B	1.26 A	1.48 B	2.38 B
T 200 (T4)	Control (M0)	0.03 c	0.31 c	0.38 e	2.10 b
	AMF (M1)	47.2 ab	1.36 ab	1.15 cd	2.24 b
T4 Ort.		23.6 C	0.83 B	0.76 C	2.17 B
AMF Treatment	Control (M0)	44.1 B	0.99	1.42	3.68 A
	AMF (M1)	50.2 A	1.12	1.48	2.37 B
Salt doses (T)		**	**	**	**
AMF		**	ns	ns	**
T x AMF		**	**	**	**
CV (%)		13.95	18.65	14.04	16.07
LSD (0.05)		14.64	1.07	0.42	1.09

*Significant at P<0.05 level, **Significant at P<0.01 level. ns: not significant.

As seen in Table 6, AMF was determined to be insignificant on Ni content, while Salt and T × AMF interaction were determined to be significant at 1% and 5% levels. As a result of salt doses, the highest Ni accumulation was measured as 1.97, 1.83, and 1.81 mg kg⁻¹ in T1, T2, and T0 applications, respectively, and these values were found to be in the same group. The lowest value was measured as 0.99 mg kg⁻¹ in the T4 dose. As a result of AMF applications, Ni content varied between 1.60–1.65 mg kg⁻¹. In the T × AMF interaction, the highest Ni content was obtained as 2.34 mg kg⁻¹ from the T2 × M0 interaction, while the lowest value was measured as 0.23 mg kg⁻¹ in the T4 × M0 application. At the end of the experiment, it was determined that Salt and T × AMF interaction were significant at the 1% level for Pb content. As a result of salt doses, the highest Pb accumulation was obtained as 0.12 mg kg⁻¹ from T1 applications, and the lowest value was obtained as 0.02 mg kg⁻¹ from the T4 dose. In the T × AMF interaction, the highest Pb content was obtained as 0.19 mg kg⁻¹ from the T1 × M1 interaction, while the lowest value was measured as 0.01 mg kg⁻¹ in the T4 × M0 application (Table 6). In this study, AMF, Salt, and T × AMF interaction were determined to be significant at the 1% level for Cd content. As a result of salt doses, the highest Cd accumulation was measured as 0.13 mg kg⁻¹ from the T2 application, and the lowest value was measured as 0.06 mg kg⁻¹ in the T4 doses. As a result of AMF applications, the lowest Cd content was observed as 0.07 mg kg⁻¹ in M0 and the highest as 0.09 mg kg⁻¹

in M1 application. In the $T \times AMF$ interaction, the highest Cd content was obtained as 0.23 mg kg^{-1} from the $T2 \times M0$ interaction, while the lowest value was measured as 0.04 mg kg^{-1} in the $T3 \times M0$ application.

AMF was determined to be insignificant on Co content, while Salt and $T \times AMF$ interaction were determined to be significant at the 1% level. As a result of salt doses, the highest Co accumulation was measured as 0.08 mg kg^{-1} from the T1 application, while the lowest value was measured as 0.06 mg kg^{-1} in the T4 doses. As a result of AMF applications, Co content was measured as 0.07 mg kg^{-1} in both applications, with similar values observed. In the $T \times AMF$ interaction, the highest Co content was obtained as 0.09 mg kg^{-1} from the $T1 \times M0$ interaction, while the lowest value was measured as 0.04 mg kg^{-1} in the $T4 \times M0$ application. As seen in Table 6, it was determined that AMF, Salt, and $T \times AMF$ interaction were significant at the 1% level for Cr content. As a result of salt doses, the highest Cr accumulation was measured in the T1 application with 1.48 mg kg^{-1} , while the lowest value was measured in the T4 doses with 0.78 mg kg^{-1} . As a result of AMF applications, the lowest Cr content was observed in the M1 application with 1.14 mg kg^{-1} , while the highest was observed in the M0 application with 1.29 mg kg^{-1} . In the $T \times AMF$ interaction, the highest Cr content was obtained as 1.60 mg kg^{-1} from the $T1 \times M0$ interaction, while the lowest value was measured in the $T4 \times M0$ application with 0.45 mg kg^{-1} .

Table 6. Effect of mycorrhiza applications on some mineral changes in soybean plants grown under salt stress conditions

Salt doses	Tretament					
	AMF Teratment	Ni (mg kg^{-1})	Pb (mg kg^{-1})	Cd (mg kg^{-1})	Co (mg kg^{-1})	Cr (mg kg^{-1})
T0	Control (M0)	1.70 ab	0.08 abc	0.12 ab	0.07 bc	1.65 a
	AMF (M1)	1.92 ab	0.10 ab	0.06 c	0.06 bcd	0.78 c
T0 Ort.		1.81 A	0.09 B	0.09 C	0.07 B	1.21 B
T1 50 (T1)	Control (M0)	2.09 ab	0.11 ab	0.08 b	0.09 a	1.60 a
	AMF (M1)	1.86 ab	0.19 a	0.06 c	0.08 ab	1.36 ab
T1 Ort.		1.97 A	0.12 A	0.07 C	0.08 A	1.48 A
T 100 (T2)	Control (M0)	2.34 a	0.09 abc	0.23 a	0.08 ab	1.25 ab
	AMF (M1)	1.33 bc	0.10 ab	0.04 b	0.06 cd	1.14 ab
T2 Ort.		1.83 A	0.10 B	0.13 A	0.07 B	1.20 B
T150 (T3)	Control (M0)	1.91 ab	0.06 abc	0.04 d	0.06 bcd	1.44 ab
	AMF (M1)	1.15 bc	0.08 abc	0.12 ab	0.08 ab	1.35 ab
T3 Ort.		1.53 B	0.07 C	0.08 B	0.07 B	1.39 AB
T 200 (T4)	Control (M0)	0.23 d	0.01 c	0.05 cd	0.04 d	0.45 d
	AMF (M1)	1.76 ab	0.03 bc	0.06 c	0.07 abc	1.12 ab
T4 Ort.		0.99 C	0.02 D	0.06 D	0.06 C	0.78 C
AMF Treatment	Control (M0)	1.60	0.07	0.07 B	0.07	1.29 A
	AMF (M1)	1.65	0.08	0.09 A	0.07	1.14 B
Salt doses (T)		**	**	**	**	**
AMF		ns	ns	**	ns	**
T x AMF		*	**	**	**	**
CV (%)		19.4	21.6	19.8	14.2	21.8
LSD (0.05)		1.07	0.12	0.37	0.03	0.86

*Significant at $P < 0.05$ level, **Significant at $P < 0.01$ level. ns: not significant.

4. Discussion

Salt stress negatively affects plant development in various ways (Kereçin and Öztürk, 2024). Although some soybean species show high salt tolerance, it has been reported that salt negatively affects the life cycle of soybean (Özçınar et al., 2022). In parallel with increasing salt doses, chlorophyll and its derivatives, which play a primary role in photosynthesis, are negatively impacted (Table 2). In a similar study, a decrease in chlorophyll content, leaf area yellowing, drying, and YAI values were observed as a result of drought stress in soybean (Oral et al., 2021). Kurt et al. (2023) found that, under different salt doses, the highest chlorophyll ratio in the “İlksoy” soybean variety was obtained from the control group plants with 41.45, while the lowest value was recorded in the 300 mM salt concentration with 38.46. In their study on *Cupressus arizonica* (Blue cypress), Akat et al. (2020) reported a 22.47% decrease in total

chlorophyll content under salt stress conditions, with a significant increase in chlorophyll levels due to an increase in mycorrhiza dose. Additionally, it was noted that leaf chlorophyll content decreased at higher salt doses (Zamani et al., 2014). Plant pigments are responsible for the bright red, yellow, and orange tones in many fruits and vegetables. Contrary to expectations, there was no sudden decrease in carotenoid content with increasing salt doses (Table 2). Ruiz-Lozano (2003), Kaya et al. (2009), and Geren et al. (2011) reported that mycorrhizal applications promoted chlorophyll formation under salt stress conditions. Carotenoid content decreased by 15.4% under salt stress, and although mycorrhizal applications and the salt \times mycorrhiza interaction increased carotenoid levels, this effect was not statistically significant. Proline is known to play a crucial role in maintaining osmotic balance and ensuring cell membrane integrity (Tuna and Eroğlu, 2017). The increase in proline synthesis despite increasing salt doses in our study indicates that proline synthesis plays an essential role as a defense mechanism against salt stress in soybean. Similarly, Manaf and Zayed (2015) reported that the amount of proline in cowpea increased as the salinity of irrigation water increased. While there was some fluctuation in proline content with mycorrhizal applications, the general trend was an increase. In many studies conducted under saline conditions, it has been reported that there is a positive correlation between the increase in salt dose and the accumulation of biochemical contents such as MDA and proline. It is well known that stress conditions typically lead to an increase in the levels of biochemical markers such as MDA and proline (Ruiz-Lozano, 2003; Kaya et al., 2009; Taibi et al., 2016). However, some stress-reducing applications can mitigate or balance this change. Kurt et al. (2023) observed an increase in MDA levels as the salt dose applied increased, with the highest value (0.75 nmol/g TA) recorded from the 300 mM salt treatment (Bahjat et al., 2023). The lowest MDA value (0.50 nmol/g TA) was found in the control pots. Similarly, in a study by Akat et al. (2020), mycorrhiza applications at a dose of 200 g da⁻¹ reduced proline content by 40% and MDA levels by 33% compared to pots without mycorrhiza application under saline conditions. A similar pattern was observed by Zamani et al. (2014), where leaf proline levels increased under higher salt doses. These results are consistent with the findings of our study.

Beneficial elements play a significant role in improving plant resistance to both abiotic and biotic stresses at low concentrations. However, the effective ranges for these beneficial elements are relatively narrow, and higher doses can be detrimental to plant metabolism (Singhal et al., 2023). Calcium, in particular, plays a vital role in maintaining the structural and functional integrity of plant cell membranes. It has been reported to stabilize cell wall structures, regulate ion transport and selectivity, and control cell wall enzyme activity and ion exchange (Hadi and Karimi, 2012). The ability of plants to absorb calcium from the soil during salt stress indicates that they can maintain cell membrane integrity. Salt stress inhibits plant growth and development by disrupting osmotic and ion balance. During the ion stress phase that follows osmotic stress, increased concentrations of Na and Cl ions in the environment compete with essential nutrients such as K, Ca, and NO₃, leading to nutrient deficiencies (Oral et al., 2021). The results of this study align with our findings. As salt stress intensified, we observed an increase in K and Na accumulation in the plant (Table 3). Patel et al. (2010) also reported that salt application caused Na and K accumulation in the leaves of cowpea. Similarly, Trouvelet et al. (1986) found that Na content in plant tissues increased as a result of salt stress in sage (*Salvia officinalis* L.). These findings further support our results.

Upon examining the findings, it was observed that phosphorus (P) content decreases following salt stress. This trend is consistent with other studies, which have shown that P content tends to decrease under increasing salt stress conditions. In our study, however, we observed that mycorrhizal activity has a positive effect on P content in plants facing stress. In their natural environment, plants interact with numerous microorganisms and form symbiotic relationships with some of them. Arbuscular mycorrhizal fungi (AMF) are capable of establishing symbiotic relationships with over 80% of terrestrial plant species (Smith and Read, 1997). Some studies have also indicated that magnesium (Mg) content, similar to phosphorus, decreases under abiotic stress (Balliu et al., 2015). Magnesium, being the central atom of chlorophyll, is essential for photosynthesis. Therefore, magnesium deficiency leads to a decrease in chlorophyll content, resulting in reduced photosynthetic activity. Consequently, this limits plant growth and leads to product loss. Magnesium is highly mobile in plants, and its deficiency is one of the most common occurrences under stress conditions (Oral et al., 2021).

Zinc (Zn) acts as a catalyst in plants and participates in cellular oxidation processes. It is essential for plant growth and development and plays a key role in carbohydrate metabolism.

Additionally, zinc enhances chlorophyll production and regulates sugar synthesis. In this study, Zn content decreased with increasing salt stress (Table 4). Previous research has indicated that zinc, being a less mobile element, tends to decrease under stress conditions (Al-Karaki, 2000).

In this study, the K-Ca/Na ratio decreased with increasing stress doses (Table 4). As the severity of NaCl stress increases, the K/Na ratio in plants typically decreases (López-Aguilar et al., 2012). Our findings also indicated that mycorrhizal applications had a reducing effect on both K/Na and Ca/Na content compared to the control, which are indicators of salt tolerance. Excess Na⁺ in the soil inhibits potassium (K) uptake by plants (Çulha and Çakırlar, 2011). Turan et al. (2010) reported that salt stress reduced the K ratio in both the root and stem of corn. Similarly, Kuşvuran et al. (2008) found that the K ratio decreased in some melon genotypes while it increased in others under salt stress. Since K functions as an osmolyte within the cell (Çulha and Çakırlar, 2011), its uptake is crucial for maintaining leaf relative water content and supporting other biochemical processes involving K. The plant's ability to continue absorbing K under salt stress helps mitigate the negative effects of Na⁺ (Daşgan et al., 2002). Our study also found that K content decreased as stress doses increased, and the K/Na and Ca/Na ratios tended to decline under increasing stress conditions. Similar studies have shown that mineral and element contents, including Mn, Mo, Cu, As, Ni, Pb, Cd, Co, and Cr are negatively affected by abiotic stressors like drought and salt, leading to decreases in plant concentrations (Kurt et al., 2023). These elements are critical for plant development, photosynthesis, hormone production, and enzyme activity. In recent years, research has emphasized the importance of compounds that mitigate the negative effects of salt stress and enhance plant stress tolerance. Studies conducted over the past 15-20 years have shown that certain treatments can help regulate the reduction in macro- and micronutrient levels under stress conditions (Hasanuzzaman et al., 2013).

Conclusion

In this study, the K-Ca/Na ratio decreased as stress doses increased (Table 4). As NaCl stress intensity increases, the K/Na ratio in plants typically declines (López-Aguilar et al., 2012). Our findings also indicated that mycorrhizal applications reduced both K/Na and Ca/Na content compared to the control, suggesting a potential role in enhancing salt tolerance. Excess Na⁺ in the soil inhibits potassium (K) uptake by plants (Çulha and Çakırlar, 2011). Turan et al. (2010) reported that salt stress reduced the K ratio in both roots and stems of corn. Similarly, Kuşvuran et al. (2008) observed that the K ratio decreased in some melon genotypes, while it increased in others under salt stress. Since K acts as an osmolyte within the cell (Çulha and Çakırlar, 2011), its uptake is crucial for maintaining leaf relative water content and supporting other biochemical processes. The plant's ability to continue absorbing K under salt stress helps mitigate the detrimental effects of Na⁺ (Daşgan et al., 2002). Our study also found that K content decreased as stress doses increased, with a corresponding decline in K/Na and Ca/Na ratios under increasing stress conditions. Similar studies have shown that the contents of minerals and elements such as Mn, Mo, Cu, As, Ni, Pb, Cd, Co, and Cr are negatively affected by abiotic stressors like drought and salt, leading to reduced concentrations in plants (Kurt et al., 2023). These elements are vital for plant development, photosynthesis, hormone production, and enzyme activity. In recent years, research has highlighted the importance of compounds that mitigate the negative effects of salt stress and enhance plant stress tolerance. Studies over the past 15-20 years have demonstrated that certain treatments can help regulate reductions in macro- and micronutrient levels under stress conditions (Hasanuzzaman et al., 2013).

Ethical Statement

Ethical approval is not required for this study.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Author Contributions

Authors contributed equally.

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