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The Effect of Biochar Amendment on Physiological and Biochemical Properties and Nutrient Content of Lettuce in Saline Water Irrigation Conditions

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ABSTRACT

Salinity often increases osmotic stress, reducing plant water uptake and inhibiting the absorption of nutrients and minerals. This imbalance situation causes physiological, biochemical disorders, and nutrient deficiencies in plants. In this study, the effects of biochar application on the physiological properties, nutrient contents and antioxidant enzyme activities of lettuce were investigated under saline irrigation water conditions. For this purpose, four different biochar doses and different irrigation water salinity levels were applied to the lettuce plant. In the study, biochar application under salt stress conditions decreased the Na, Fe, Zn content and antioxidant enzyme activity of the plant. Leaf relative water content, chlorophyll content (SPAD) and some nutrients (Ca, K, Mg, P, Cu and Mn) also increased. Therefore, biochar applied under salt irrigated water conditions offers good potential to reduce the severity of plant exposure to salinity stress. In addition, the biochar amendment helped the plant uptake of nutrients.

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Introduction

Salinity negatively affects productivity and plant growth as it negatively affects both soil physical properties and microbial activity (Parkash and Singh 2020). Moreover, high salinity in the soil leads to oxidative stress by increasing reactive oxygen species (ROS) (Mansoor et al. 2022). Fortunately, plants have developed several protective mechanisms to reduce or eliminate ROS. Antioxidants are at the forefront of these mechanisms. Antioxidants (scavenging systems of ROS) can contribute to plant development under stress (Das and Roychoudhury 2014; Guzel et al., 2018; Krupodorova et al., 2022). Antioxidative defense mechanisms are capable of scavenging ROS molecules under steady-state conditions (Akgül et al., 2022). The equilibrium relationship between ROS production and scavenging can be disrupted by diverse biotic and abiotic stress factors, such as pathogens, salinity, drought or extreme temperatures (Das et al., 2016; Sahin et al., 2016; Gündoğdu et al., 2019; Kına et al., 2021; Mohammed et al., 2022). The enzymatic antioxidant system is one of the protective mechanisms such as catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD), SOD enzyme, responding with the superoxide anion (O2) to ROS. This reaction causes H_2O_2 accumulation and is scavenged by CAT and peroxidase enzymes (Sevindik et al., 2017; Mehdizadeh et al., 2019; Uysal et al., 2021).

Renewable materials of vegetable origin are used to reduce abiotic stress in the plant (drought, salinity, etc.). Biochar is a material with high organic carbon (C) content, mostly formed by the pyrolysis of vegetable wastes at high temperatures (>250°C) and anaerobic conditions. Biochar application increases the yield of the crop, while at the same time increasing the organic matter, water holding capacity and productivity of the soil (Cooper et al. 2020, Çakmakci et al., 2021; Farhangi-Abriz et al. 2021, Cakmakci and Sahin 2022; Yerli et al., 2022). Kul et al. (2021) reported that biochar application decreased the negative effect of salinity and increased plant growth by balancing some physiological and biochemical mechanisms in tomato.

Many studies have investigated the effects of biochar addition on drought, soil physical properties and crop yield. However, it has been determined that studies that provide more available information on plant physiology in improvement with biochar of the effect of salinity in lettuce are limited. For this reason, the relationships between the use of biochar in irrigation conditions with salty water and lettuce plant stress management, plant nutrient content and antioxidant enzyme activity should be further examined. The hypothesis of this study is that (I) the stress effect of saline irrigation water on the physiological and biochemical properties of the plant can be alleviated by the use of biochar (2) the use of biochar will help plant nutrient absorption, and (3) biochar amendment to the soil will decrease the plant antioxidant enzyme activity despite the negative effect of salt. Based on this information, in this study, it was aimed to investigate the effects of biochar application on the physiological and biochemical properties, nutrient content and antioxidant enzyme activities of lettuce under saline irrigation conditions.

Material and Methods

Materials and Experiment Area

Lettuce (*Lactuva sativa* L.) was used as plant material in the experiment. From the seeds obtained from a commercial company, seedlings were grown in viols and transferred to trial pots (volume of 3.0 litres and dimensions of 200 × 180 mm). The lettuce variety used as a trial material is suitable for production in late autumn and early spring. The vegetation period is 60-80 days. Biochar obtained from a commercial firm. NaCl was used as the salt source. The research was carried out in the plastic greenhouse of Van Yüzüncü Yıl University Faculty of Agriculture in the 2021 growing season. Temperature and humidity values inside the greenhouse were recorded hourly with the Hobo device (HOBO, Campbell Scientific Inc., USA) (Figure 1).

Applications

The study was carried out according to a randomized plot design with four replications in a total of 64 pots. In the study, four different irrigation water qualities (S0, S1, S2 and S3) and four different biochar doses (B0, B1, B2 and B3) were applied. Soil samples were dried and sieved with a 4 mm sieve. The biochar particle size was < 2 mm after sieving (Edenborn et al. 2015). The control application was only filled with soil (sandy loam) (0%, B0). Biochar was weighed at rates of 1% (B1), 2% (B2) and 3% (B3) (w:w) by soil weight, mixed thoroughly with soil, and then used to fill the pots. In the study, irrigation water with 4 different salinities with electrical conductivity (ECw) values of S0: 0.72 dS m^{-1}, S1: 0.9 dS m^{-1}, S2: 1.8 dS m^{-1} and S3: 2.7 dS m^{-1} was used. The salinity levels of the waters were controlled before each irrigation.

At the beginning of the experiment, all pots were irrigated without stress with the same amounts of water (S0) considering the field capacity level of pot soil as the upper limit until the irrigation period with salty waters. Then, irrigation continued with the amounts of salty water enough to complete the water deficit in the pot soils considering the field capacity determined for each of the different salty water treatments (S0, S1, S2 and S3) throughout the experiment.

Physiological, Biochemical and Nutrient Analyses

Fresh leaf samples at the harvest period were used to determine the physiological and biochemical properties and ion contents, and the leaves were stored at -80°C for CAT, SOD, APX and MDA analysis.

Chlorophyll content was measured at three different parts of the plant leaf with Chlorophyll meter (SPAD-502) before harvest, and the average of these data was recorded as the SPAD value for each plant. The fresh weights (LFW) of leaf samples taken with a diameter of 10 mm before harvesting to determine leaf relative water content (LRWC) were weighed on a precision scale, and the leaves were kept in distilled water for 4 hours. Leaf turgor weights (LTW) were determined by weighing, and leaf dry weights (LDW) were determined by drying the same samples in an oven set at 65°C for approximately 48 hours and weighing.

The LRWC was calculated from the weights obtained with the help of the following equation (Smart and Barss 1973, Ors et al. 2021):

$$LRWC\ (\%) = 100 \times (LFW - LDW) / (LTW - LDW)$$

Membrane injury index (MII) was calculated by measuring the electrolyte coming out of the leaf cells (Shi et al., 2006). Disc-shaped (10 mm diameter) samples were taken from each plant leaf before harvest. After the leaf discs were kept in 30 mL deionized water in 50 mLtubes at room temperature for 24 hours, the electrical conductivity of the water in the tube was measured and recorded as the EC1 value. Then, the tubes were kept in a water bath at 95°C for 20 minutes, cooled to room temperature, and the electrical conductivity was measured again and the EC2 value was found. MII value was determined using the following equation.

$$MII = (EC_1/EC_2) \times 100$$

The enzyme extraction process of the plant samples was carried out at +4°C. Superoxide dismutase (SOD) was detected with modified method of Jebara et al. (2010). Reduction of 50% of NBT as a unit was determined as SOD activity. Catalase (CAT) enzyme activity was analyzed by recording the disappearance of H_2O_2 at a wavelength of 240 nm (Çakmak and Marschner, 1992). Ascorbate peroxidase (APX) activity was determined according to Nakano and Asada (1981). The absorbance value was measured at 290 nm immediately after the extract was added. Lipid peroxidation (MDA) was determined with method of Heath and Packer (1968). The absorbance value of the mixture was determined at 532 and 600 nm wavelengths, and the MDA content was calculated with a molar absorption coefficient of 155 mM/cm.

Determination of macro micro nutrients in plant leaves was made by the dry combustion method. Leaves were dried in oven at 65°C for approximately 48 hours and crushed with the help of a porcelain mortar. The dry combustion process was performed by taking 0.5 grams from the ground samples, and 10 mL of 1 N H_2SO_4 was used in the washing process. The K, Ca, Na, Mg, Fe and Zn values were detected by atomic absorption spectrophotometry, and the P, Cu and Mn values were determined by ICP–OES.

Data Analysis

The data obtained in the study were tested with the General Linear Model approach using SPSS (version 23.0) software. Between the means significance level (0.05) was analyzed by Duncan's multiple range test (Duncan 1955). Correlograms with a scatterplot, correlation coefficient, and variable distribution were created by the RStudio package to determine the relationships between LRWC, SPAD, MDI, CAT, SOD, APX and MDA values and plant macro micro nutrient contents.

Results and Discussion

Salinity among abiotic stress factors is considered the more effective limiting plant growth in agricultural production. Lettuce is known as a plant resistant to moderate salinity (Adhikari et al 2019). Recently, many studies have investigated the effects of biochar applications to reduce the detrimental effects of salinity on plant productivity (Elshaikh and She 2018; Farhangi-Abriz and Torabian 2018; Parkash and Singh 2020). Salty water and biochar treatments significantly affected the LRWC, chlorophyll content (SPAD), MDI, CAT, SOD, APX and MDA values, and plant nutrient contents (P<0.01) (Table 1).

The LRWC values decreased with increasing salinity levels; thus, plants provided more LWRC (89.7%) under nonsaline conditions (S0), while a lower LWRC (74.8%) was determined at the high salinity level (S3), which indicates a decline of 16.6%. Biochar treatment positively affected LRWC compared with B0, and the value was the highest in B3 (85.2%) (Figure 2). SPAD value declined significantly with the effect of increasing salinity levels (P<0.01) (Figure 2). The S3 treatment decreased the SPAD by 21.2% compared to S0. However, increasing doses of biochar treatments increased the SPAD, and the B3 treatment resulted in a higher value by 13.4% compared to the treatment without biochar. Considering the interaction between biochar and salinity levels, the highest SPAD value (43.7%) was determined in the B3-S0 treatment, while the lowest value (28.8%) was found in the B0-S3 treatment. In this study, salty irrigation water significantly reduced the LRWC and chlorophyll content (SPAD). Under saline conditions, low LRWC value is associated with increased solute content in plant cells exposed to salinity (Munns et al., 2006). Therefore, irrigation with salty water in this study might cause a decrease in water uptake by roots and thus a decline in LRWC. However, the findings showed that biochar amendment alleviated the adverse effects of salinity stress by improving LRWC and stimulating chlorophyll content. A significant linear correlation in the study was found between LRWC and SPAD (Figure 6). Previous studies have also proven that salinity stress reduces the LRWC and chlorophyll content (Garrido et al. 2014, Kanwal et al. 2018, Kul et al. 2021). However, biochar positively alters the soil's physical structure and air capacity and has a beneficial effect on available water retention (Laird et al. 2010). Akhtar et al. (2014) also reported that biochar significantly increases chlorophyll content, stomatal conductivity, photosynthesis rate, water use efficiency and relative water content under stress conditions.

Table 1. The variance analysis results for LRWC, SPAD, MDI, CAT, SOD, APX, MDA values and plant macro-micro nutrients

		LRWC		SPAD		MII		CAT	
Source	df	Mean square	P	Mean square	P	Mean square	P	Mean square	P
Biochar (B)	3	58.529	0.000	68.144	0.000	42.032	0.000	8.793	0.000
Salinity (S)	3	682.518	0.000	217.694	0.000	2165.490	0.000	218.501	0.000
B×S	9	2.982	0.070	2.228	0.004	4.482	0.000	1.709	0.166
Error	48	1.539		0.704		0.675		1.088	
		SOD		APX		MDA		P	
Source	df	Mean square	P	Mean square	P	Mean square	P	Mean square	P
Biochar (B)	3	78.164	0.000	0.007	0.000	4.526	0.000	14700.845	0.000
Salinity (S)	3	30.262	0.000	0.008	0.000	1.029	0.024	795.466	0.000
B×S	9	1.245	0.364	0.005	0.000	0.153	0.822	462.921	0.000
Error	48	1.092		0.000		0.277		40.280	
		K		Ca		Mg		Na	
Source	df	Mean square	P	Mean square	P	Mean square	P	Mean square	P
Biochar (B)	3	1.959	0.000	0.356	0.000	0.169	0.000	0.248	0.000
Salinity (S)	3	0.409	0.000	0.167	0.000	0.200	0.000	0.663	0.000
B×S	9	0.015	0.554	0.117	0.000	0.083	0.000	0.037	0.000
Error	48	0.017		0.009		0.007		0.005	
		Fe		Mn		Zn		Cu	
Source	df	Mean square	P	Mean square	P	Mean square	P	Mean square	P
Biochar (B)	3	6569.695	0.000	6,067	0,000	1050.874	0.000	0.265	0.000
Salinity (S)	3	9291.699	0.000	1,081	0,000	1460.116	0.000	0.150	0.000
B×S	9	2233.741	0.000	0,634	0,000	390.235	0.000	0.049	0.000
Error	48	324.914		0,129		34.637		0.008	

LRWC: leaf relative water content, SPAD: Chlorophyll value reading, MII: Membrane injury index, CAT: Katalaz, SOD:Süperoksit dismutaz, APX: Askorbat peroksidaz, MDA: Malondialdehit

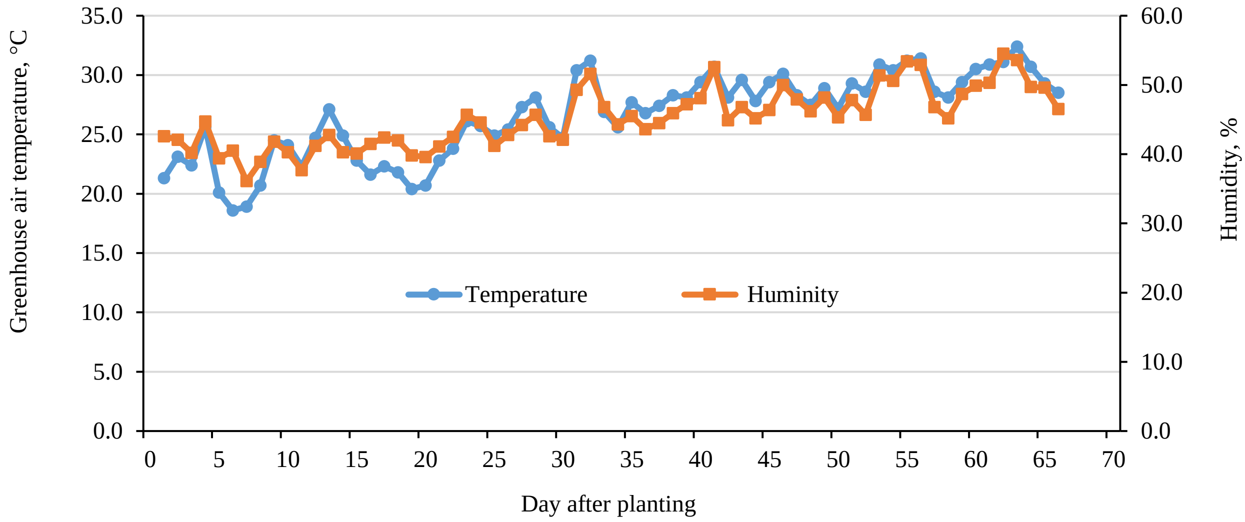

Figure 1. Daily air temperature and humidity values in greenhouse throughout the growing period.

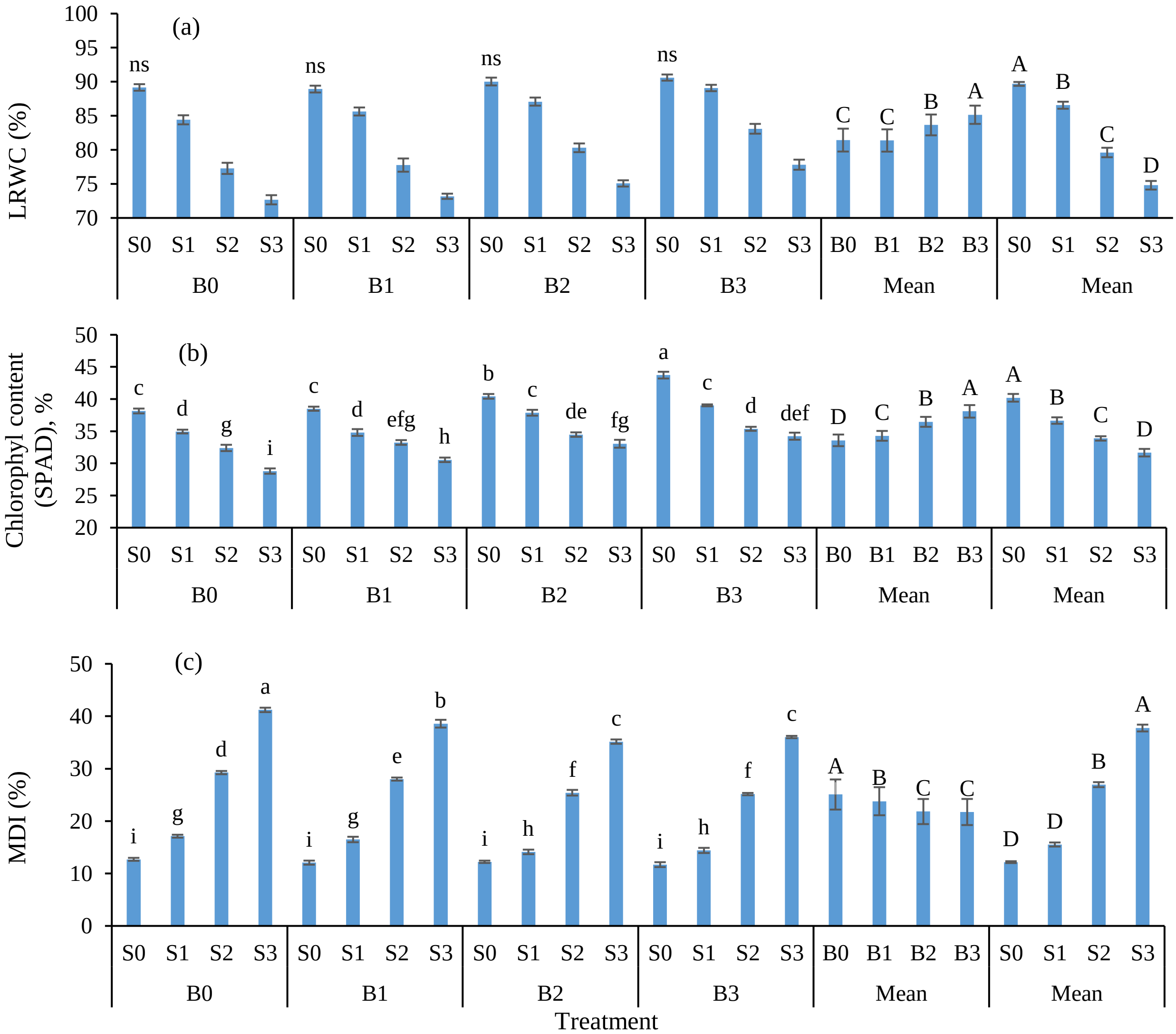

Figure 2. Effect of salt water irrigation to (a) leaf relative water content (LRWC), (b) chlorophyl content, and (c) membrane damage index (MDI) under biochar applied conditions.

B0: Non- biochar, B1: 1% biochar, B2: 2% biochar, B3: 3% biochar, S0: control, S1: irrigation water electrical conductivity 0.9 dS m^{-1}, S2: irrigation water electrical conductivity 1.8 dS m^{-1}, S3: irrigation water electrical conductivity 2.7 dS m^{-1}, The means marked with different lowercases are differ at the level of P<0.01, ns: not significant

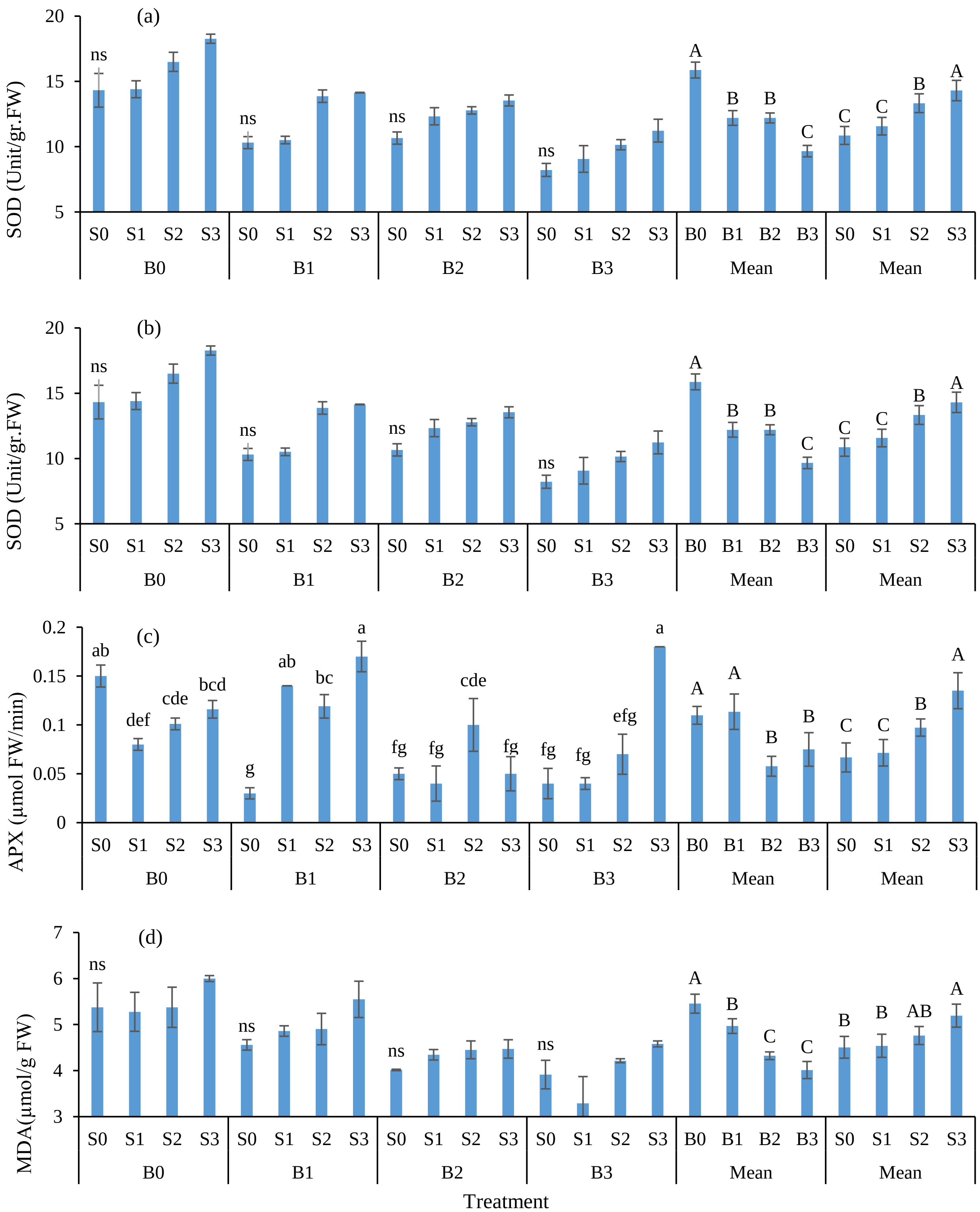

Figure 3. Effect of salt water irrigation to (a) CAT activity, (b) SOD activity, (c) APX activity, (d) MDA activity under biochar applied conditions.

B0: Non- biochar, B1: 1% biochar, B2: 2% biochar, B3: 3% biochar, S0: control, S1: irrigation water electrical conductivity 0.9 dS m^{-1}, S2: irrigation water electrical conductivity 1.8 dS m^{-1}, S3: irrigation water electrical conductivity 2.7 dS m^{-1}, The means marked with different lowercases are differ at the level of $P<0.01$, ns: not significant

Decreasing the K content in plants reduces photosynthetic activity by causing oxidative stress under saline conditions. Reduced K in soil nutrient solution significantly decreased the relative chlorophyll content (SPAD) in lettuce (Zhang et al. 2017). Mg is a building block in chlorophyll formation (Afzai et al. 2022). Mn, Fe and Cu also help in the formation of chlorophyll (Bolat and Kara 2017). The study findings showed that the correlations of SPAD with plant K, Mg and micronutrient (Fe, Mn, Cu) contents were significantly positively linear (Figure 6). Afzai et al. (2022) indicated that the increase in SPAD by biochar application could be attributed to better absorption of K and Mg.

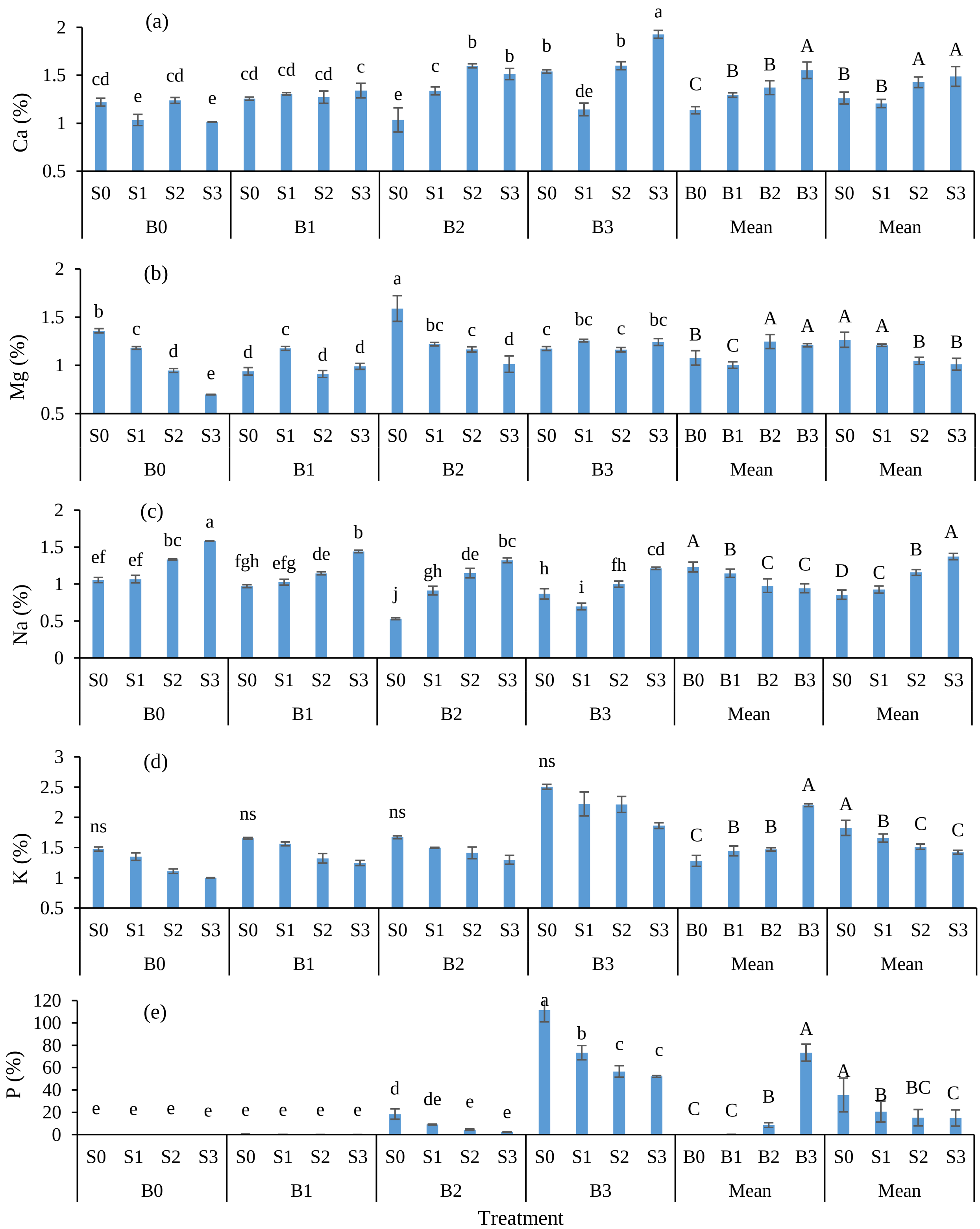

Figure 4. Effect of salt water irrigation to (a) Ca, (b) Mg, (c) Na, (d) K, and (e) P content under biochar applied conditions.

B0: Non- biochar, B1: 1% biochar, B2: 2% biochar, B3: 3% biochar, S0: control, S1: irrigation water electrical conductivity 0.9 dS m^{-1}, S2: irrigation water electrical conductivity 1.8 dS m^{-1}, S3: irrigation water electrical conductivity 2.7 dS m^{-1}, The means marked with different lowercases are differ at the level of P<0.01.

Although Zn application increases SPAD under drought stress (Ma et al. 2017) and improves the chlorophyll index (SPAD) under salinity stress (Tolay 2021), it was determined that SPAD values decreased with increasing plant Zn content (Figure 6). This could be explained by the fact that while the Zn content increased with increasing salinity, it decreased with increasing biochar dose (Figure 5), which resulted in low SPAD values.

The highest level in salinity (S3) increased the membrane injury index (MII) approximately 3-fold compared to the control (S0). However, biochar treatment noticeably alleviated membrane injury index.

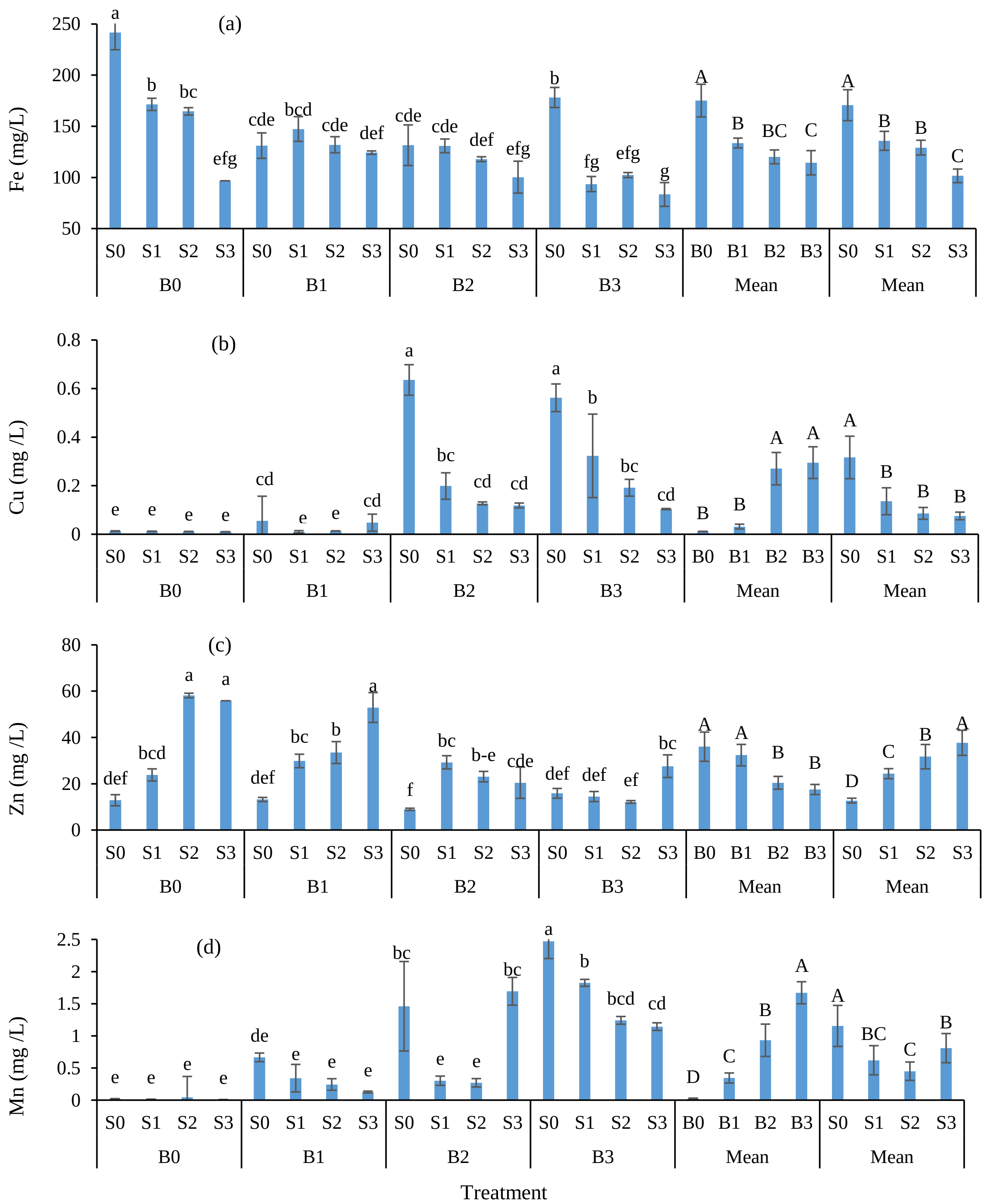

Figure 5. Effect of salt water irrigation to (a) Fe, (b) Cu, (c) Zn, and (d) Mn content under biochar applied conditions.
B0: Non- biochar, B1: 1% biochar, B2: 2% biochar, B3: 3% biochar, S0: control, S1: irrigation water electrical conductivity 0.9 dS m^{-1}, S2: irrigation water electrical conductivity 1.8 dS m^{-1}, S3: irrigation water electrical conductivity 2.7 dS m^{-1}, The means marked with different lowercases are differ at the level of P<0.01

On average, the B3 treatment decreased the MII by 13.3% compared to the treatment without biochar (Figure 2). A significant (P<0.01) interaction was also determined between the biochar and salt-water treatments.

The S3 treatment increased the CAT, SOD, APX and MDA values 4.3, 1.3, 2.0 and 1.2-fold compared to the S0, respectively, while the B3 treatment decreased these activities by 25.3%, 39.1%, 31.8% and 26.4% compared to the B0 values (Figure 3). Considering the significant interaction between treatments for APX, the highest (0.18 mmol gr FW/min) value was determined in the B3-S3 treatment, while the lowest value (0.03 mmol gr FW/min) was determined in the B0-S3 treatment. Salty water induced a significant increase in antioxidant enzymatic activity (SOD, CAT and APX), lipid peroxidation (MDA), and membrane injury index (MII) with a defense mechanism against the detrimental effects of salinity.

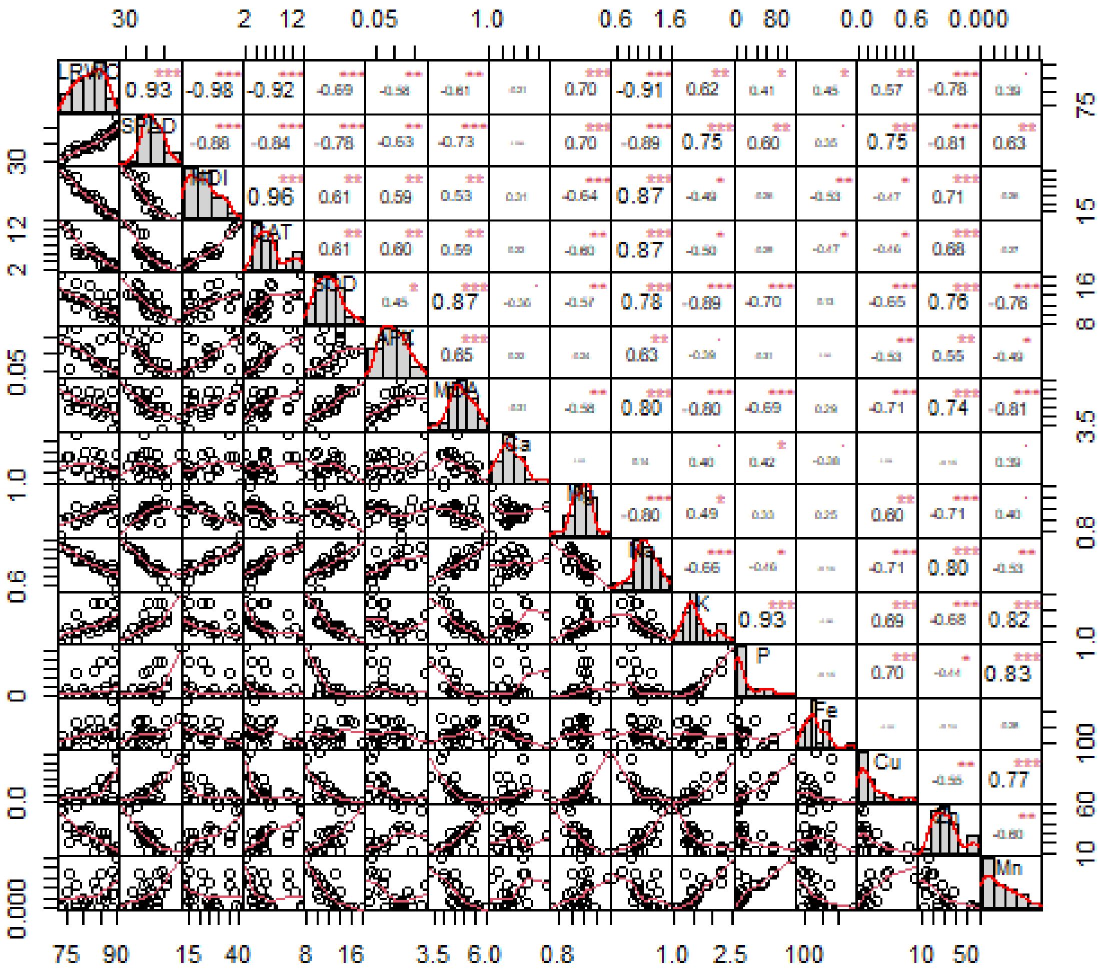

Figure 6. Correlation matrix for LRWC, SPAD, MDI, CAT, SOD, APX and MDA values, and plant macro-micro nutrient contents.

LRWC: leaf relative water content, SPAD: Chlorophyll value reading, MII: Membrane injury index, CAT: Katalaz, SOD:Süperoksit dismutaz, APX: Askorbat peroksidaz, MDA: Malondialdehit, ***, **, *: Significant at the level of 0.001, 0.01 and 0.05, respectively

Similar results were obtained in the studies conducted by Gharib et al. (2016) and Sattar et al. (2019). ROS production leads to oxidative damage (protein degradation, membrane damage, esc.), ultimately resulting in cell death under stress conditions (Bano et al. 2021, Zhang et al. 2021, Aslam et al. 2022). The increase in antioxidant enzyme activity under salinity stress reduces oxidative stress by decreasing ROS. On the other hand, the antioxidant enzyme activity, MDA accumulation and MII decreased with biochar amendment. Organic matter treatment can alleviate the negative effects of salt stress by regulating the activity of antioxidant enzymes in plants (Tartoura et al. 2014). Previous studies reported that the antioxidant enzyme activities increased under salinity stress in rice (Zhang et al. 2012), tomatoes (Kim et al. 2016) and soybean (Farhangi-Abriz and Torabian 2018) and decreased with biochar applications. Organic amendments improve salinity tolerance by increasing leaf water content, photosynthetic activity and osmolyte accumulation (Afzai et al. 2022). The significant negative correlations of SPAD with MDA, MII and antioxidant enzymes were found to be valuable to support this approach (Figure 6).

The P, K, Mg, Fe, Cu and Mn contents in lettuce crops decreased with increasing salinity of water applied, while the Ca, Na and Zn contents increased (Figures 4 and 5). The increased biochar dose increased the P, K, Ca, Mg, Cu and Mn contents and decreased the Na, Fe and Zn contents. High Na accumulation in plants causes specific ion toxicity that inhibits osmotic regulation and disrupts nutrient balance (Katerji et al. 2004; Safdar et al. 2019). This ionic stress caused by salt can involve serious physiological damage and increase the aging or death of mature leaves, causing sharp reductions in plant growth and photosynthetic CO_2 assimilation (Munns et al. 2006, El-Hendawy et al. 2019). In our study, Na accumulation increased with increasing salinity, and a significant decrease in K content was observed. Increased Na significantly decreased the SPAD value by decreasing K (Figure 6). The decrease in the K concentration in the plant is the result of the exchange of K with Na ions under salinity. The reduction in K content inhibited by Na is a competitive mechanism exhibited by plants under saline conditions (Ashraf et al. 2015, Ashraf et al. 2018, Afzai et al. 2022). Moreover, the salty irrigation water also reduced the uptake of Fe, Mg, P, Cu and Mn nutrients in this study.

Salinity not only inhibits the uptake of other nutrients but also increases the out-of-root Na concentration, which damages root selectivity (Ashraf et al. 2018). Therefore, the intake of useful nutrients decreases in saline soil conditions. However, biochar amendments contributed to the uptake of nutrients, such as K, Ca, Mg, P, Cu and Mn, while decreasing the Na content. It is known that biochar can be used to improve salinity stress by reducing Na uptake in plants and has a high salt absorption potential (Laghari et al. 2015). A previous study reported that biochar increases the uptake of nutrients such as Ca, Mg and K into the soil solution (Masud et al. 2014, Inal et al. 2015). Another study determined that biochar plays an important role in regulating nutrient availability by affecting soil pH (Van Zwieten et al. 2010). Moreover, many studies have reported that biochar application can increase nutrient availability and nutrient uptake in soil by increasing soil cation exchange capacity and surface area (Xiao et al. 2016, Haider et al. 2017).

Conclusion

The study showed that biochar could reduce the negative effects of salinity on lettuce growth by improving physiological and biochemical activities. In the salinity irrigation water, the addition (B3) of biochar to the soil increased the chlorophyll content (%13.43) of the plants and the leaf relative water content (%4.57) compared to the control treatment. Biochar amendment considerably alleviated the negative effects on membrane injury index of salty irrigation water by providing a lower leaf Na/K ratio. Biochar amendment improved the physiological and biochemical properties of lettuce by reducing Na adsorption. Considering that biochar application reduces the antioxidant enzyme activity (CAT, SOD, APX) and lipid peroxidation in the plant under irrigation water salinity stress conditions, it has been determined that the plant alleviates salt stress. It was clearly seen that the highest abiotic stress in lettuce was in the treatment with 2.7 dS/m salinity, and the most effective 3% biochar application in reducing stress. Considering the inadequacy of existing water resources, future studies should focus on integrated management (biochar, etc.) for the use of salt-water resources.

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