Impact of Biochar on Growth, Physiology and Antioxidant Activity of Common Bean Subjected to Salinity Stress

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Abstract: A pot study was carried out to evaluate the impacts of biochar applications on the growth, physiological properties, and antioxidant enzyme activity of common beans under salinity stress. The experiment was conducted in a completely randomized design with two salinity levels of NaCl [S0 (control, 0 mM NaCl) and S1 (100 mM NaCl)] and three biochar levels [BC-0 (control, non-biochar), BC-1 (2.5%) and BC-2 (5 %)]. Results of the study revealed that plant growth, relative leaf water content (LRWC), and chlorophyll reading value (CRV) of common bean decreased significantly, while malondialdehyde (MDA), hydrogen peroxide (H_2O_2), proline, and sucrose content increased significantly with salinity stress. Biochar applications mitigated the negative impact of salinity stress on plant growth and physiological characteristics of common beans. The salinity tolerance due to biochar applications could be associated with a significant reduction of antioxidant activity, MDA and H_2O_2, and an increase of LRWC and chlorophyll content. Therefore, it can be concluded that biochar could be used to reduce the negative impacts of salinity stress in common bean.

Keywords: Biochar, Mitigation, Salty stress, Soil amendment.

INTRODUCTION

According to the International Biochar Initiative (IBI) ''biochar is a solid material obtained from thermochemical conversion of biomass in an oxygen-limited environment''. Physical characteristics of biochar include particle density, surface area, and pore-size distributions, whereas the main chemical characteristics include pH, total C, and total N, conductivity, cation exchange capacity, and selected nutrient and contaminant trace elements. Characteristics of biochar materials will vary depending on the biochar feedstock and pyrolysis conditions [1, 2].

Various studies have shown that biochar increases crop yield and improves soil quality [3, 4]. Also, biochar as a soil amendment can sequester carbon from the atmosphere [5, 6]. In addition, biochar has the characteristics of higher stability against decomposition and excellent ability to absorb ions as compared to other forms of soil organic matter [7, 8].

Salt stress is reported to be one of the important agricultural problems limiting crop production, especially in arid and semi-arid regions. Even at relatively low levels of its presence significantly reduced the yield of common bean (*Phaseolus vulgaris* L.) because it's a salt-sensitive crop. Biochar is attracting attention in recent years as a potential soil amendment under stress conditions. Biochar balances water holding capacity and air porosity in soils, thus biochar promotes benefits in plant growth in saline soils through the reduction in oxidation stress and osmotic stress [9].

Several studies observed that the application of biochar is effective in reducing salinity stress by improving soil physicochemical and biological properties directly related to Na removals [10, 11]. It was reported that incorporation of biochar into salt-affected soil could alleviate salinity stress in potatoes mainly because of its high salt (Na^+) adsorption potential [12].

There is little information about the effect of biochar on growth, physiology, and antioxidant activity of common bean subjected to salinity stress. Therefore, the study is conducted to investigate the role of biochar as a soil amendment in mitigating the effect of salinity in bean seedlings grown in saline conditions.

MATERIALS AND METHODS

The study was conducted as a pot experiment under greenhouse conditions in Department of Horticulture of Atatürk University in Turkey. In the experiment, "Gina" common bean (*Phaseolus vulgaris*

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L.) variety was used as plant material. The average greenhouse temperature and humidity measured with digital temperature and humidity indicator were about 27 ± 3 ° C and 54 ± 5%, respectively.

The biochar used in the study was produced by the thermal conversion process of 60% sewage sludge and 40% household waste such as biological solids and green wastes. Some chemical properties of biochar used in the experiment is given in Table **1**. Biochar at the rate of 0% (BC-0), 2.5 % (BC-1) and 5% (BC-2) by weight and mixed thoroughly with sandy loam soil before filling the pot. Then, the pots (21 x 17 cm/ 3.5 L) were filled equally weighted (3 kg) and were placed randomly on the benches in the greenhouse. Three bean seeds were sown in the annealed soil by irrigating it until reached to the field capacity.

After seedling emergence, only one seedling was left for each pot. All groups of biochar applications were watered with two levels of salt applications including non-saline water (S0 =Tap water-0 mM NaCl) and saline water (S1 = 100 mM NaCl). Approximately fifty days after sowing, measurements and analyses were carried out by harvesting. Photo showing the effects of biochar as a soil amendment to mitigate the damage of salt stress in bean seedlings is presented in Figure **1**.

At harvest, plants from each replication were taken to measure stem diameter, plant height, leaf number, seedling fresh and dry weight. For dry weight measurements, the plant material was kept at 70 °C for 48 h. To determine the content of proline, sucrose, MDA, H_2O_2, and antioxidant enzyme activity, roughly 20 g of fresh leaves were frozen in liquid nitrogen and then stored at -80 °C.

Table 1: Chemical Characteristics of Biochar Produced from Urban Wastes

Properties	Unit	Analysis Results
pH	-	7.8
EC	dS m^{-1}	0.38
Total (humic + fulvic)	%	4.9
Organic Nitrogen	%	1.6
C	%	21.54
H	%	1.26
N	%	1.38
O	%	2.1
Pb	mg kg^{-1}	162
Cd	mg kg^{-1}	10
Cu	mg kg^{-1}	393
Ni	mg kg^{-1}	310
Zn	mg kg^{-1}	1187
Cr	mg kg^{-1}	449
Mn	mg kg^{-1}	549
K	mg kg^{-1}	10290
P	mg kg^{-1}	22980
Mg	mg kg^{-1}	7372
Ca	mg kg^{-1}	57500
Fe	mg kg^{-1}	25680

The chlorophyll content of the plant leaves was determined by a chlorophyll meter (SPAD – 502, Konica Minolta Sensing, Inc., Japan). The area of the leaves was quantified as cm^2 with a leaf area meter (LI-3100, LI-COR). LRWC was determined according to [13]. H_2O_2 was determined according to Velikova *et al*. [14]. The content of H_2O_2 was calculated by using a

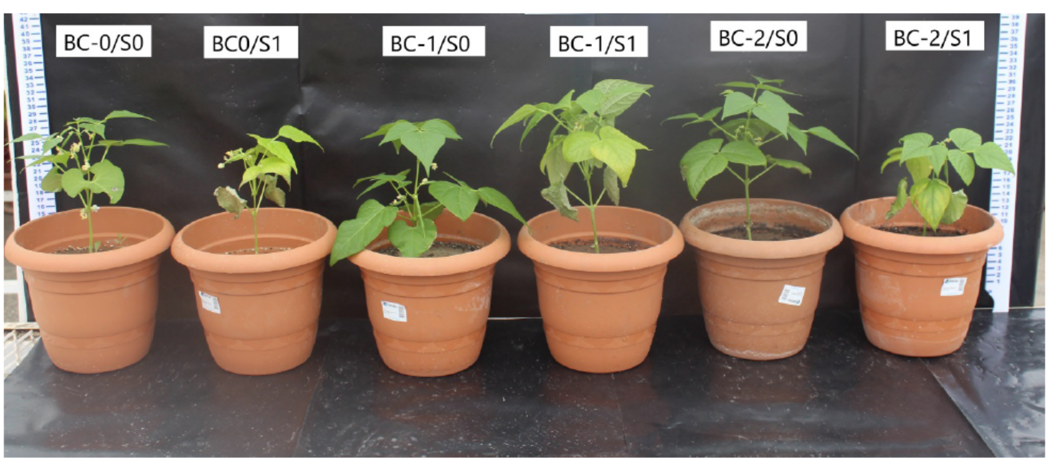

Figure 1: The mitigating effect of biochar on bean seedlings grown under salt stress conditions.

standard calibration curve previously made by using different concentrations of H_2O_2. Thiobarbituric acid-reactive substances were measured as MDA, a degraded product of the lipid, which determines the lipid peroxidation. The concentration of MDA was determined from the absorbance curve, by using an extinction coefficient of 155 mmol l^{-1} cm^{-1}. Sucrose concentration was measured by a method given by Chopra *et al*. [15]. Proline concentration was assayed spectrophotometrically at 520 nm [16].

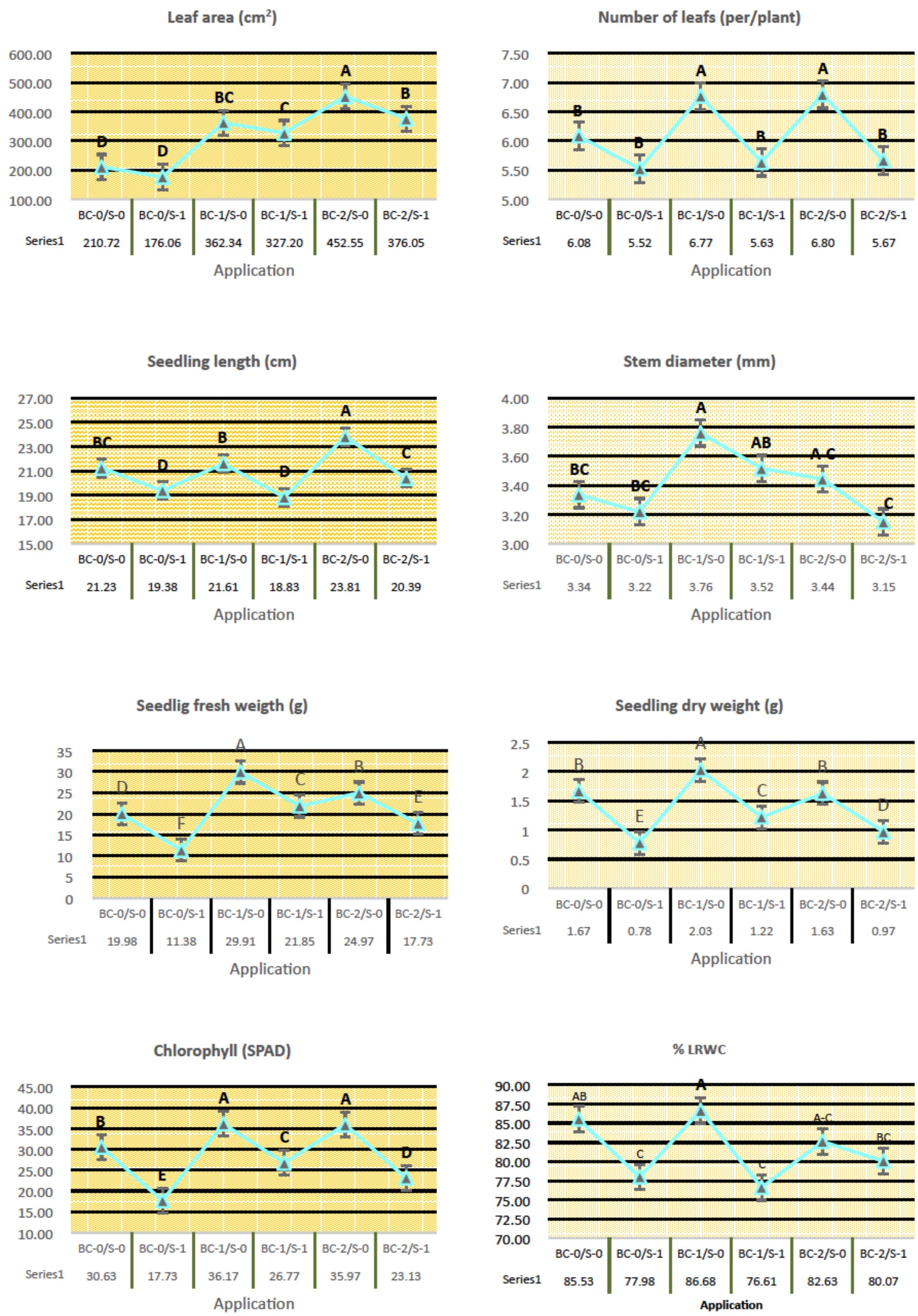

Figure 2: The effect of biochar applications on the growth properties chlorophyll (SPAD) and %LRWC of bean seedlings grown under salt stress conditions. S0: 0 mM NaCl/S1: 100 mM NaCl; BC-0: %0 biochar / BC-1: 2.5% biochar/BC-2: 5 % biochar.

The study was conducted in three replications, keeping 5 pots in each replication as according to the randomized plot design. The data obtained from the study arranged according to a randomized complete block design was analyzed by using the SPSS 25 package program. Different letters indicate significant differences tested by Duncan's multiple comparisons ($P < 0.05$).

RESULTS

The results show that all of the examined growth characters of bean were decreased at salt stress treatments compared to salt-free applications. However, biochar applied to the soil enhanced the growth components except for stem diameter and leaf number in comparison with the non-biochar treatment. Also, the BC-1 was more effective on the fresh and dry weight of common bean than the other treatments (Figure **2**).

Effect of biochar on H_2O_2 and MDA content of common bean under salinity stress is presented in Figure **3**. The results also indicated that salinity increased H_2O_2 and MDA content. However, biochar application significantly resulted in decreasing H_2O_2 and MDA content. Additionally, saline conditions significantly did not change SOD, CAT and POD activity whereas biochar application significantly decreased SOD, CAT and POD activity (Figure **3**).

Figure **3** presents effect of biochar on sucrose and proline content of common bean under salinity stress. Both salinity and biochar applications significantly affected sucrose and proline content of common bean. In addition, it is observed that salt application increases in proline and sucrose contents in plants. However, the addition of biochar in salty conditions decreased the proline and sucrose content of the plants (Figure **3**).

DISCUSSION AND CONCLUSION

Salinity negatively affects plant growth, which is one of the most major abiotic stresses, limiting crop productivity. Climate changes are expected to increase the salinity risk of soils. The present study pointed out that salinity stress caused to reduce growth of common bean. Salt stress limits the growth of plants by osmotic and ionic stress [17]. The salinity stress can cause the synthesis of active oxygen species (ROS) impairing membrane function, DNA, protein, and chlorophyll [18]. Biochar mitigated salinity stress on the growth of common bean. Biochar can improve the physical, chemical, and biological characteristics of soil [19]. Biochar increased the growth and yield of some crops under salinity stress [20, 21].

Biochar added to the soil reduced LRWC in bean leaves. Salt stress triggers the change of lipid composition in the membrane structure and causes membrane damage [22]. Biochar has been reported to increase soil water holding capacity, therefore improving water use efficiency [23]. Biochar as a soil amendment preserves the water balance and leaf turgidity of plants grown under salinity, greatly reducing water waste through stomatal closure and transpiration [24]. Biochar has high adsorption capacity, which helps to mitigate the detrimental impact of salinity by minimizing the uptake of Na^+ [24].

Salinity stress negatively affected CRV of common bean (Figure **2**). Salinity stress causes the enrichment of active oxygen species in plants that disrupt the structure of chlorophyll [17]. However, biochar applications enhanced the CRV. Akhtar *et al*. [24] suggested that biochar increased chlorophyll content because of an enhanced level of N in leaves.

In the study, it was determined that salinity stress elevated leaf H_2O_2, MDA, proline, and sucrose content of common bean. Salt stress has been shown to increase the production of Reactive Oxygen Species (ROS). MDA is an indicator of tissue damage in plants against salt stress. Studies have shown a positive relationship between the synthesis of organic substances such as sucrose and proline and stress tolerance [25]. Biochar applications reduced the H2O2, MDA, proline, and sucrose content of common bean under salinity stress.

In the study, salinity stress enhanced CAT and SOD of eggplant seedlings but it is statsitically non significant (Figure **3**). Studies have reported that salt stress increases CAT and SOD enzyme activity in many plants [25]. Various Abiotic stresses including salt stress result in the generation of reactive oxygen species (ROS) in plants.

As a result, the effectiveness of 2.5 % biochar was superior to the 5% treatment in terms of evaluated parameters, especially under saline conditions. The produced results supported the idea that biochar can contribute to protecting common bean against NaCl stress by regulating the antioxidant enzyme system,

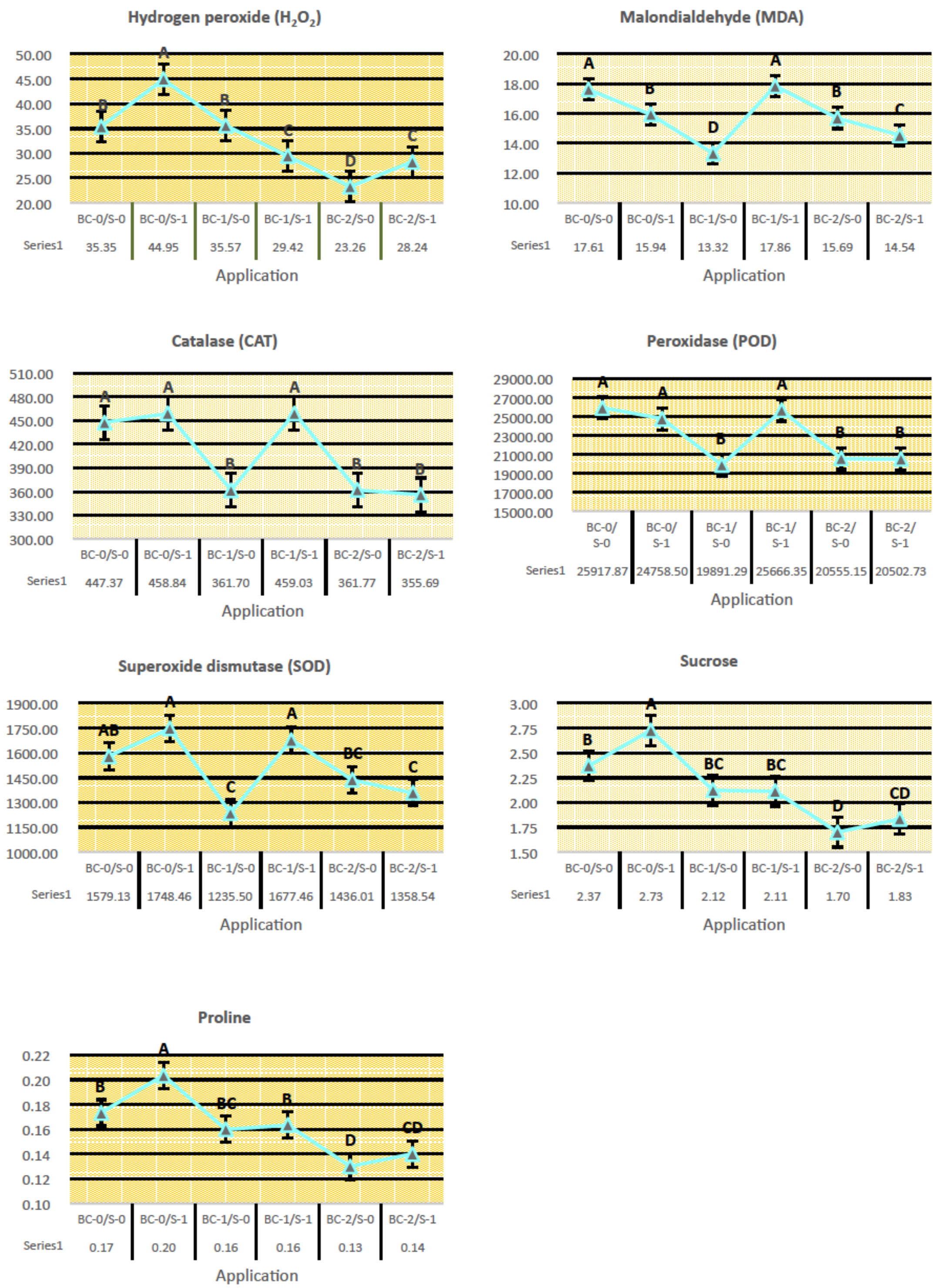

Figure 3: The effect of biochar applications on the antioxidant enzymes (CAT, POD, SOD), proline and sucrose concentrations of bean seedlings grown under salt stress conditions. S0: 0 mM NaCl/S1: 100 mM NaCl; BC-0: %0 biochar / BC-1: 2.5% biochar/BC-2: 5 % biochar.

the improvement of growth hormones, and alleviating of oxidative stress.

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