

# Biochar and Halotolerant Bacteria in The Improvement of Saline-Sodic Soil Health and Wheat Growth

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**Abstract**— Salt toxicity is a brutal environmental factor affecting crop production worldwide. Biochar, a byproduct of the process of pyrolysis, is a carbon-rich material which has the potential to ease the harmful effects of salinity. Use of microbial inoculants to counteract salinity stress is also a sound option to increase crop growth and yield under stress environments. The present study was conducted to access the combined effect of cotton biochar and salt-tolerant bacteria (PGPBs) to improve wheat growth and the quality of salt-affected soil. Biochar was applied at 10 and 20 t ha<sup>-1</sup> rates. The results of the study showed the significant positive effect of applied amendments on wheat growth parameters. The highest values for all the parameters were obtained with the combined application of 20 tha<sup>-1</sup> biochar and halotolerant. Sole application of halotolerant also had a positive effect on improving wheat growth under salinity stress. In terms of soil properties, a substantial decrease in soil pH, EC and Na<sup>+</sup> was observed after crop harvest for all treatments. Whereas, a significant increase in soil organic matter (OM), soil total nitrogen (TN) and exchangeable cations (Ca<sup>2+</sup>, Mg<sup>2+</sup> and K<sup>+</sup>) were recorded. In conclusion, it is stated that the combined use of cotton biochar and halotolerant could be a wise approach for improving the soil quality and crop growth under salt stress.

**Index Terms**— cotton biochar, halotolerant bacteria, salt-stressed soils, wheat growth

## 1 INTRODUCTION

The major environmental stresses like extreme temperature, soil salinity/sodicity, drought and flooding have affected the development of crops. The soil salinization issue is a scourge for global agricultural productivity. Worldwide, around 1/5th of the irrigated agricultural lands are severely salt-affected negatively influencing plant growth both in the plant and cellular levels [1]. A good number of agro-nomic crops are rather salt susceptible and cannot survive even a small amount of salinity. Hence, the magnitude of the salinity issue is the gap between production and demand for food all over the world. Salts amassment in the soil not only hamper plant growth but also deteriorate soil status by disturbing soil physical, chemical and biological environment [2] [3].

Remediation of salt-stressed soils is, therefore, necessary to favour crop development as well as to improve overall soil health. Various practices are applied to modify these soils, however, the efficacy of organic amendments like compost, manure and mulch for reclamation of soils is a focus of researchers now for their soil remediation efficiency. Addition of organic matter in salt-affected soils is highly recommended because of its ability to decrease electrical conductivity and exchangeable sodium percentage and to increase soil water holding capacity, aggregate stability and leaching of salts [4]. A study conducted by a researcher reported an improvement in the physical properties of soil with the use of municipal solid waste under saline-water irrigation Lax et al. [5].

Recently, the use of biochar as an organic amendment in the reclamation of salt-affected soil has gained much attention. Various scientists have documented that adding biochar amendments to soils not only increased yield but also helped plants to survive numerous stresses caused by pesticides [6] heavy met-

als [7] and toxic compounds or drought [8, 9]. Application of biochar to salt-affected soils resulted in improved physico-chemical and biological soil properties [10, 11, 12]. Biochar could be a potential source of different nutrient elements in the soils, mainly the cations (e.g., N, K, Ca, Mg, Zn, Mn) [13, 14] thus meeting the nutrient requirements of plants in deteriorated soils. As biochar can last in the soil for years, its use would be immensely valuable in treating contaminated soils as compared to the other fast degrading organic amendments [15].

Another approach to minimize salt stress is the use of biological agents such as plant growth-promoting bacteria (PGPBs) which are well known for their potential to increase crop growth and yield under stress environments [16]. Use of microbial inoculants to counteract salinity stress is a sound option over developing salt-tolerant crops, which is not only time-consuming but a difficult and uneconomical strategy for sustainable agriculture [17]. Studies conducted on various crops, including maize, wheat, rice, peas and tomato showed the beneficial effect of salt-tolerant PGPBs strains under saline conditions [18, 19]. Mechanisms by which plant growth-promoting bacteria improves plant productivity under salt stress includes the production of Osmoprotectants, hydraulic conductance, and presence of Aminocyclopropane-1-Carboxylate (ACC). Consequently, lowering ethylene production and translocation of Na<sup>+</sup> ions, increasing the biosynthesis of antioxidative enzymes, stomatal conductance and photosynthetic activities. Hence, seed co-inoculation with diverse species of PGPB, such as *Rhizobium* and *Azospirillum* could be a sensible method to alleviate the injurious effects of salt stress on many crops [20].

Considering the beneficial effects of PGPBs on varieties of

agronomic crops, the present study was conducted to evaluate the interactive effect of biochar and salt-tolerant bacteria on growth parameters of wheat, as well as on the post-harvest properties of salt-affected soil.

## 2 MATERIALS AND METHODS

An experiment was conducted in the glass-house of Department of Genetics and Bioengineering, Yeditepe University, Turkey to assess the interactive effect of biochar and halotolerant on wheat growth parameters as well as post-harvest changes in the chemical properties of saline-sodic soil. Cotton biochar used in the study was prepared by the carbonization method as described by Sadaka et al. [21]. Biochar was applied at two rates, i.e. 5 and 10 t ha<sup>-1</sup>. The unpyrolyzed cotton stalk was used as a positive control at the same rates as biochar. The treatment plan used in the study is presented in Table. 1.

TABLE 1  
TREATMENTS PLAN

Treatments	Description
T1	No biochar (Negative control)
T2	Cotton stalk (CS) @10t ha <sup>-1</sup> (Positive control)
T3	Cotton stalk (CS) @20t ha <sup>-1</sup> (Positive control)
T4	Cotton stalk (CS) @10t ha <sup>-1</sup> + Halotolerant
T5	Cotton stalk (CS) @20t ha <sup>-1</sup> + Halotolerant
T6	Cotton stalk biochar (CB) @10t ha <sup>-1</sup>
T7	Cotton stalk biochar (CB) @20t ha <sup>-1</sup>
T8	Halotolerant
T9	Cotton biochar (CB) @10t ha <sup>-1</sup> + Halotolerant
T10	Cotton biochar (CB) @20t ha <sup>-1</sup> + Halotolerant

### 2.1 Leaching

As the saline-sodic to be used in the study was higher in EC and pH, leaching was done to decrease the salinity of the soil. Good quality irrigation water was applied for this purpose. Leaching was continued until the EC reached to the level that crop to be grown could tolerate. The post leaching electrical conductivity was 5.1 dSm<sup>-1</sup>.

### 2.2 Experimental Procedure

Plastic pots with a capacity of about 4 kg were taken and filled with the already collected saline-sodic soil. After labelling with respective treatments, pots were arranged in a completely randomized order and irrigated with distilled water. All the treatments were well mixed into their respective pots, except the control and the one receiving solely halotolerant as an inoculum. A basal dose of NPK (175:60:90 kg ha<sup>-1</sup>) was also ap-

plied depending on the crop.

### 2.3 inoculum preparation

Bacterial strains were grown in sterilized DF minimal salt medium, containing ACC as substrate (N source) with a working volume of 150 ml in Erlenmeyer flasks of 250 ml and incubated for 72 hrs at 28 ± 1 °C and 100 rpm. After incubation, a spectrophotometer was used to measure the optical density of the medium and uniform population (OD540 = 0.45; 107-108 CFU ml<sup>-1</sup>) was achieved by diluting with sterilized water before use.

### 2.4 Sowing

Wheat seeds to be inoculated according to the respective treatments were the first surface sterilized, moistened with cool concentrated sugar solution and coated with the inoculum, thereafter. Depending on the treatment plan, six healthy inoculated, as well as non-inoculated seeds, were sown in pots. Where necessary, all regular cultural practices and irrigation/watering were performed. At the vegetative stage, the crop was sampled to measure growth parameters (shoot and root length, fresh and dry weights).

### 2.5 Post Harvest Soil Analysis

After crop harvest soil in the pots was used to analyze some chemical properties (EC, pH, N, OM, Na, K, Ca and Mg).

### 2.6 Statistical Analysis

All data from the greenhouse study was analyzed according to the completely randomized design (CRD), using statistix 10 software. LSD values were used to indicate the significant variance between the mean values of both treatments and time intervals. The probability value ( $P \leq 0.05$ ) given in the text indicated the significance of treatments and their correlation.

## 3 RESULTS

### 3.1 Growth Parameters

A significant positive effect of applied amendments on wheat growth parameters (shoot and root length, shoot fresh and dry weight) was seen (Figure. 1, 2). All the amendments significantly increased the growth parameters when compared to the control, however, their combinations with halotolerant were highly effective. The un-pyrolyzed cotton stalk applied alone was less effective in increasing growth performance than the sole application of biochar levels. However, when combined with halotolerant, cotton stalk showed better results in comparison to the only biochar application rates. The highest values for all the parameters were obtained with the combined application of 20 t ha<sup>-1</sup> biochar and halotolerant (T10), followed by T9. Sole application of halotolerant (T8) also had a positive effect on improving wheat growth under salinity stress.

When comparing the application rates, though both levels significantly affected the growth performance, 20 t ha<sup>-1</sup> rates exhibited better results.

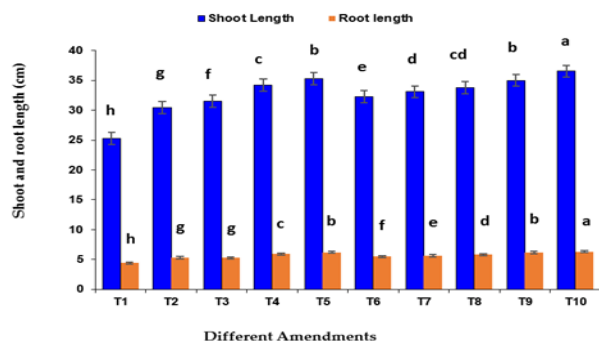


Fig. 1. Effect of different treatments on shoot and root length of the wheat crop. The letters on each bar indicate the statistical differences among the treatments with  $p \leq 0.05$ .

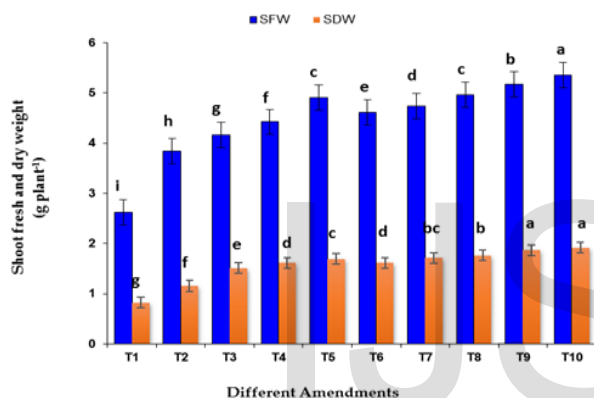


Fig. 2. Effect of different treatments on the shoot fresh and dry weight (SFW, SDW) of the wheat crop. The letters on each bar indicate the statistical differences among the treatments with  $p \leq 0.05$ .

### 3.2 Soil Properties After Crop Harvest

The soil in the pots was analysed for certain chemical properties after the harvest. Analysis of variance had a substantial effect of amendments on the selected soil properties (Table 2, 3).

For all treatments, a substantial decrease in soil pH was observed after crop harvest. Control soil had the highest pH (10.347) while the lowest (10.280) was recorded in T6 where 10 t ha<sup>-1</sup> biochar was applied. The reduction in pH varied between 10.280 to 10.342 among different amendments. Lower the application rates, lower were the pH. All treatments of biochar significantly lowered the soil pH relative to the control however, alone application of biochar was more effective than its combination with halotolerant. The almost same trend was seen for cotton stalk applications (Table. 2).

Soil electrical conductivity (EC) was also significantly reduced in comparison to the control. The difference among treatments was also significant and the lowest EC value (4.23

dSm<sup>-1</sup>) was recorded in the treatment receiving 20 t ha<sup>-1</sup> CS (T6), followed by 4.52 dSm<sup>-1</sup> in halotolerant treatment (T8). In the case of CS, soils amended at higher application rates (20 t ha<sup>-1</sup>) had lower EC, regardless of whether used alone or combined with halotolerant. While results were opposite when coming to biochar. Again, as observed with pH, co-application of treatments with halotolerant had higher EC compared to their sole applications. Comparing biochar with its feedstock, feedstock was more effective in lowering the soil EC (Table. 2).

Both cotton stalk (CS) and its biochar (CB) as well as the sole application of halotolerant improved the OM significantly relative to the control. Soil OM's concentration varied between 9.62-14.13 g kg<sup>-1</sup> among different amendments. The OM content increased with increasing the application rates and the maximum value was observed in the treatment receiving 20 t ha<sup>-1</sup> CS + Halotolerant (T5), followed by T10 (Table. 2).

Soil TN concentration in the control was 0.35g and was increased to a maximum of 1.3g with the combine application of halotolerant PGPBs and 20 t ha<sup>-1</sup> biochar (T10). Effect of CB applied alone or together with halotolerant, showed higher values than all CS treatments. Sole halotolerant application was less efficient in increasing soil TN content compared to other treatments (Table. 2).

TABLE 2

EFFECT OF DIFFERENT TREATMENTS ON POST-HARVEST SOIL CHEMICAL PROPERTIES

Treatments	pH	EC (dSm <sup>-1</sup> )	OM (g/kg)	TN (g/kg)
T1	10.347a	5.31a	7.58 i	0.35g
T2	10.301f	4.63f	10.41f	0.90de
T3	10.308de	4.23h	11.29c	0.80e
T4	10.342b	5.05b	9.78g	0.90de
T5	10.322c	4.88e	14.13a	0.95cd
T6	10.280g	4.92de	9.83g	1.1bc
T7	10.305ef	4.94d	10.57e	1.2b
T8	10.313d	4.52g	9.62h	0.55f
T9	10.306ef	4.94cd	10.69d	1.2b
T10	10.326c	4.97c	11.62b	1.3a
LSD	0.0053	0.0462	0.062	0.012

Exchangeable cations were also greatly affected by the amendments. An increase in Ca<sup>2+</sup>, Mg<sup>2+</sup> and K<sup>+</sup> contents were recorded, while on the other hand Na<sup>+</sup> level was significantly reduced (Table. 3). Though all the amendments significantly reduced the soil Na<sup>+</sup> level, the sole application of halotolerant was the most effective amongst them. Results indicated that Na<sup>+</sup> concentration was 68.44 Cmol (+) kg<sup>-1</sup> in control treatment

and was decreased to the smallest point of 51.46 Cmol (+) kg<sup>-1</sup> showing a comparative reduction of 25% (Table. 3).

Increasing the application rates led to an increased concentration of K<sup>+</sup> and Mg<sup>2+</sup>. Biochar at 20 t ha<sup>-1</sup> applied together with halotolerant (T10) had the highest values of 3.04 Cmol (+) kg<sup>-1</sup> and 1.38 Cmol (+) kg<sup>-1</sup> for K<sup>+</sup> and Mg<sup>2+</sup>, respectively. However, the result was opposite in the case of Ca<sup>2+</sup>, where soil amended with a lower rate of both CS and CB had the maximum Ca<sup>2+</sup> level (Table. 3).

Overall, when comparing the cotton biochar with the feedstock, biochar was more efficient in improving the nutrient status of saline-sodic soil. Undoubtedly, the sole application of amendments had a positive effect on soil health, but the effect was more obvious to their combination with halotolerant.

TABLE 3

EFFECT OF DIFFERENT TREATMENTS ON POST-HARVEST SOIL EXCHANGEABLE CATIONS

Treatments	Na <sup>+</sup>	K <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>
Cmol +/kg				
T1	68.44a	1.88h	7.05j	0.86e
T2	63.29b	2.22f	9.87e	1.14bc
T3	60.84c	2.41d	8.32g	1.12c
T4	60.15e	2.26f	8.24h	1.04d
T5	60.93c	2.35e	8.17i	1.06d
T6	59.37f	2.89b	9.75f	1.15bc
T7	56.49h	2.56f	9.93d	1.17b
T8	51.46i	2.41d	11.14b	1.07d
T9	60.62d	2.65c	11.81a	1.35a
T10	57.10g	3.04a	10.34c	1.38a
LSD	0.14	0.047	0.062	0.043

## 4 DISCUSSION

### Growth Parameters

Cotton biochar either applied alone or together with halotolerant bacteria significantly increased the growth parameters and the results obtained in our study are supported by many other researchers [11, 12, 22-24]. Kanwal *et al.* in their research concluded an improvement in the germination and growth of wheat under salinity stress amended with 1 and 2% biochar [25]. Similarly, Akhtar *et al.* documented that a combined application of biochar and plant growth-promoting bacteria mitigated the adverse effect of salinity on maize [26]. Again, Akhtar *et al.* in a pot experiment concluded a positive effect of biochar amendments on wheat performance under salinity stress

[12]. Usman *et al.* in an experiment documented that Conocarpus biochar significantly increased the tomato yield under saline water irrigation [27]. Likewise, Agbna *et al.* conducted a greenhouse experiment with biochar and concluded an overall improvement in tomato growth and yield under saline soil condition [28]. Improvements in crop germination, growth and yield with biochar under salinity stress maybe because of the Na<sup>+</sup> sorption by biochar, thereby limiting the Na<sup>+</sup> uptake by the plants, hence protecting them against salt stress [29] [30] [12]. Biochar indirectly enhances crop performance by improving soil physio-chemical and biological properties of saline soil, thus providing the conditions favourable for crop establishment [31], and results of the soil properties in our study confirm this.

Halotolerant PGPBs safeguard related crops against damaging salinity impacts. Production of exopolysaccharide (EPS) can be beneficial in the attachment of bacterial cells to biotic surfaces like plants. Inoculation of EPS-producing bacteria would be an appreciated approach for amelioration and improving crop productivity of the salt-affected soils [32, 33] EPS under salinity stress can bind sodium ions and alleviate its toxic effect in the soil [34]. Salt-free soil thus favours the plant growth by providing sufficient nutrients in the soil [35]. Production of ammonia and HCN may participate in the inhibition of many plant pathogens and metalloenzyme under salinity stress [36]. Moreover, a siderophore produced by bacteria is considered biocontrol agent as they sequester the iron from the pathogen needed for their growth [37], thus protecting the plants from numerous fungal or bacterial diseases [38, 39].

### Soil Properties

Reduction in the pH of saline-sodic soil with the biochar application documented in our results is in accordance with the findings of Zhang *et al.* who reported that adding biochar in black soils reduced pH by 0.5 units [39]. According to the findings of some researchers, adding low pH biochar into high pH soils either does not affect or decrease the pH of that soil, predominantly in saline-sodic and sodic soils [40, 10, 27]. As the pH of the biochar used in our study was lower than the soil, thus justifying the findings.

A significant reduction in the soil EC was observed in our study. Some scientists also noted a reduction in EC of saline-sodic soils with biochar enrichment [28, 41]. This can be ascribed to the biochar's fine pore structure, which allows the adsorption of different materials by physically trapping them in the pores [42, 43]. But the formation of the pore relies on the biochar's manufacturing temperature. Biochar manufactured at elevated temperatures has a larger surface area and microporosity [44], leading into increased salt absorption in the functional groups on the biochar surface [45, 46].

We found that biochar significantly increased the OM and TN content of the soil. These results are consistent with other researchers' findings [47-49]. Abbasi *et al.* recorded a substantial rise in TN and OM, following the addition of white clover residue and poultry manure biochar on a loam soil [46]. Biochar could be a direct source of many nutrients, depending on the nature of the feedstock and pyrolysis. Recent studies have shown significant improvements in the nutrient status of bio-



char modified salt-affected soils [47, 46, 50]. For instance, Abdullaeva et al. recorded a considerable rise in OM and TN when apple wood chip biochar was added to a saline-alkaline soil [51]. All the biochar treatments significantly increased the OM and TN content, however, their combination with halotolerant had the highest values. This could be attributed to the ability of bacteria to immobilize mineral nutrients, fix nitrogen as well as synthesis and mineralize soil organic matter (SOM), thereby enhancing the efficiency of added amendments. Since biochar is a carbon, organic matter and nutrients rich material, its addition to the soil increases TN and organic matter [44].

A considerable increase in the concentration of exchangeable cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{K}^{+}$ ) recorded in this study is affirmed by other researchers who also reported increased cation exchange capacity (CEC) and exchangeable cations by adding different biochar types in different soils [28, 52, 48]. For example, Silva et al. concluded that exchangeable  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{K}^{+}$  levels increased with increasing biochar application [42]. Biochar having high CEC when added to the soils, improve the soil CEC. The reason for the increased soil CEC upon biochar addition could be for the high porosity and high surface area of biochar [52-54, 30]. Besides, an increase in CEC could also result from slow oxidation of biochar in soils, thus enhancing the nutrient retention potential of the soil [55-58]. Results showed a reduction in the concentration of exchangeable  $\text{Na}^{+}$ . These findings are in line with the research of Alcívar et al. who stated that soil exchangeable  $\text{Na}^{+}$  levels reduced significantly when biochar and gypsum were mixed applied [59]. Reduction in  $\text{Na}^{+}$  could be the direct result of increased soil  $\text{Ca}^{2+}$  level with biochar addition. Saline-sodic soils are high in exchangeable  $\text{Na}^{+}$ , which adversely affects the soil physical, chemical and biological properties. The incorporation of organic amendments in these soils improves the  $\text{Ca}^{2+}$  concentration and encourages the removal of adsorbed  $\text{Na}^{+}$ , which improves soil structure by increasing soil aggregation and hydraulic conductivity, thereby improving the overall soil health [24].

## 5 CONCLUSIONS

High pH and EC of Salt affected soils make them unsuitable for crop production. Biochar and halotolerant used in our study significantly lowered these soil parameters. Besides, salt stressed soils are low in OM and essential plant nutrients and the results of this study showed a considerable increase in both OM and TN, thus encouraging plant growth. Biochar and its combination with halotolerant resulted in increased  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{K}^{+}$ , and decreased  $\text{Na}^{+}$  on the other hand. Saline-sodic soils, mostly have deteriorated structure because of elevated levels of exchangeable  $\text{Na}^{+}$  which causes the dispersion of soil particles. Instead  $\text{Ca}^{2+}$  act as a binding agent to form soil aggregates, which as a result improves soil structure, soil porosity, soil aeration, soil water holding capacity and overall soil quality. Thus, improvement in soil health with biochar and halotolerant application resulted in improved wheat growth parameters.

In the light of the results it is concluded that, since salt-affected soils are highly degraded and need special attention for their rec-

lamation, combined application of halotolerant bacteria and biochar could be a sensible approach to help restore the soil's fertility and productivity, thereby improving the crop yields.

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