9 *Egypt. J. Soil Sci.* **Vol. 65**, No. 1, pp: 121 - 134 (2025)

Egyptian Journal of Soil Science

http://ejss.journals.ekb.eg/

Enhancing Wheat Productivity in Salt-Affected Soils UsingTraditional and Acidified Biochars: A Sustainable Solution

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SOIL salinity constitutes a significant threat to crops. Probably, adding some soil additives such as biochar could alleviate salt stress and , at the same time, improve soil characteristics. To test this hypothesis, crop residues were collected and subjected to pyrolysis to prepare normal biochar. Portions of this product were acidified with sulfuric acid, while the other portions were left non-acidified. Then, a pot experiment of a complete randomized design was established comprising 5 treatments, with three replicates each, i.e. either 5 or 10 g kg^{-1} of each of acid modified biochar and the non-acidified one, beside of the non-amended control soil (EC= 5.94 dS m^{-1}) which was deemed a control one. Wheat was sown in all pots and soil moisture was sustained at field capacity using well water (EC = 0.59 dSm^{-1}) throughout the duration of the experiment. After a period of approximately 50 days post-germination , proline and chlorophyll contents were assessed in shoots. At physiological maturity stage, whole plants were harvested and soil samples were collected from the rhizosphere of each pot. The plant stress osmoprotectant named proline decreased significantly in plants amended with biochar as if these plants suffer less from salinity stress. Moreover, plant biomass improved significantly due to biochar application, with superiority for the acidified one. This additive (acid-modified biochar) also augmented grain to shoot ratio; number of plants per pot, number of spikes per plant and plant height, while lessened root-to-shoot ratio. In this regard, the most significant enhancements in the aforementioned plant growth parameters were observed at the higher application rate of biochar, specifically 10 g kg^{-1}. The improvements in plant growth parameters exhibited a substantial correlation with the concomitant increases in nitrogen, phosphorus, and potassium (NPK) uptake by the plants. Notably, K concentrations in the shoots were sufficiently high, suggesting a potential role in osmoregulation. On the other hand, no significant differences were detected in leaf chlorophy (SPAD) among the investigated treatments. Concerning soil organic carbon content, a markable increase was noted in soil following the application of biochar, especially the non-acidified biochar when being applied at a rate of 10 g kg^{-1}. Nonetheless, the latter application rate raised significantly soil salinity, while acid modified biochar declined soil EC. In conclusion, the improvements in characters of salt affected soils due to intensive cropping and enhancement of wheat growth as noted for the application of acidified biochar may, at times, be more effective than the effect of the soil amendment itself, which is remarked for application of non-acidified biochar.

Keywords: biochar; salt affected soil; wheat; proline; NPK uptake.

1. Introduction

Wheat is a strategic crop in many countries around the world (Dianatmanesh *et al*., 2022; Lalarukh *et al*., 2022a, b & c). In Egypt , it is a strategic commodity crop (Hussein *et al*., 2022; Abdalla *et al*., 2023; Abd El-Aty *et al*. 2024; Rashwan *et al.* 2024), which provides the population with 1/3 of their daily caloric intake (bread *baladi*) (Abdalla *et al*., 2023).

Soil salinity poses a threat to food production and security around the world (Iqbal *et al*., 2014; EL Sabagh *et al*., 2021; Parveen *et al*., 2024), especially in arid and semi-arid regions (Gorji *et al*., 2015). This problem expanses

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Received:01/10/2024; Accepted: 28/10/2024
DOI: 10.21608/EJSS.2024.325183.1869

over 100 countries and diminishes the quality of about one billion of hectares (Singh, 2022), which represents approximately 7% of the earth's land surface (Hopmans *et al.*, 2021). Expected yields in such area do not exceed 20% to 50% of the optimum yield (Shrivastava and Kumar, 2015). This is because of osmotic and ionic stresses in plants, e.g. lessen plant growth, decrease in water potential, increase in active oxygen species and imbalance in nutrients uptake (Ahmed *et al*., 2021). Thus, salt-stressed soils should be managed properly to achieve the Sustainable Development Goals (SDGs) of UN, specially the one named “zero huger” (Singh, 2021).

Egypt is one of the arid countries which suffer from soil salinity (Fahd *et al*., 2023). This salinity altimately causes farmland deterioration (Abdullahi *et al*., 2023) and harshly distresses the sustainability of food production thereon (Mohamed *et al*., 2023).

Biochar is an organic additive that may help to ameliorate the salinity stress in salt-affected soils (Saifullah *et al.*, 2018; Kul *et al.*, 2021). This product results from the pyrolysis of organic residues in absence of oxygen (Bassouny and Abbas, 2019; Elshony *et al.*, 2019; Abdelhafez *et al.*, 2021; Asaad *et al.*, 2022; Farid *et al.*, 2022; Tolba, 2021; Gyanwali et al., 2024; Mohamed *et al.*, 2024). It is then added to salt affected soils to increase their contents of stable water aggregates (Saifullah *et al.*, 2018) in order to allow better salt leaching (Huang et al., 2019b).

This organic product also enhances the cation exchange capacity (CEC) of the soil, while diminishes the levels of soluble salts (Ahmed *et al.* 2021). Furthermore, it elevates the exchangeable potassium (K^+) content in soil and improves the K^+/Na^+ ratio in plants, thereby decreasing electrolyte leakage (Kul et al., 2021) and increase plant tolerance to salinity stress (Wu et al., 2024). It is worth noting that plants exhibit an increase in an endogenous osmolyte as a self-defense mechanism under stress (Koc et al., 2024) while biochar application declined this osmolyte in shoots, which indicates that plants become less stressed (Shahzadi et al., 2024)

One of the main drawbacks of using biochar in arid soils is its alkaline nature (Abdelhafez *et al.*, 2014), which lessen the availability of many soil nutrients in soil and therefore plants grown on such soils suffer from nutrient deficiency (Khalil *et al.*, 2023). Alternatively, acid modified biochar can be used effectively (El-Sharkawy *et al.*, 2022). Although this additive exhibits higher surface area than normal biochar (El-Sharkawy *et al.*, 2022); yet its stability in soil could be lower than that of normal biochar.

The present investigation evaluates the effectiveness of both normal alkaline biochar and the acid-modified one as soil amendments, applied at rates of 5 and 10 g kg^{-1} of soil, in order to improve the characteristics of salt-affected soils and enhance growth of wheat cultivated thereon. To assess these impacts on the grown plants, a range of morphological (including plant dry weights, root-to-shoot ratio, and grain-to-shoot ratio), physiological (NPK uptake), and biochemical indicators (specifically proline content in shoots) have been measured in this study. Also, their impacts on some soil characteristics (soil organic matter, soil pH and EC) by the end of the growing season were also a matter of concern.

Generally, the current study anticipates that non-acidified biochar could effectively hinder soil EC thus increase wheat growth and productivity (hypothesis I). Acid modified biochar could; on the other hand, increase soil EC temporarily; nevertheless because of its relatively high degradation rate versus normal biochar, it enriches plants with nutrients needed for proper wheat growth and productivity (hypothesis II). Overall, the improvement in characteristics of salt affected soils under intensive cropping practices and application of acidified biochar may occasionally be more effective than the application of the soil amendment alone, which is remarked, in this study for application of non-acidified biochar (hypothesis III).

2. Materials and methods

2.1. Materials of study

Surface soil samples (0-30 cm) were collected from Agricultural Research Center, EL-Sabhya Station, Alexandria. These samples were mixed together to form a composite sample, then air dried, crushed and sieved to pass through a 2-mm sieve, and their chemical and physical characteristics were determined as outlined by Sparks *et al.* (2020) and Klute (1986), respectively, The obtained results are presented in Table 1.

Table 1. Soil chemical and physical properties.

Character	Soil pH	Soil EC (dS m^{-1})	Calcium carbonate content (g kg^{-1})	Soil organic matter (g kg^{-1})	Field capacity (%)	Particle size distribution (%)				
						Fine sand	Coarse sand	Silt	clay	Textural class
Value	7.88	5.94	147.1	17.2	32.50	13.79	26.50	23.66	36.05	Clay loam

*Soil pH was determined in soil:water suspension (1:2.5), soil EC was measured in soil paste extract

In this study, maize stover was collected Egypt. These residues from farms that are situated in Moshtoohr, Benha, were oven-dried at 65°C overnight, then pulverized and sieved. Afterwards, these residues were heated in a muffle furnace at 550°C for 2 h under limited aeration conditions (pyrolysis). The product *wa*s divided into two halves, the first half was used for preparation of acid modified biochar as mentioned by El-Sharkawy *et al*. (2022) via shaken with 0.1 M H_2SO_4 (1:100 w/v) for 4 h at 150 rpm then filtered and rinsed in tap water followed by double distilled water to remove chemicals. Thereafter, plant residues were oven dried at 70 °C. The other half was left non-acidified (normal biochar)

Table 2. Chemical characteristics of the produced biochars.

Character	pH	EC	C	N	H	O	S	Yield	Ash
		dS m^{-1}				(%)			
Normal biochar	8.57	1.28	78.02	0.52	0.75	5.82	0.33	41.56	14.56
Acid modified biochar	4.23	2.86	68.45	0.41	3.25	12.28	1.05	38.95	14.56

2.2. Methods of study.

Fifteen plastic pots of 25 cm diameter ×19 cm depth were uniformly packed with the investigated samples (equivalent to 4 kg), then germinated with 10 seeds of wheat (Giza 171) on the 25th November 2023. All pots were then fertilized with the recommended doses of N and P fertilizers, i.e. 75.6g urea (460 g kg) kg^{-1} soil (equivalent to 302 g N per pot) and also received 31.5 g calcium super phosphate(156 g P_2O_5 kg^{-1} equivalent to 126 g P_2O_5 per pot).

Pots were then distributed under the greenhouse conditions in a complete randomized design. Soil moisture was monitored periodically using a Tensiometer (model Theta-θ-Probe ML2x) and crops were irrigated with well water (EC=0.59 dS m^{-1}) to keep soil moisture always at field capacity. Chlorophyll content in wheat shoots (SPAD) were estimated after 50 days of cultivation using CM-402 Chlorophyll Meter. On 18th April, plant height, number of plants per pot and spikes per plant were determined. Whole plants were harvested at the physiological maturity stage and placed on sieves, washed with tap water to remove dusts followed by rinsing in distilled water twice.

2.3. Soil and Plant analyses.

Soil samples were collected from the rhizosphere of each pot to measure their contents of organic matter according to Walkley and Black method as outlined in Sparks *et al.* (2020), soil pH by pH meter (model Consory C3210) in soil:water suspension prepared at 1:2.5 and EC using EC meter (model WTW inolab Cond 720). Plants were divided into roots, shoots and grains then oven dried at 65 °C for 48 h. Afterwards, 0.5 g portions of the plant materials were wet digested using perchloric and sulfuric acids on a sandy hot plate at 250 °C according to Cottenie *et al*. (1982). In plant digest, nitrogen was determined by micro Kjeldahl apparatus, phosphorus was measured photometrically by JENWAY 6405 UV/VIS. following molybdate-ascorbic acid method and potassium was assessed by flame photometer (model JENWAY PFP7). Proline was determined in shoots after 50 days of cultivation according to Bates *et al.* (1973).

2.4. Data processing.

All chemicals, which were utilized in this research, were of analytical grade. The data were analyzed using one-way ANOVA and Dunnett’s test through SPSS statistical software version 18. Graphs were created using Sigma Plot version 10. Root-to-shoot ratio was calculated as the ratio of root dry masses to shoot dry masses (Ma *et al*., 2010; Fayed *et al*., 2018), whereas the grain-to-shoot ratio was computed as the ratio of grain dry masses to shoot masses.

3. Results

3.1 Chemical characteristics of the produced biochars

Data presented in Table 2 provides an intricate comparison of the properties of normal and acidified biochar, outlining various distinct parameters. The pH level of the acidified biochar was 4.23, while the non-acidified (normal) one was 8.57. Eectrical conductivity (EC) of the acidified biochar (2.86 dS m^{-1}) was notably higher than that of normal biochar (1.28 dS m^{-1}). Carbon content decreased from 78.02% in normal biochar to 68.45% in the acidified one, while nitrogen content decreased slightly from 0.52% to 0.41%. Remarkably, the acidified biochar contained a greater hydrogen content of 3.25%, compared to only 0.75% in normal biochar, and also exhibited an increase in oxygen content from 5.82% to 12.28%. Additionally, sulfur content was higher in acidified biochar, upgraded from 0.33% to 1.05%. On the other hand, the yield of acidified biochar is slightly lower (38.95%) than that of normal biochar (41.56%), and both contained equivalent ash contents of 14.56%.

3.2. Effects of applied biochars on soil chemical characteristics

Amending the investigated salt-affected soil with either acid-modified or normal biochars raised significantly organic C content in soil. Such increases were notable with increasing the application rate from 5 to 10 g kg^{-1}, with superiority for the non-acidified biochar versus the acidified one (Fig 1A).

No significant variations were found in soil pH owing to the application of any of the two types of biochar (Fig 1B). Regarding soil EC, application of non-acidified biochar raised significantly soil salinity only when being applied at the highest rate (10 g kg-1), while the application of the acid modified biochar decreased significantly this parameter. In this concern, there were no significant variations in soil EC with increasing the rate of applied acidified biochar from 5 to 10 g kg^{-1} (Fig 1C).

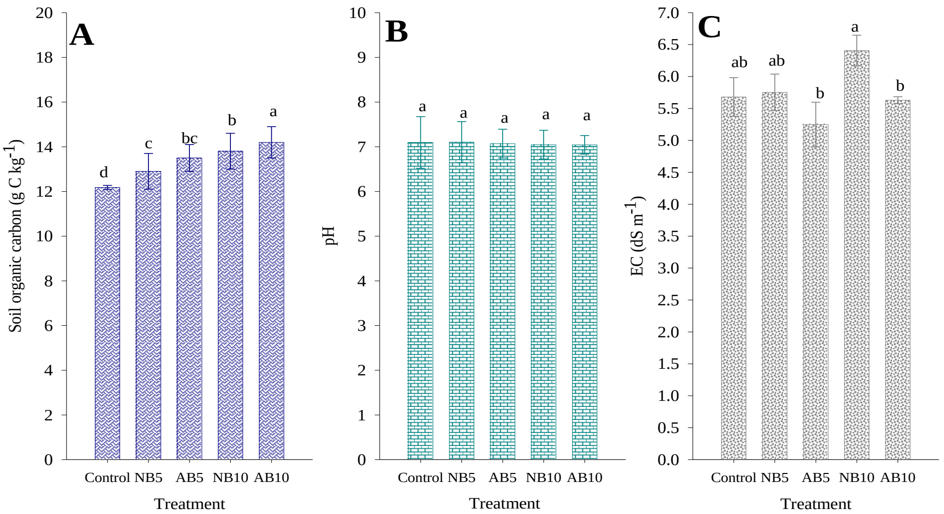

Fig. 1. Soil chemical characteristics (means ± standard deviations) as affected by application of acidified and non-acidified biochars.

Note: NB 5: non acidified biochar at a rate of 5 g kg^{-1}, AB 5: acidified biochar at a rate of 5 g kg^{-1}, NB 10: non acidified biochar at a rate of 10 g kg^{-1}, AB 10: acidified biochar at a rate of 10 g kg^{-1}. Similar Dunkan's letters indicate no significant variations among treatments

3.3. Effects of applied biochars on plant growth parameters

Application of either acidified or non-acidified biochar enhanced noteworthy the dry weights of wheat shoots and grains, as illustrated in Figure 2A. Such increases were more pronounced when acidified biochar was utilized, especially at its higher application rate. Additionally, both amendments promoted root development, but this significance was observed only when they were being applied at a concentration of 10 g kg^{-1}, where acidified biochar demonstrated a distinct advantage. Even so, the stressed plants continued to experience higher root-to-shoot ratios, with decline in grain to shoot ratio.

Incorporation of biochar into the soil decreased the ratio of root-to-shoot, while simultaneously augmented grain-to-shoot ratio. This effect was particularly pronounced when adding the acid-modified biochar at a rate of 10 g kg^{-1}. There was a slight decline in the grain-to-shoot ratio for plants that received the biochar at the higher rate of 10 g kg^{-1} compared to those amended with 5 g kg^{-1}; nevertheless these reductions were significant.

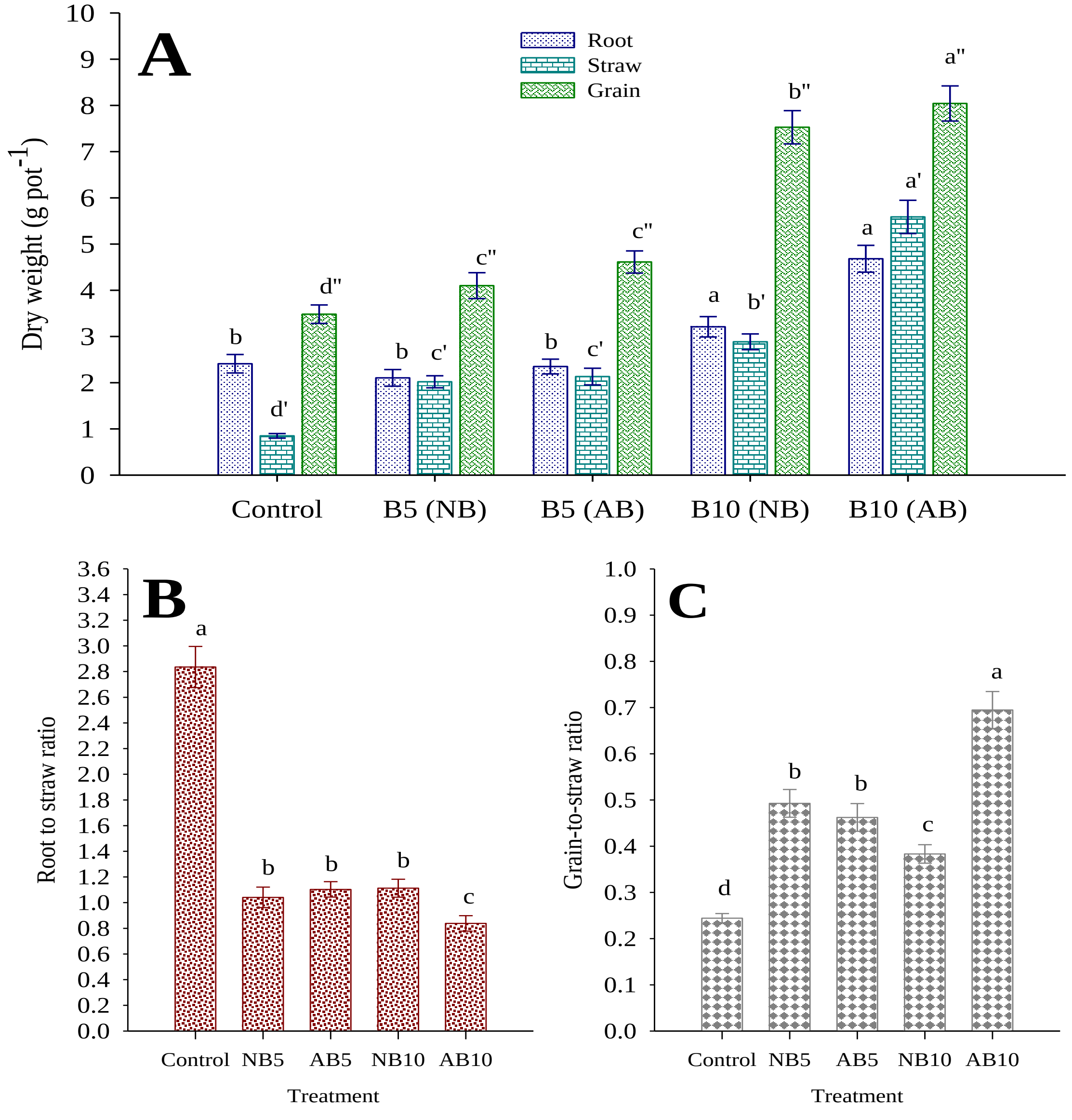

Fig. 2. Plant dry weights (A), root-to-shoot ratios (B) and grain-to-shoot ratios (C) (means ± standard deviations) as affected by application of acidified and non-acidified biochars. See footnote Fig 1.Similar Dunkan's letters indicate no significant variations among treatments

Likewise, number of plants per pot, number of spikes per individual plant, and the overall plant height showed a noteworthy enhancement when being cultivated on the soil enriched with biochar compared to the corresponding ones grown on the non-amended control soil (Fig 3). The highest increases were observed in the soil treated with

10 g kg^{-1} as acidified biochar. On the other hand, no significant variations were remarked for application of the two types of biochars on chlorophyll content within plant shoots.

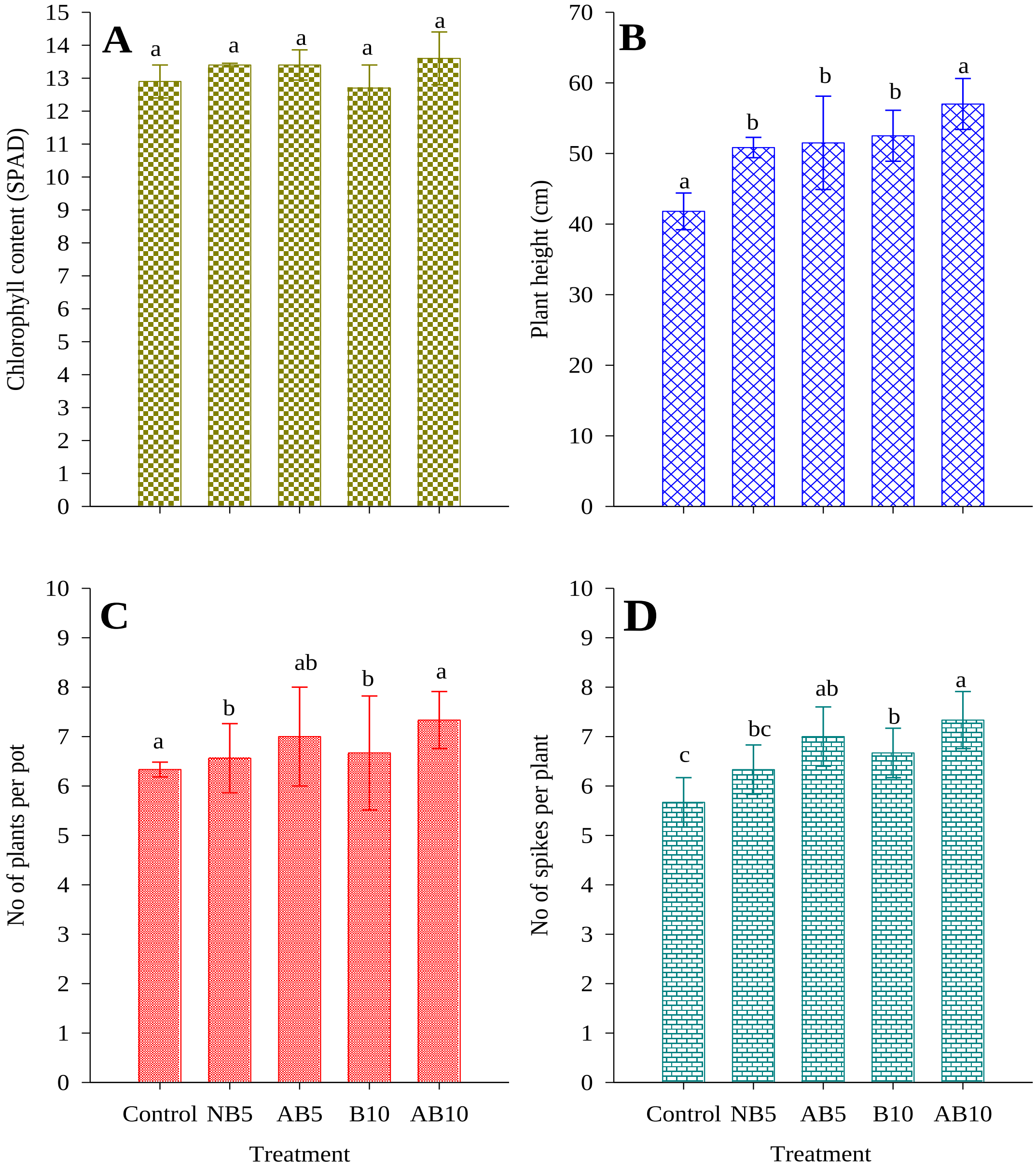

Fig. 3. Plant growth parameters (means ± standard deviations) as affected by application of acidified and non-acidified biochars. See footnote Fig 1. Similar Dunkan's letters indicate no significant variations among treatments.

3.4. Effects of applied biochars on nutrients availability and uptake by plants

The obtained results revealed that there were no notable differences in nitrogen (N) concentrations within the different parts of wheat plant, i.e. roots, shoots, and grains, regardless of the rate of biochar application (Fig 4). Concerning phosphorus (P) concentrations in wheat parts, the most significant increases were found in roots of the plants treated with non-acidified biochar at a dosage of 10 g kg^{-1}. The highest increases in P within the aboveground tissues were for acidified biochar applied at a rate of 10 g kg^{-1}. Similarly, this particular treatment recorded the highest increases in K content within wheat grains.

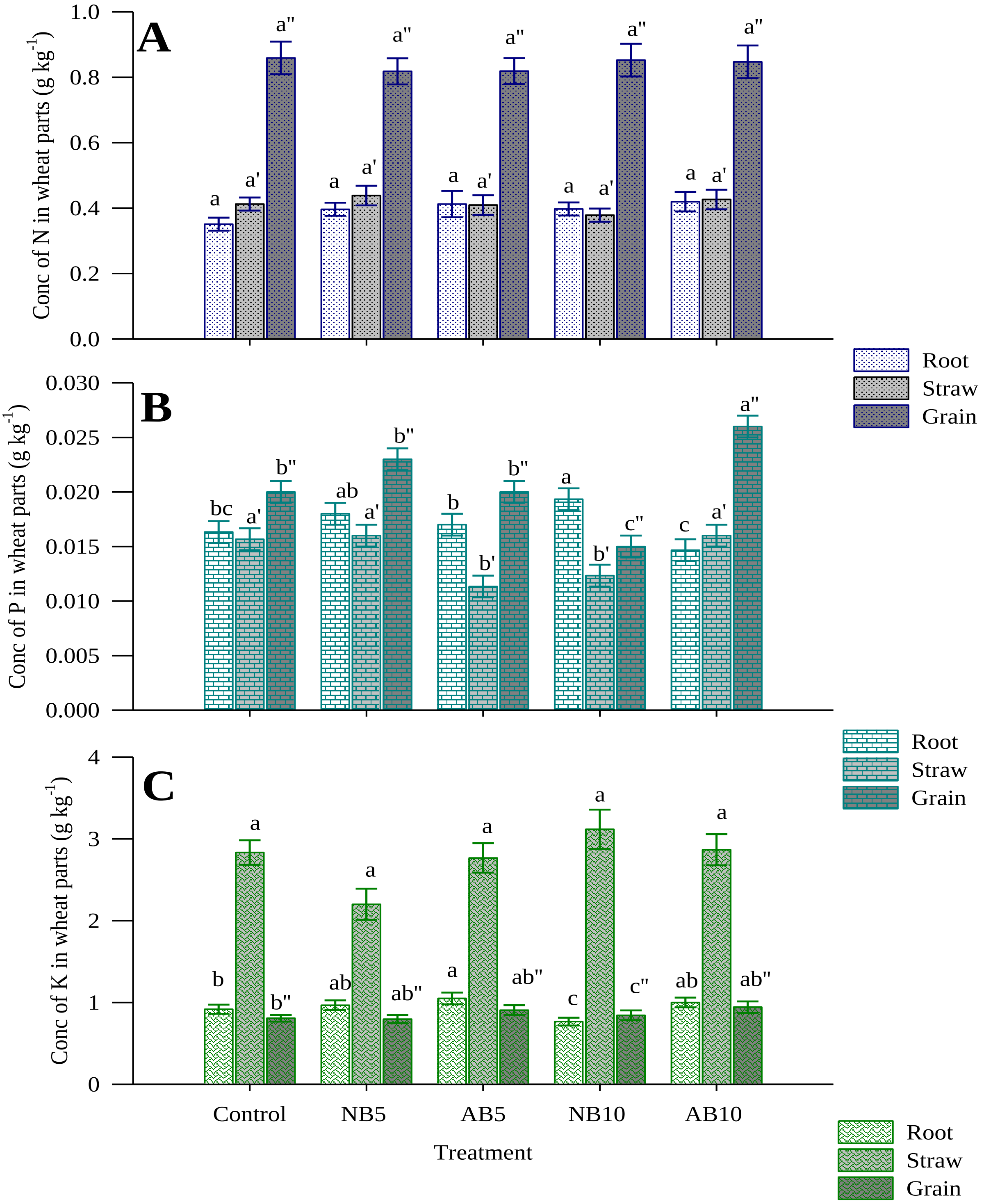

Fig. 4. Concentrations of NPK (means ± standard deviations) within different wheat parts as affected by application of acidified and non-acidified biochars. See footnote Fig. 1. Similar Dunkan's letters indicate no significant variations among treatments.

Regarding NPK uptake by wheat plants (multiplication of nutrient concentrations by dry matter yield), it was observed that these essential nutrients increased significantly within the various parts of plant as a result of biochar application. Notably, such increases became more pronounced with higher doses of biochar application. Specifically, the most considerable increases were noted for the acidified biochar versus to the non-acidified one.

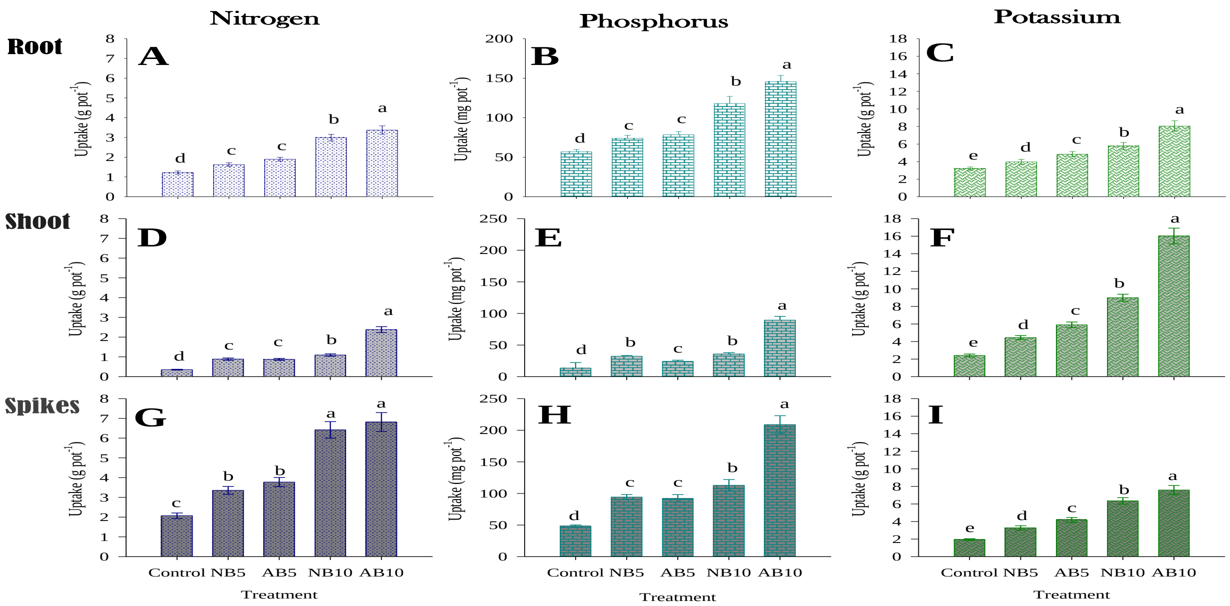

Fig. 5. Uptake of NPK (means ± standard deviations) by different wheat parts as affected by application of acidified and non-acidified biochars. See footnote Fig 1. Similar Dunkan's letters indicate no significant variations among treatments.

3.5. Proline as an indicator of salinity stress imposed on wheat plants

Results presented in Fig 6 reveal that proline content decreased significantly in shoots of wheat plants grown on a soil amended with any of the two types of biochars. The highest reductions occurred in plants that received acidified biochar, which were 33.4% in plants amended with 5 g AB kg^{-1} (AB 5), and 36.6% when plants received 10 g AB kg^{-1} (AB 10). It seems that increasing the application rate of both types of biochar from 5 to 10 mg g^{-1} did not affect significantly this stress indicator.

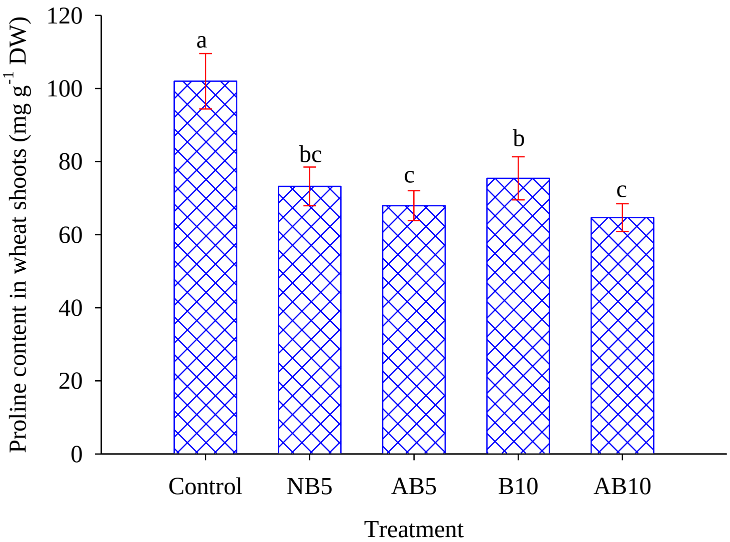

Fig. 6. Proline content (means ± standard deviations) in wheat shoots as affected by application of acidified and non-acidified biochars. See footnote Fig 1. Similar Dunkan's letters indicate no significant variations among treatments.

3.6 Root, straw and grain yields of wheat plants affected by proline content in shoots and the total NPK uptake by plants

Significant positive correlations were detected among the dry weights of wheat roots, straw and grain yields. These dry weights were, at the same time, significantly correlated with their uptake of NPK nutrients. Likewise, significant correlations existed among the uptake values of these three nutrients by plants. Shoot and grain biomasses of wheat (but not root dry weights) were correlated significantly and negatively with the proline content in shoots. It seems that this proline content did not only affect grain-to-shoot ratio but also raised root-to-shoot ratio of the grown plants

Table 3. Correlations between growth parameters and productivity of wheat plants grown on a salt affected soil as affected by proline content in straw and their total NPK uptake by plant.

	Root dry weight	Straw dry weight	Grain dry weight	Root/shoot ratio	Grain/shoot ratio	Proline content	N-uptake	P-uptake	K-uptake
Root dry weight		0.931**	0.885**	-0.363	0.696**	-0.388	0.892**	0.939**	0.943**
Straw dry weight			0.874**	-0.657**	0.889**	-0.673**	0.933**	0.998**	0.981**
Grain dry weight				-0.578*	0.619*	-0.536*	0.985**	0.891**	0.944**
Root/shoot ratio					-0.765**	0.957**	-0.680**	-0.644**	-0.644**
Grain/shoot ratio						-0.793**	0.738**	0.876**	0.810**
Proline content							-0.649**	-0.649**	-0.652**
N-uptake								0.944**	0.977**
P-uptake									0.983**
K-uptake									

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

4. Discussion

4.1. Wheat growth and productivity under salinity conditions

The least growth parameters and productivity of wheat were noticed in the control plants subjected to salinity stress. Their root biomasses increased relative to their shoots to exhibit the highest root-to-shoot ratio. Probably, such increases in root biomasses were to access new areas that contained non-saline water (Bazihizina *et al.*, 2012); yet these biomasses were overall less than the corresponding ones of non-stressed plants (Zeeshan *et al.*, 2020).

The vegetative growth of stressed plants also decreased in response to soil salinity (Khataar *et al.*, 2018) as the majority of carbon products in plants and energy acquired by photosynthesis were re-used in cell maintenance (Munns and Gilliham, 2015). Besides, a drop occurred in C-compounds and energy which are essential for floral development and grain filling (Munns and Gilliham, 2015). Thus, the grain-to-shoot ratio diminished, whereas the root-to-shoot ratio increased. Anyhow, these reductions in wheat shoots may help to minimize water transpiration under such stressful conditions (Harris *et al.*, 2010). Under these stress conditions, further declines in wheat growth parameters were evident. For instance, number of plants per pot decreased, which became stunted and of shorter spike length.

Application of biochar alleviated salinity stress on plants as denoted by the significant reductions that occurred in proline content within shoots. This low-molecular solute increase salinity tolerance in plants (Iqbal *et al.*, 2014) via preserving osmotic adjustment which keeps membranes from damage (Shafi *et al.*, 2019). Thus, application of biochar enhanced root, shoot and grain yields. Similar results were reported by Akhtar *et al.* (2015), Kanwal *et al.* (2018) and Huang *et al.* (2019a). In this concern, the highest increase in grain-to-shoot was calculated for plants that received the higher doses of acid modified biochar (AB 10). Also these plants demonstrated the least root-to-shoot ratio. A point to note is that the acidified biochar decreased the stress marker named proline to concentrations beyond those attained for normal biochar. Despite the salinity stress, chlorophyll content did not vary significantly among plant shoots, irrespective of the type and rate of applied biochar.

4.2. NPK concentrations and uptake by wheat plants under salinity conditions

Nirogen content did not vary significantly within the studied plant parts (roots, traw and grains) owing to the applied biochar treatments. This nutrient is thought to be incorporated in biosynthesis of amino acids and proteins (Krapp, 2015), which increased plant growth and development (Kraiser *et al.*, 2011) rather than being accumulated in high concentrations within plant tissues. Unlike N, P influx needs metabolic energy and high-affinity transporters (Smith *et al*., 2011). This nutrient (P) accumulated in higher concentrations in plants that were amended with biochar, as P is needed for root and shoot development (Betencourt *et al.*, 2012) as well as grain productivity (Fischer, 2011).

Concentration of potassium (K) also increased in various sections of plants treated with biochar. This phenomenon probably decreased sodium-to-potassium (Na/K) ratio, which in turn resulted in a notable improvement in both growth and yield (Ahanger and Agarwal, 2017). It is important to highlight that the highest concentration of potassium was observed in shoots of plants, corresponding to other plant parts, suggesting its role in osmoregulation and enhancement of antioxidative capabilities to mitigate reactive oxygen species (ROS) (Sehar *et al*., 2021). Overall, uptake of nitrogen, phosphorus, and potassium (NPK) were higher in wheat plants supplemented with biochar, particularly the ones that received the higher application rate of 10 g kg^{-1} of acidified biochar.

4.3. Effect of applied biochars on soil chemical characteristics

Application of biochars (acid-modified and normal ones) raised significantly soil organic carbon in soil. This is because of their high stability in soil (Gross et al., 2021) which may last for more than 11 years in the top soil (Gross et al., 2021). Besides, biochar augmented the humification of the dissolved organic matter in soil (Feng *et al.*, 2021). In particular, acid modified biochar is more easily biodegradable in soil than the normal one (Khalil *et al.*, 2023); thus its residual content by the end of the growing season could be relatively lower as found herein. Acid modification of biochar also raised its CEC while decreased its pH (Murtaza et al., 2022), and this (high CEC) in turn immobilized partially soluble salts in soil; thus decreased soil EC (El-Sharkawy *et al.*, 2022). Generally, the investigated soil is characterized by its high buffering capacity because of its relatively high content of clay (36.05%), so no significant changes in soil pH were noticed owing to the application of the two types of biochar.

Overall, the above results confirm the feasibility of the two types of biochar as soil additives to ameliorate a salt affected soil and therefore, the 1st and 2nd hypotheses become valid. Because the decline in soil EC and the increases that took place in wheat growth parameters were more noticeable in the soil amended with acid modified biochar versus the non-acidified one; thus the third assumption was also supported. This assumption indicates that "amelioration of saline soils under high cropping (using acidified biochar) could sometimes be more functioning than the action of the soil amendment itself(using non-acidified biochar)".

5. Conclusion and Future Prospective

Application of biochar decreased the stress marker named proline within wheat shoots. Moreover, its application enhanced plant growth and productivity, i.e. root, shoot and grain dry weights. In addition, application of biochar boosted other plant growth parameters, i.e. grain to shoot ratio; number of plants per pot, number of spikes per plant and plant height. The highest increases in wheat growth parameters and productivity were attained for the acid modified biochar treatment when being added at a rate of 10 g kg^{-1}. Such increases were probably the consequences of increasing NPK uptake by wheat. In particular, acid modified biochar was more easily biodegradable and released more nutrients than normal biochar. This probably raised initially soil EC, while lessened residual organic carbon by the end of the growing season versus normal biochar. On the other hand, its high CEC retained many salts in soil (Ahmed *et al.*, 2021); accordingly soil EC re-decline by the end of the growing season to values lower than that of the control.

Future perspectives are needed to find out the feasibility of using these additives for alleviating salt stress in different crops under field conditions on the long run.

Declarations

Ethics approval and consent to participate

Consent for publication: The article contains no such material that may be unlawful, defamatory, or which would, if published, in any way whatsoever, violate the terms and conditions as laid down in the agreement.

Availability of data and material: Not applicable.

Competing interests: The authors declare that they have no conflict of interest in the publication.

Funding: Not applicable.

Authors' contributions: Authors contributed equally in writing the original draft, editing and finalizing the manuscript. They read and agreed for submission of manuscript to the journal.

Acknowledgments:

The authors would like to thank Prof Dr Hassan H. Abbas and Prof Dr Ihab M. Farid (Soils and Water Department) as well as Prof Dr Ahmed AA El Hosary (head of Agronomy Department), Faculty of Agriculture, Benha University, Egypt for their help during conducting this study.

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