



Mitigating the Harmful Effect of Salinity on Maize Plants Using Fish Waste-Derived Biochar

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was conducted to determine if applying biochar made from fish waste to the soil can alleviate the adverse impacts of salinity stress on maize (*Zea mays* L.) seedling growth.

Materials and Methods: Maize plants were cultivated in two groups of pots; the first group had the soil without any additions, and the second group had the soil mixed with biochar (1% w/w). Each group was irrigated with saline water (0, 50 and 150 mM NaCl).

Results: According to the findings, *Zea mays* exposed to salt stress showed a significant decrease in growth traits such as shoot and root length, fresh weight, and dry weight of shoot and root, compared to untreated control. The addition of biochar significantly enhanced these attributes. As salinity levels increased, the value of photosynthetic pigments gradually declined. Applying biochar to the soil significantly increased the amounts of Chl a, Chl b, and carotenoid. Salt-stressed seedlings treated with biochar have lower levels of soluble sugars, soluble proteins, and total free amino acids at 150 mM NaCl + FWB of the shoot. The findings demonstrate that applying biochar

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to salt-stressed seedlings caused their proline content to increase noticeably at the highest salinity level (150 mM NaCl). The contents of Na^+ and Cl^- were positively affected by increasing salt stress. Increasing salt stress had a deleterious impact on K^+ , Ca^{2+} , and Mg^{2+} levels. On the other hand, applying FWB raised the content of K^+ , Ca^{2+} , and Mg^{2+} while decreasing the amounts of Na^+ and Cl^- .

Conclusion: Biochar made from fish waste has the potential to reduce salinity stress significantly.

Keywords: Saline water; biochar; photosynthesis; plant growth; maize seedlings.

1. INTRODUCTION

Egypt, like many other developing countries, has arid and semi-arid regions facing a significant problem: insufficient freshwater resources to irrigate different crops [1]. Lower-quality water, such as saline water, is widely used to overcome this shortage. However, employing saline water disrupts the region's ecological balance and the physicochemical characteristics of the soil. [2]. The physical qualities of the soil, such as soil aggregate stability, porosity, permeability, and infiltration, are also affected by an excess of Na^+ in the soil because it induces the dispersion of soil colloids. [3]. Salinity affects not only the qualities of the soil but also the ecological balance of a region, lowering crop productivity and yields. The plant faces osmotic and ionic stress as a result of increasing salinity conditions. Osmotic stress affects plants when there is an increase in salt accumulation in the soil solution in the root zone, which inhibits the roots' ability to absorb water. Root and shoot growth deteriorates as a result of decreased root water intake. Ionic stress occurs when ion accumulation in "plant" tissues exceeds the threshold levels at which the ions induce harmful effects. Ionic stress causes a reduction in cellular metabolic processes, including photosynthetic activity, chlorosis, necrosis, and early leaf senescence [4]. "About 30 agricultural plants currently provide 90% of the plant-based food consumed by humans. The bulk of these crops, glycophytes, are neither salt tolerant nor salt sensitive" [5]. The difference between maize output and consumption in Egypt is thought to be around 45%. Egypt imports 45% of the maize, consuming and producing 55%, making the total consumption equal 10,265,817 tons [6]. "It is necessary to boost maize plant yields and mitigate salinity's negative effects on the development of maize plants. Studies have been done on several approaches to decrease the negative effects of salt stress on plants, including the use of various irrigation systems, scraping, flushing, and leaching to drain the additional salt from the plant's root zone and improving the

tolerance of plants to salt, use of various irrigation methods and enhancement of plants' salt tolerance" [7,8]. However, these methods may not effectively resolve the salinization issue because they are expensive and labor requirements. So, it is crucial to look for an efficient strategy to reduce salinity's harmful effects on crop yield, which includes using organic amendments. Compost, animal manure, poultry litter, crop residues, and biochar are some examples of different organic materials that have frequently been investigated for their potential to reduce the effect of salinity on crop growth [9].

"Biochar is produced by pyrolysis (thermal degradation) of biomass in the absence or limited presence of oxygen" [10]. "The conditions of the pyrolysis process and the kind of raw biomass materials determine biochar's physical and chemical characteristics" [11]. "Common biomass sources include agricultural waste, bioenergy crops, forest residues, food waste, sewage sludge, and animal waste" [12]. "The biomass is transformed into a mostly stable and recalcitrant organic carbon (C) compound when heated to high temperatures, between 300°C and 1000°C. Biochar enhances the physical, chemical, and biological characteristics of salt-affected soils" [13]. "According to reports, adding biochar to saline soils increases their capacity to hold water and lowers oxidative and osmotic stresses" [14]. "Additionally, biochar amendment encourages plant development by raising quantities of organic carbon and nutrients, cation exchange capacity, and hydraulic conductivity" [15,13].

This study aimed to comprehend how fish waste-derived biochar could mitigate the negative impacts of salt stress on corn crops.

2. MATERIALS AND METHODS

2.1 Preparation and Characterization of Fish Waste-Derived Biochar (FWB)

The fish waste, like fish heads, tails, and bones, was gathered from fish restaurants in Qena,

Egypt, and air-dried before being crushed. The dried wastes were crushed into less than two mm-sized particles after being pyrolyzed at 300°C for 4 hours in a muffle furnace. Organic matter was determined in the FWB samples by loss on ignition—the method proposed by Sparks et al. [16]. FWB pH was determined in a 1:2.5 ratio (biochar: water) suspension using a pH meter. According to the procedure described in Burt [17], salinity was measured using an electrical conductivity meter in a 1:2.5 ratio (biochar: water) extract. A mixture of H₂SO₄ and H₂O₂ digested a sample of biochar (2gm) based on the technique of Parkinson and Allen [18]. Then nitrogen, potassium, magnesium, and calcium were determined in the digested sample. The primary chemical attributes of the biochar made from fish waste are displayed in Table 1.

2.2 Experimental Setup and Treatments

Present work was carried out in the experimental farm of Botany Department, Faculty of Science, South Valley University, Qena, Egypt, under field conditions, during the growing season 2023. Two groups of pots were prepared; each group was represented by nine plastic pots (25 cm diameter and 30 cm depth). Each one contained 3 kg of dried soil, the first group had the soil without any additions. The second group had the soil mixed with biochar (1% w/w). Thereafter, pots in each group were sub-divided in to three divisions which represent the salt concentrations, i.e., 0, 50, and 150 mM NaCl, three replicates were prepared for each treatment. Maize seeds (*zea mays* white hybrid), supplied by the Agronomy Department, Faculty of Agriculture, South Valley University. were sterilized in 5% sodium hypochlorite (NaOCl) solution for 5 min to avert contamination. An equal number of seeds (15 seeds/pot) of maize were sown in pots. Seven days after germination, seedlings were thinned to eight per pot. Then, treatment of plants with saline solutions began, the pots were irrigated with a constant amount of different salt concentrations, i.e., 0, 50 and 150 mM NaCl for the two groups, saline solution was added to the soil in such a way that, the soil solution acquires the assigned salinization level at field capacity for 30 days (seedling stage). The seedlings were separated into shoots and roots for carrying out the measurements of growth parameters (shoot length, root length, fresh and dry weights of shoot and root).

2.3 Growth Measurement

The shoot and root lengths were determined using a manual scale. After recording their fresh weights, the shoot and root were oven-dried to constant weight in an aerated oven at 80 °C for their dry weight.

2.4 Determination of Photosynthetic Pigments

The photosynthetic pigments were extracted from a known fresh weight (0.1 g) of leaves in a definite volume of 85% aqueous acetone, then (chlorophyll a, chlorophyll b, and carotenoids) were determined using spectrophotometric method recommended by Metzner et al. [19]. The absorbance of the extract was measured at the wavelengths of 663, 644 and 452.5 nm, against a blank of pure 85% acetone.

2.5 Determination of Osmolytes

The amount of soluble sugar in dry shoots and roots was measured by the enthrone sulphuric acid method, as described by Badour [20].

“Water-soluble protein was determined according to the method of Bradford” [21]. “Total free amino acid contents were measured by Lee and Takahashi” [22]. Free proline content was determined according to Bates et al. [23].

2.6 Determination of Mineral Ions

Estimation of Na⁺ and K⁺ was done by using a flame photometer [24]. “The versene (disodium dihydrogen ethylene-diamine-tetraacetic acid) titration method was employed for calcium and magnesium determinations, as described by Bower and Hatcher” [25]. Chlorides were determined by silver nitrate (AgNO₃) titration method as described by Cotlove [26].

Effect of salt stress and application of fish waste derived biochar (FWB) on shoot length (cm plant⁻¹), root length (cm plant⁻¹), fresh weight (F.W.) of shoot (g plant⁻¹), fresh weight (F.W.) of root (g plant⁻¹), dry weight (D.W.) of shoot (g plant⁻¹) and dry weight (D.W.) of root (g plant⁻¹) of 5- week old corn plants (*Zea mays*). Data presented are means ± S.D. (n=3). Data followed by similar letters are not significantly different by Duncan's multiple range test at P ≤ 0.05.

Table 1. Chemical characterization of fish waste-derived biochar

N mg/gm	K mg/gm	Ca mg/gm	Mg mg/gm	O.C. (%)	O.M. (%)	pH 1:2.5	EC 1:2.5 (dS m⁻¹)
1.731	4.05	4.8	3.6	38.3	65	6.37	0.818

O.C. (organic carbon), O.M. (organic matter)

pH and EC (electrical conductivity) were determined in 1:2.5 biochar:water suspension and its extract, respectively

3. RESULTS

The chemical analysis of biochar made from fish waste is shown in Table 1, indicating that organic matter and carbon content were generally high, as well as the potassium and nitrogen content.

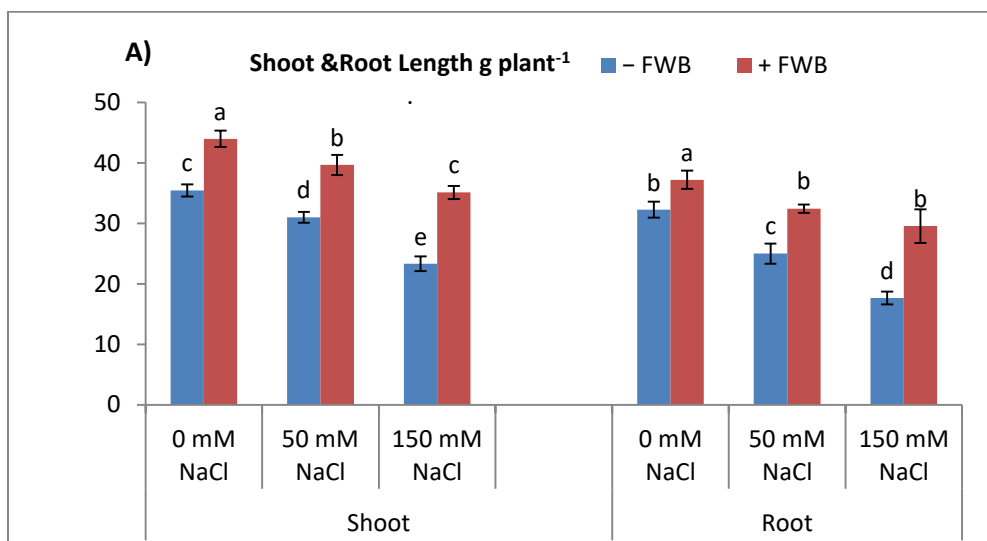
3.1 Growth Criteria

According to the findings in Figs. 1(A), (B), and (C), *Zea mays* exposed to salt stress experienced a significant decline in growth characteristics such as shoot and root length, fresh weight (F.W.), and dry weight (D.W.) of shoot and root. Relative to untreated control, 150 mM NaCl decreases the length of shoot and root by 34.17% and 45.25%, respectively, as well as the shoot and root's F.W. by (24.9% and 29.64%) and D.W. by (36.52% and 47.62%) respectively. However, adding biochar considerably improved these attributes and lessened the adverse effects of salt stress. In non-stressed plants, by application of FWB The percentage rise in length, F.W., and D.W. by 24.14%, 26%, and 34.78%, respectively, in shoot and 15.36%, 27.71%, and 57.14%, in root as compared to untreated control (Fig. 1). In treatment 150 mM NaCl + FWB, the percent

increase in length, F.W. and D.W. of shoot was 33.23%, 18.73% and 16.09%, respectively and 36.58 %, 24.34% and 11.91%, respectively in root, over the plants that were exposed to 150 mM NaCl alone (Fig. 1).

3.2 Photosynthetic Pigments

The values of photosynthetic pigments gradually decreased in contrast to the control as salinity levels increased (Fig. 2). The highest decrease in chlorophyll a (74.48%), chlorophyll b (80%), or carotenoids (52.56%) was detected at 150 mM NaCl. However, adding biochar to the soil reduced the negative effects of salinity on photosynthetic pigments compared to stressed plants. Chlorophyll pigments (Chl a and Chl b) and carotenoid concentrations significantly increased due to the application of biochar, i.e. 50 mM NaCl + FWB and 150 mM NaCl + FWB significantly enhanced Chl a (70.88% and 96.64%), Chl b (86.48% and 95.88%) and carotenoids (68.88% and 82.66%), respectively when compared to salinized plants alone (Fig. 2). However, the application of biochar alone the percentage increase to 69.33%, 60% and 77.55%, respectively for Chl a, Chl b and carotenoids over the untreated control (Fig. 2).



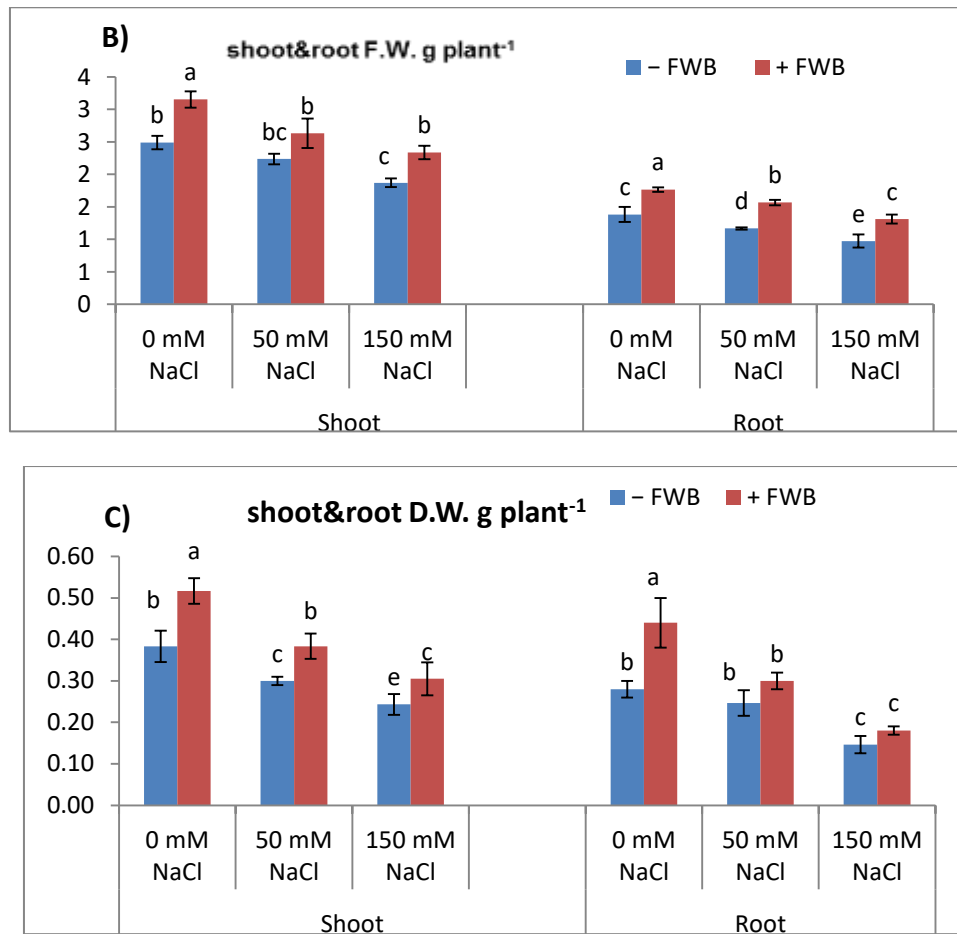


Fig. 1. Effect of salt stress and application of fish waste-derived biochar (FWB) on shoot and root length (cm plant⁻¹) (A), fresh weight (F.W.) of shoot and root (g plant⁻¹) (B), dry weight (D.W.) of shoot and root (g plant⁻¹) (C) of 5- week old maize plants
 Data presented are means \pm S.D. (n=3). Data followed by similar letters are not significantly different by Duncan's multiple range test at $P \leq 0.05$

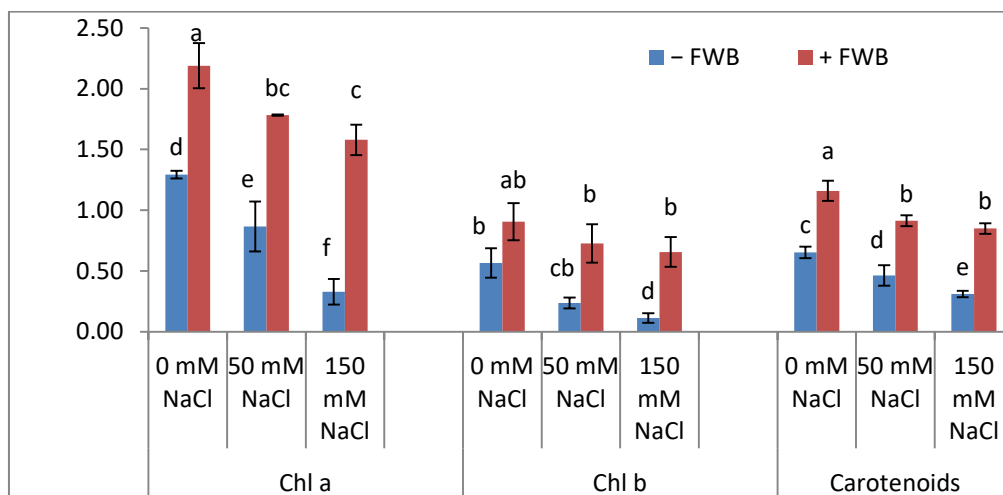
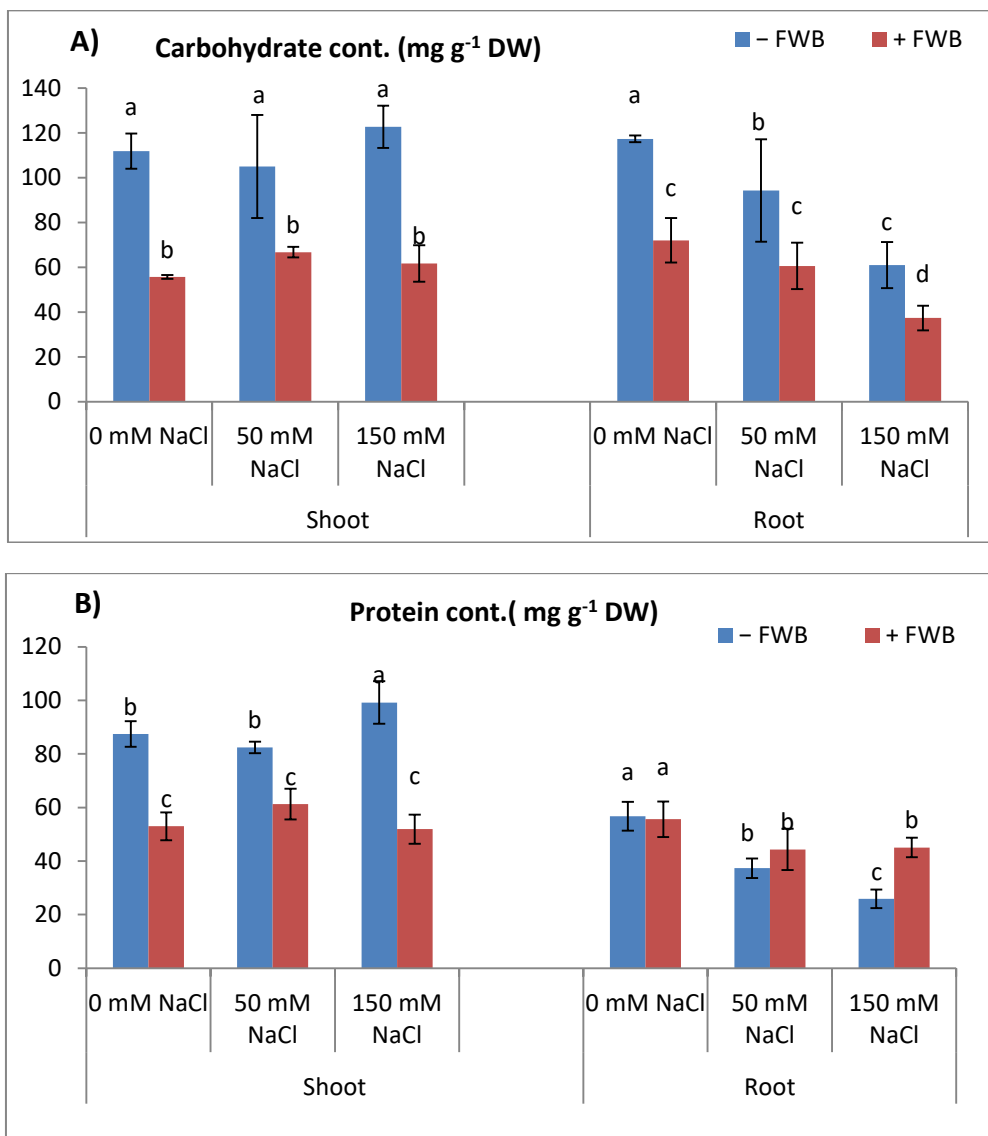


Fig. 2. Effect of salt stress and application of fish waste-derived biochar (FWB) on photosynthetic pigments content of maize leaves
 Data presented are the means \pm S.D. (n=3). Data followed by similar letters are not significantly different by Duncan's multiple range test at $P \leq 0.05$

3.3 Total Soluble Carbohydrate, Soluble Protein, and Total Free Amino Acids Content

The amount of soluble sugars, soluble proteins, and total free amino acids significantly increased when *Zea mays* were exposed to salt stress. The concentration of 150 mM NaCl recorded the highest increase in soluble sugars (9.66%), soluble proteins (13.44%), and total free amino acids (33.76%) in the shoot, as compared to the corresponding values of the untreated control (Fig. 3. (A), (B) and (C)). On the other hand, the root of salt-stressed seedlings showed the opposite pattern, with a notable decrease in soluble sugars, soluble proteins, and total free amino acids contents by 48.08%, 54.39%, and

24.41%, respectively, in comparison to matching controls. When biochar was used as a sole treatment, the negative effects of salinity stress were significantly mitigated, leading to a notable decrease in soluble sugars, soluble proteins, and total free amino acids contents of salt-stressed seedlings at 150 mM NaCl + FWB by 54.49%, 54.08%, and 52.86% of the shoot, respectively, compared to corresponding controls. In comparison, the total soluble sugar in the root was significantly reduced when biochar was applied by (20.1%). In contrast, dramatically accumulated soluble proteins and total free amino acids contents with the highest accumulation at 150 mM NaCl (33.82% and 25.59%), respectively, compared to the corresponding control, have occurred (Fig. 3).



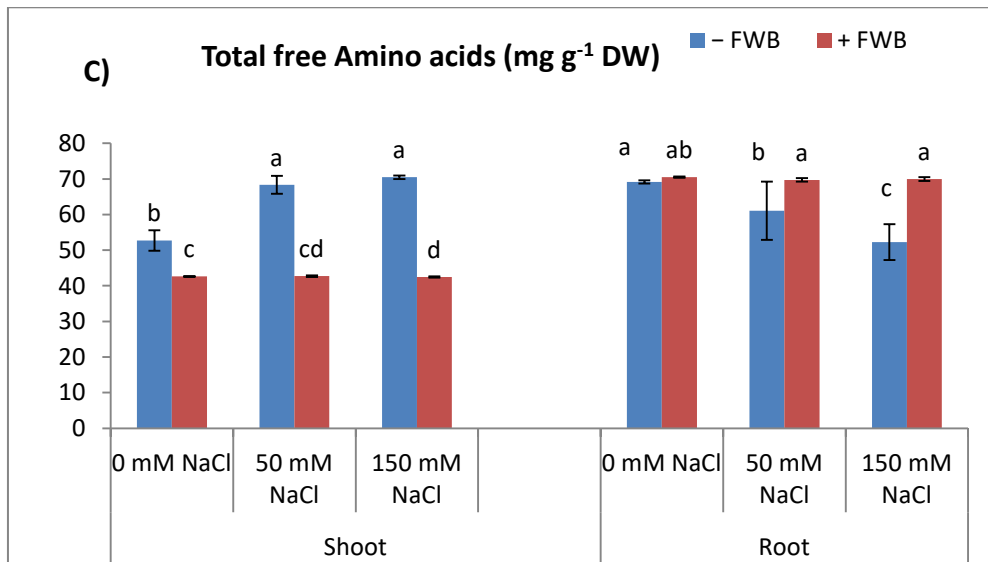


Fig. 3. Effect of salt stress and application of fish waste-derived biochar (FWB) on the content of soluble sugars (A), soluble proteins (B), and total free amino acids (mg g⁻¹ D.W.) (C) of maize shoots and roots

Data presented are the means \pm S.D. (n=3). Data followed by similar letters are not significantly different by Duncan's multiple range test at $P \leq 0.05$

3.4 Proline Content

The Proline content of the shoot and root of 5-week-old *Zea mays L.* seedlings is affected by salinity stress, as shown in Fig. 4. According to the findings, salt stress at (150 mM NaCl) led to a slight reduction in both shoot and root proline by 12.14 % and 2.8%, respectively, in comparison to controls. However, the application of biochar as the only treatment revealed a substantial increase in the amount of proline at the maximum salinity level (150 mM NaCl) of salt-stressed seedlings by 129.04% and 9.16% of the shoot and root, respectively, compared with matching controls.

3.5 Mineral Ions

The data in Tables 2 and 3 indicated that Salinity stress significantly increased the contents of Na⁺ and Cl⁻ by increasing salt stress. Reversibly, as salt stress increased, the K⁺, Ca²⁺, and Mg²⁺ levels decreased until they reached their lowest values at the maximum salt concentration (150 mM NaCl). However, compared to the matching -FWB untreated plants, the application of FWB decreased Na⁺ and Cl⁻ levels and raised the content of K⁺, Ca²⁺, and Mg²⁺.

4. DISCUSSION

The findings of the present study showed that all assessed growth parameters, including plant

length, fresh and dry weights, reduced under salinity stress in maize seedlings, which is in agreement with those of Kaya et al. [27] on maize seedlings. Limiting water intake under stress circumstances during germination and emergence is the cause of the reduction in seedling length under salinity stress [28]. Additionally, ion cytotoxicity and osmotic stress, which result in nutritional shortages and metabolic imbalance, maybe the source of the negative effects on plant growth. [29]. The addition of biochar to the soil in the current study results in improvement of all growth parameters of maize seedlings. This improvement in plant growth can be due to biochar's high sorption capacity, which reduces the detrimental effects of salinity on plant growth [30]. Additionally, increased plant absorption of nutritional elements resulted from the availability of nutrients in soil caused by adding biochar Abiven et al. [31] or increasing water field capacity for plant development [32].

According to the findings, salinity decreased the amount of pigments needed in photosynthetic processes. These results align with those of Kaya et al. [27] on maize. "The photochemical efficiency of PSII (Fv/ Fm) can be used as a criterion for evaluating plant performance under stressful conditions" [33]. "Reduced water content due to osmotic stress, altered mineral uptake, and down-regulation of the activities of

vital photosynthetic enzymes like δ -aminolevulinic acid dehydratase and protochlorophyllide reductase all contribute to reduced pigment synthesis” [34]. The increase in chlorophyllase, which turns chlorophyll into chlorophyllide and phytol, damages the photosynthetic apparatus by inhibiting pigment-protein complexes (proteins of PSII) made in the chloroplasts, down-regulating CO₂ fixation and stomatal closure, may also be responsible for the decrease in Chl a and Chl b levels [35]. Osmotic stress, leading to damaging chloroplast layers and increasing membrane penetrability or loss of membrane uprightness, is another reason the amount of chlorophyll may be declining [36].

Our findings demonstrated that adding biochar to salt-stressed environments significantly boosted the chlorophyll concentration. These results are consistent with the research by Kanwal et al. [37] and Akhtar, Andersen, and Liu [38]. Similarly, according to a study by Karabay et al. [39], biochar additions (5 t ha⁻¹ and 15 t ha⁻¹) considerably mitigated the negative effects of saline irrigation on the total leaf chlorophyll and carotenoid content of beans. “With the addition of biochar, it was discovered that the increase in photosynthetic rate and efficiency was caused by a larger buildup of the intermediates and metabolites needed for chlorophyll production under salinity stress” [40].

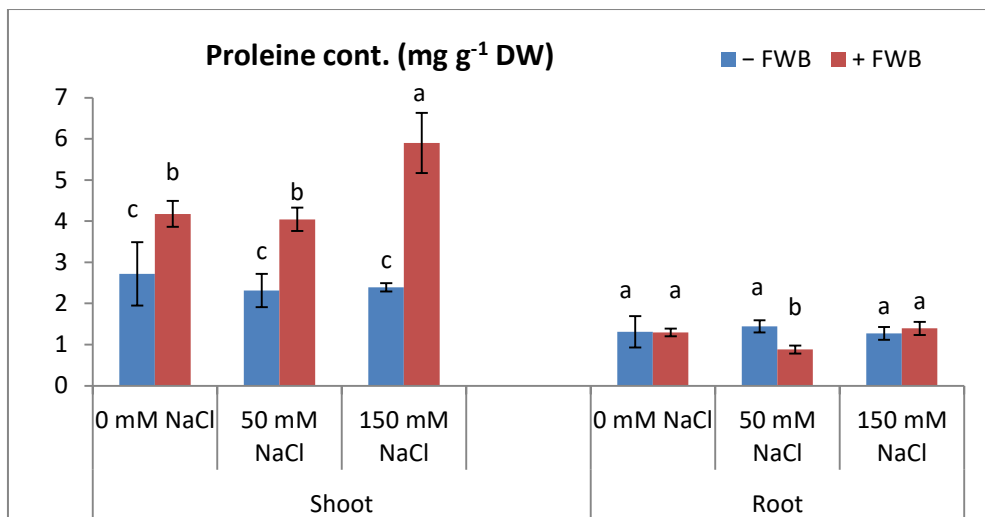


Fig. 4. Effect of salt stress and application of fish waste-derived biochar (FWB) on the content of proline (mg g⁻¹ D.W.) of maize shoot and root

Data presented are the means \pm S.D. (n=3). Data followed by similar letters are not significantly different by Duncan's multiple range test at $P \leq 0.05$

Table 2. Effect of salt stress and application of fish waste-derived biochar (FWB) on Cl⁻, Na⁺, K⁺, Ca²⁺ and Mg²⁺ contents each as mg g⁻¹ D.W. of maize shoot

Treatments (NaCl; mM)	FWB	Cl ⁻	Na ⁺	K ⁺	Ca ²⁺	Mg ²⁺
0	- FWB	10.93 \pm 0.59 ^c	5.99 \pm 0.8 ^c	25.61 \pm 1.19 ^b	7.33 \pm 2.31 ^{ab}	6.17 \pm 0.76 ^{ab}
	+ FWB	8.10 \pm 0.72 ^d	3.37 \pm 0.08 ^d	37.96 \pm 2.00 ^a	8.33 \pm 2.31 ^a	5.97 \pm 0.46 ^{ab}
50	- FWB	9.36 \pm 0.85 ^b	7.39 \pm 0.84 ^b	25.91 \pm 1.99 ^b	5.33 \pm 0.58 ^c	5.00 \pm 1.00 ^b
	+ FWB	13.89 \pm 1.85 ^c	5.36 \pm 0.08 ^c	37.44 \pm 4.43 ^a	7.67 \pm 1.15 ^a	7.70 \pm 1.21 ^a
150	- FWB	15.76 \pm 2.08 ^a	9.46 \pm 0.46 ^a	20.67 \pm 3.05 ^c	5.67 \pm 1.53 ^b	5.33 \pm 1.01 ^b
	+ FWB	15.07 \pm 2.86 ^c	6.07 \pm 0.2 ^c	30.03 \pm 0.68 ^b	7.67 \pm 0.58 ^a	7.20 \pm 2.16 ^a

Data presented are the means \pm S.D. (n=3). Data followed by similar letters are not significantly different by Duncan's multiple range test at $P \leq 0.05$

Table 3. Effect of salt stress and application of fish waste-derived biochar (FWB) on Cl⁻, Na⁺, K⁺, Ca²⁺ and Mg²⁺ contents each as mg g⁻¹ D.W. of maize root

Treatments (NaCl; mM)	FWB	Cl ⁻	Na ⁺	K ⁺	Ca ²⁺	Mg ²⁺
0	- FWB	6.20 ± 0.51 ^b	7.09 ± 1.01 ^b	13.91 ± 1.58 ^{ab}	6.33 ± 0.58 ^{ab}	7.33 ± 4.07 ^b
	+ FWB	8.08 ± 0.75 ^a	7.61 ± 0.34 ^{ab}	16.77 ± 2.34 ^a	7.33 ± 0.58 ^a	10.67 ± 1.15 ^a
50	- FWB	7.19 ± 1.12 ^b	7.10 ± 0.39 ^b	13.00 ± 2.15 ^b	4.00 ± 0.00 ^{cd}	5.17 ± 0.58 ^c
	+ FWB	6.40 ± 0.85 ^b	7.46 ± 0.56 ^{ab}	13.52 ± 2.15 ^b	5.33 ± 0.58 ^b	11.67 ± 2.08 ^a
150	- FWB	7.19 ± 1.12 ^b	8.28 ± 0.68 ^a	8.25 ± 0.31 ^c	2.67 ± 0.58 ^d	2.83 ± 0.29 ^d
	+ FWB	7.88 ± 0.85 ^{ab}	7.31 ± 0.59 ^b	9.36 ± 1.03 ^c	6.00 ± 3.46 ^b	12.00 ± 1.00 ^a

Data presented are the means ± S.D. (n=3). Data followed by similar letters are not significantly different by 'Duncan's multiple range test at P ≤ 0.05

The current findings demonstrated increased organic constituents (total soluble sugars, proteins, and amino acids) in maize shoots under salt stress. These outcomes are compatible with those of Kapoor and Srivastava [41]. These organic solutes let plants tolerate and adapt to more stress [42], make osmotic balance, regulate water influx (decrease outflow), and permit turgor maintenance in salinity [43]. The observed increase in protein content might result from its interaction with cellular macromolecules to detoxify ROS and stabilize their structure or from their protective function of the cell under stress by balancing the osmotic strength of the cytosol with that of the vacuole and the surrounding environment [44].

Adding biochar resulted in a significant increase in the content of soluble carbohydrates, proteins, and amino acids of stress-exposed plants' roots compared to the control plant. These findings are consistent with those of Desoky et al. [45]. However, in the case of shoot, applying biochar as a sole treatment resulted in a highly significant amelioration of the harmful effects induced by salinity stress, causing a remarkable reduction in total soluble proteins and total soluble carbohydrate contents of salt-stressed seedlings. These results are in concurrence with the findings of Osman et al. [46].

According to the results, biochar considerably raised the amount of proline in plant tissue. Generally, reactive oxygen species (ROS), produced by proline under stress conditions, significantly contribute to the stability of membranes and other cellular components.

Furthermore, it maintains the pH and turgor of the cell. Additionally, the rise in proline concentration may be related to its vital role in plant defense by activating stress-associated proteins [47]. Our findings concur with those of Desoky et al. [45].

The current study also showed improved Na⁺ uptake, which had a concomitantly detrimental impact on the uptake of other ions like K⁺, Ca²⁺, and Mg²⁺. Our findings of decreased absorption of vital mineral ions due to salt stress are consistent with those of Iqbal et al. [48].

Our findings showed that adding biochar increased the uptake of important mineral elements like K⁺, Ca²⁺, and Mg²⁺ while reducing the amount of Na⁺ in plants. These findings concur with earlier research [30,49]. Biochar can immobilize Na⁺ from the soil solution because of its high sorption capability. As a result, it can enhance plant growth in saline conditions by releasing mineral nutrients and reduce Na⁺ uptake and lowering its toxicity to plants [50].

5. CONCLUSION

Biochar could significantly mitigate salinity stress, thus ultimately improving the growth and physiological processes of corn plants, as shown in Fig. 5 (A and B). This could be mainly attributed to enhancing photosynthetic pigments, osmolyte levels, and K⁺, Ca²⁺, and Mg²⁺ content. Therefore, incorporating biochar may be a novel approach for improving crop productivity under salinity stress where it may alleviate the negative impacts of salt stress on crops.

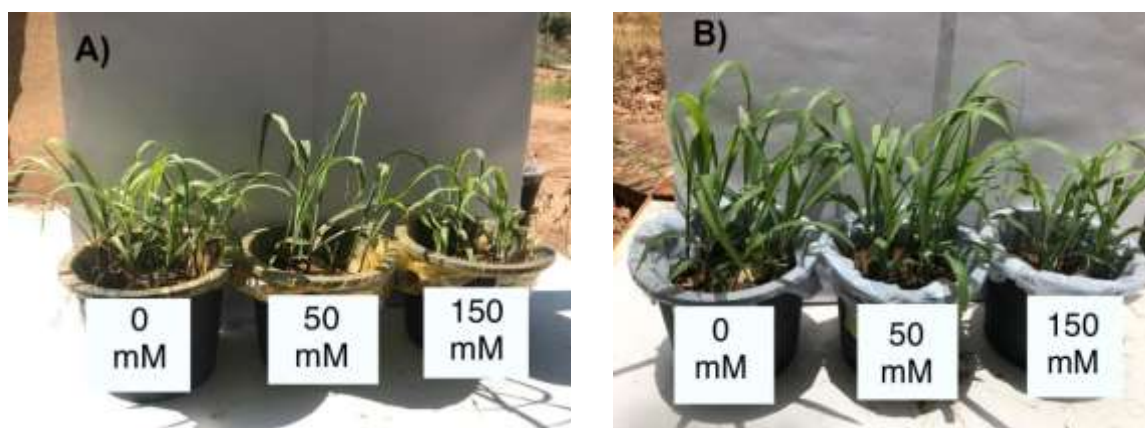


Fig. 5. Effect of different levels of NaCl (0, 50 and 150 mM) and application of fish waste-derived biochar, -FWB (A) and +FWB (B) on the growth of maize plants

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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