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Investigating the Influence of Biochar and Salicylic Acid on Maize (Zea Mays L.) Performance and Physiological Responses Under Salinity Stress Conditions

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Abstract

This study examined the ways in which salicylic acid and biochar can improve maize development in the face of salt stress. Three replicates were used in the factorial, fully randomized design of the greenhouse experiment. For each factor, three levels of treatments were used: biochar (0%, 0.6%, and 1.2%), salicylic acid (0, 0.75, and 1.5 mM), and salinity stress (caused by NaCl at 0, 4, and 8 dS/m). The dried leaves and stems of date palms were used to make the biochar. The findings indicated that, in comparison to the control, salt stress decreased plant height by around 40%, dry weight of the shoot by 60%, dry weight of the roots by 51%, chlorophyll content of leaves by 3%, and grain production by 38%. Notably, the greatest concentrations of biochar (1.2%) & salicylic acid (1.5%) considerably enhanced all growth indices and reduced the activities of peroxidase and catalase enzymes at a salinity index of 4 dS/m, therefore mitigating the negative impacts of salinity. Salicylic acid and biochar together helped sustain elevated levels of the investigated features even in the most extreme salinity levels (8 dS/m) as compared with the treatment absent these additions. To sum up, the results suggest using 1.2% biochar in conjunction with 1.5 mM of salicylic acid to enhance maize growth in circumstances when exposed to mild stress from salinity (4 dS/m).

Keywords:

Biochar, foliar application, plant growth regulator.

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Introduction

Corn is a high-yield crop with a comparatively quick growing phase. In terms of farmed acreage, it is in third place behind rice and wheat, and second in terms of production per area of land worldwide. According to (Butcher et al., 2018), corn accounts for 20–25% of the world's supply of food, 60–75% of feed for animals, and around 5% of industry raw materials. Globally, salinity is a serious problem for agriculture (Pathak, 2000). Salt buildup in the soil's root zone, which is frequently brought on by inadequate drainage, is particularly troublesome in dry and semi-arid areas where salt leaching is restricted by little rainfall. Additional sources of soil salinity include saline deposits in deeper soil layers and fluctuations in salt concentration due to irrigation practices (Vijayvargiya & Kumar, 2011). This stress leads to osmotic challenges (Jiménez-Mejía et al., 2022) and ion toxicity from chloride and sodium (Khayyat et al., 2014). To meet growing consumer demand, enhancing the productivity of saline and arable lands is crucial (Menon & Rao, 2024). Although several methods exist to manage soil salinity—such as chemical amendments (which can cause secondary pollution), cultivating salt-tolerant varieties (a time-consuming process), and soil washing (which may move salts deeper)—each has its drawbacks (Li et al., 2010). In order to reduce soil salinity and enhance crop productivity, straightforward, natural methods are thus required. Selecting a sustainable modification is strongly related to the target area's unique physicochemical and geographic features (Narayanan & Rajan, 2024). Biochar has gained popularity as a soil supplement recently. Biochar can limit the upward flow of salts from greater depths or groundwater in saline soils, decrease transpiration and evaporation, and, because of its high ability to adsorb, lower levels of salt in the soil and soil solution (Abel et al., 2013; Wang et al., 2003). Studies have indicated that the use of biochar under salt stress enhances maize yield and growth indices (Yamato et al., 2006; Raboin et al., 2016; Calys-Tagoe et al., 2019). Also, salicylic acid is essential for improving plant defense signaling in response to biotic stressors and salinity (Verma & Agrawal, 2017; Pokotylo et al., 2021). In plant stress reactions, it functions as a signaling molecule, regulates several physiological activities, and acts as a protective agent (Rasheed et al., 2022). In addition to other species such as cucumbers (Nie et al., 2018), tomatoes (Gharbi et al., 2018), the faba bean (Souana et al., 2020), as well as alfalfa (Palma et al., 2013), the advantages of salicylic acid in enhancing resistance to salinity have been reported in cereals like wheat, rice, barley, in addition corn (El-Tayeb, 2005; Jini & Joseph, 2017). Finding substances that might enhance germination, development, and total crop output under salt stress is crucial due to the limited supply of freshwater, the utilization of saline water in the agricultural sector, and the continuous salinization of soils (Jayakannan et al., 2015). Therefore, the purpose of this study is to examine how biochar and salicylic acid might improve corn growth and output while reducing the negative impacts of salt stress in greenhouse settings (Kanwal et al., 2018; Mehta & Dutta, 2024).

Materials and Methods

Research Location

In 2022–2023, this study was carried out at the research greenhouses of the faculty's Department of Science of Soils and Water Resources in the College of Agricultural Sciences at the University of Baghdad.

Research Treatments

Three replications of a greenhouse factorial experiment were carried out in a totally randomized design to examine the effects of biochar and salicylic acid treatment on the physiological traits and performance of maize under salt stress conditions (Hütsch et al., 2014; Mehta & Sharma, 2024). Among the treatments were three concentrations of salicylic acid (0, 0.75, & 1.5 mM), three concentrations of biochar (0%, 0.6%, and 1.2% by weight per pot), & three concentrations of salinity stress brought on by NaCl (0, 4, and 8 dS/m-1).

Biochar Preparation

In this study, biochar was prepared from dried date palm leaves and stems (Helaoui et al., 2023). Table 1 reports the nutrient content of the biochar derived from palm leaves and stems.

Table 1. Characteristic of biochar used in the experiment

Mg (mg. g^{-1})	Ca (mg. g^{-1})	Na (mg. g^{-1})	K (mg. g^{-1})	P (mg. g^{-1})	C: N	N (mg. g^{-1})	C (mg. g^{-1})	PH	EC (V/V= 1:5) ds.m^{-1})
0.8	7.43	0.5	7.8	1.6	69.6	4.6	320	7.30	3.21

Research Methodology

Experimental Procedures

Soil Acquisition and Pot Preparation

The experiment's soil came from the research farm of the University of Baghdad's Faculty of Agricultural Sciences' Soil and Water Resource Department. After its collection, the soil's physical and chemical characteristics were analyzed (see Table 2), and 20 kg was allocated to each pot in the greenhouse (Kanagala et al., 2023; Musa & Mohamad, 2018). Before sowing, the soil was thoroughly blended with predetermined amounts of biochar.

Sowing and Seedling Management

Seeds of the hybrid corn variety Single Cross 704 (KSC704), obtained from the Abu Ghraib Agricultural Research Center (Seed Group), were first sterilized using Carboxin Thiram. Five seeds were sown per pot at a 3 cm depth. Once the seedlings reached the three-leaf stage, thinning was conducted to retain only three vigorous plants per pot.

Irrigation and Treatment Applications

All pots were irrigated until they reached field capacity, after which salt stress was introduced using NaCl. Foliar applications of salicylic acid were carried out every two weeks until complete wetting of the leaves was achieved, while control pots received only distilled water. Salinity stress treatments began two days after the initial salicylic acid spray and continued throughout the growing season. Water was refilled to maintain capacity in the field, plus an additional 15% to guarantee proper leaching, and pots were weighted every other day.

Harvesting and Data Collection

The crops were taken from the earth's surface four months after they were planted. Plant elevation dry weights of the roots and aerial parts, leaf area, and grain output were all measured (according to Daneshmand et al., 2012. Furthermore, the activities of the enzymes peroxidase (POX) with catalase (CAT) were evaluated by (HADWAN & KHABT, 2018), and the amount of chlorophyll in the leaves was ascertained using the technique of (Lichtenthaler & Buschmann, 2001).

Table 2. The experiment uses a physical and chemical analysis of the soil

Particle density (%)	Bulk Density ($g.cm^{-3}$)	CEC (meq $100g^{-1}$)	CO_3^{2-} ($g.kg^{-1}$ soil)	$CaSO_4$ (%)	O.M (%)	EC ($dS.m^{-1}$)	PH
24	1.45	14.8	136.5	0.64	3.15	2.66	7.41

Clay (%)	Silt (%)	Sand (%)	K ($mg.kg^{-1}$)	P ($mg.kg^{-1}$)	N ($mg.kg^{-1}$)	PWP (%)
38	40	22	163	9.0	56	12

NO_3^- ($mg.kg^{-1}$)	CO_3^{2-} ($mg.kg^{-1}$)	HCO_3^- ($mg.kg^{-1}$)	SO_4^{2-} ($mg.kg^{-1}$)	Cl^- ($mg.kg^{-1}$)	K^+ ($mg.kg^{-1}$)	Mg^{2+} ($mg.kg^{-1}$)	Na^+ ($mg.kg^{-1}$)	Ca^{2+} ($mg.kg^{-1}$)
21	35	608	209	691	25	248	677	586

Statistical Analysis

Using JMP-8 software, the study's data was statistically analyzed. Additionally, the LSD test was used to compare the mean values.

Results

According to the findings, the total weight of the aerial parts and root matter was significantly impacted by the interaction of salicylic acid, salt, and biochar at the one per cent probability level ($P \leq 0.01$). Furthermore, varying amounts of charcoal and salicylic acid also had an impact on plant height (Table 3).

Table 3. Variance analysis of seed yield, morphological characteristics, and activity of enzymes under various treatments

Source of variation	**df**	**Plant height**	**Shoot dry weight**	**Root dry weight**	**Leaf area**	**Chlorophyll content**	**Seed yield**	**Peroxidase activity**	**Catalase activity**
Salinity (S)	2	1261^{**}	52.6^{**}	931^{**}	26053069^{**}	324.5^{**}	37.6^{**}	3749^{**}	212^{**}
SA	2	567^{**}	24.2^{**}	208^{**}	5020244^{ns}	4.94^{*}	8.93^{**}	3.36^{**}	129^{**}
Biochar (B)	2	91^{**}	2.26^{**}	75.2^{**}	2659139^{ns}	0.23^{ns}	4.25^{**}	3.91^{**}	7.09^{**}
SA×S	4	184^{**}	13.3^{**}	44.8^{**}	13274060^{*}	1.80^{ns}	2.19^{**}	86.3^{**}	16.03^{**}
SA×B	4	41.6^{**}	0.37^{ns}	5.83^{**}	389708^{ns}	1.44^{ns}	0.77^{ns}	1.93^{**}	12.48^{**}
S×B	4	260^{**}	2.14^{**}	13.17^{**}	4842694^{ns}	0.97^{ns}	0.77^{ns}	6.30^{**}	1.39^{*}
SA×S×B	8	147^{**}	1.69^{**}	5.77^{**}	4056969^{ns}	1.14^{ns}	0.11^{ns}	1.38^{**}	2.21^{**}
Error	54	2.34	0.27	0.32	4418528	9.74	0.33	0.43	0.37

ns: non-significant treatment; **: Significant at 1% level of probability, *: Significant at 5% level of probability

The research results showed in Fig. 1 that salinity stress and the absence of salicylic acid and biochar significantly reduced plant height. In the treatment with 8 ds.m-1 NaCl + 0 mM SA + 0% Biochar, plant height decreased to 59 cm. Plant height in this treatment was reduced by 31% compared to the control treatment (0 $ds.m^{-1}$ NaCl + 0 mM SA + 0% Biochar). However, the use of biochar and salicylic acid significantly increased plant height at all salinity levels. The application of different levels of biochar in the presence of the highest concentration of salicylic acid (1.5 mM) under the highest salinity stress resulted in the highest recorded plant height (Table 4).

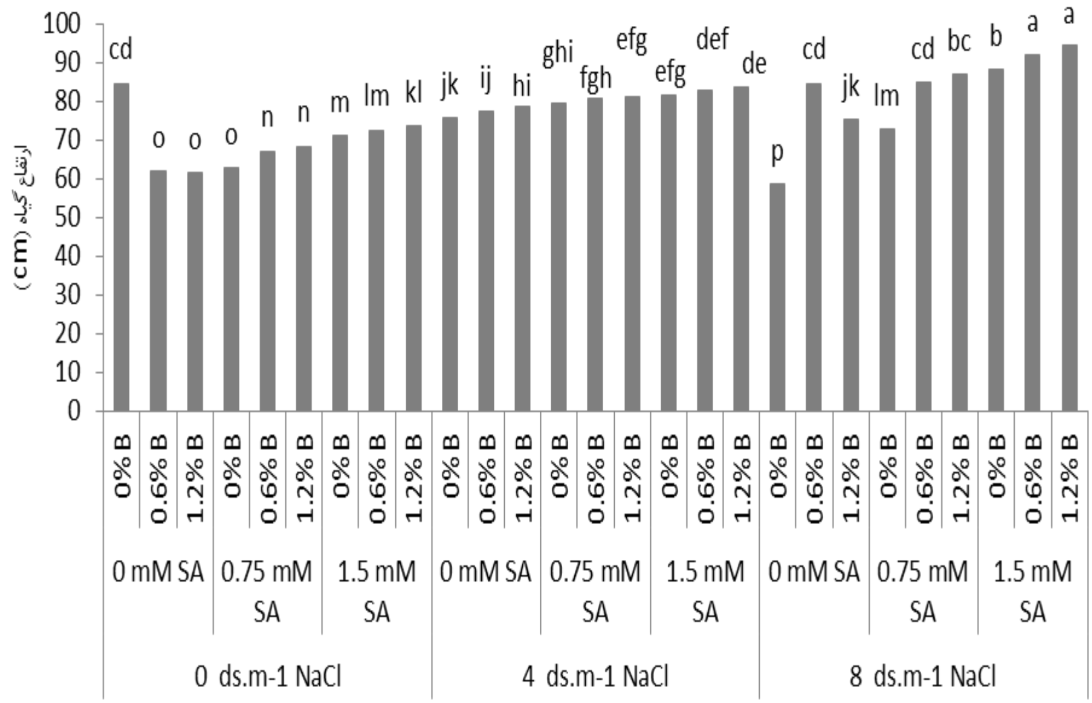

Figure 1. Effect of different concentrations of salicylic acid and biochar on plant height under saline conditions

The plant's shoot biomass was significantly impacted negatively by salinity. In a condition with 8 dS/m NaCl and no bentonite or salicylic acid in it, the total dry mass of the aerial portions was more than 50% lower than the control. When 1.5 mM salicylic acid & the maximum rate of biochar were added to the soil at an acceptable salt level of 4 dS/m NaCl, the shoot dry weight increased by 38.9% in comparison to the similar salt-stressed condition without these additions. Even at the highest salinity, adding salicylic acid as well as biochar increased shoot biomass; in particular, treatments that combined 8 dS/m NaCl along with 1.5 mM SA and 0.6% or 1.2% biochar increased dry weights by 69% and then 51%, respectively, compared for the neglected salt-stressed plants (refer to Table 4).

Root biomass was similarly affected by salt stress, with untreated plants experiencing a 50% reduction in root dry weight. But in both salinity and non-saline circumstances, foliar sprays of salicylic acid, together with the addition of biochar, greatly increased root growth. For example, administering 1.5 mM SA mixed with 1.2% biochar under non-saline circumstances increased the dry weight of roots by 80% in comparison with an untreated control, whereas the same treatment increased the biomass of roots by 52% at 4 dS/m NaCl. 1.2% biochar and 1.5 mM SA together improved root dry weight by 27% over the equivalent salt-stressed control, even at extreme saline (8 dS/m NaCl) (see Table 4).

Table 4. Plant height, shoot & root dry weight, and the effects of varying salicylic acid & biochar concentrations under saline conditions

Salinity ($dS.m^{-1}$)	**SA (mM)**	**Biochar (%)**	**Shoot dry weight (g)**	**Root dry weight (g)**
0	0	0	15.35h	14.06a
		0.6	16.17h	12.97bc
		1.2	17.79g	11.92efg
	0.75	0	19.05f	11.26ghi
		0.6	20.30de	12.01defg
		1.2	22.79bc	10.63hij
	1.5	0	19.41ef	12.36cdef
		0.6	22.09c	12.81cd
		1.2	27.64a	13.04bc
4	0	0	11.09k	9.92jk
		0.6	12.00k	10.59hij
		1.2	13.04j	10.34j
	0.75	0	14.33i	11.73fg
		0.6	15.34h	12.60cde
		1.2	22.08c	12.94bc
	1.5	0	20.03de	11.80efg
		0.6	20.70d	13.00bc
		1.2	23.34b	13.78ab
8	0	0	7.60p	6.96m
		0.6	8.58mno	8.13l
		1.2	8.18nop	8.45l
	0.75	0	9.41lm	9.43k
		0.6	9.24lm	10.15jk
		1.2	9.00lmn	10.75hij
	1.5	0	7.84op	11.37gh
		0.6	8.61mno	11.73fg
		1.2	9.66l	10.49ij

The same letters in each column are not significantly different at a probability level of 5% based on the LSD test.

Grain yield and the leaf area index are significantly correlated with the salinity-salicylic acid interaction (Table 3). Salicylic acid applied topically greatly expanded the plant's leaf area. The application of salicylic acid with both 0.75 & 1.5mM concentrations enhanced the plant's leaf area under saline conditions equal to 4 dS/m-1 NaCl. The application of an elevated amount of salicylic acid considerably decreased the leaf area at the maximum salinity level (8 dS/m-1). However, the plant's leaf area increased when 0.75 mM of salicylic acid was used. The treatment with 8 dS/m-1 NaCl Plus 0.75 mM of salicylic acid had the largest leaf area (14052 cm^2). In comparison to the control therapy (no salicylic acid in + no salt), the area of leaves in this form of therapy grew by 38% (Table 5).

Table 5. Salinity × salicylic acid's impact on seed output and leaf area

Salinity ($dS.m^{-1}$)	SA (mM)	Leaf area (cm^2)
0	0	10209.5d
	0.75	11918bcd
	1.5	12546.5abc
4	0	12521.8abc
	0.75	13142.4abc
	1.5	14051.4a
8	0	13895.2ab
	0.75	14052.8a
	1.5	11890.5cd

According to the LSD test, the same letters in each column do not differ substantially at the 5% probability level.

The amount of chlorophyll in the leaves was considerably decreased by applying varying salt levels to the plant. In particular, the leaves' chlorophyll content was lowered by more than 2% when salt was applied at the maximum amount (8 dS/m-1) (Figure. 2a). When salicylic acid was applied topically to the plant, the amount of chlorophyll in the leaves rose. When the plant with the highest levels of salicylic acid was applied topically, the amount of chlorophyll in the leaves rose by 8% in comparison to the control treatment, which did not apply any salicylic acid (Figure. 2b). Significantly less grain was produced when the corn plant was planted in salty circumstances. Nonetheless, in salty circumstances, the plant's grain production was enhanced by foliar treatment with salicylic acid. In comparison with the control treatment (there is no salinity, plus no salicylic acid application), the use of salicylic acid at a dosage of 1.5 mM enhanced grain yield by over 41 per cent at a salinity value of 4 dS/m-1 (Figure. 3a). Corn plants' grain output was also greatly increased by the addition of biochar to the soil. In comparison with the control treatment (there's no biochar application), the use of biochar with rates of 0.6% & 1.2% enhanced grain production by 15% & 22%, respectively (Figure. 3b).

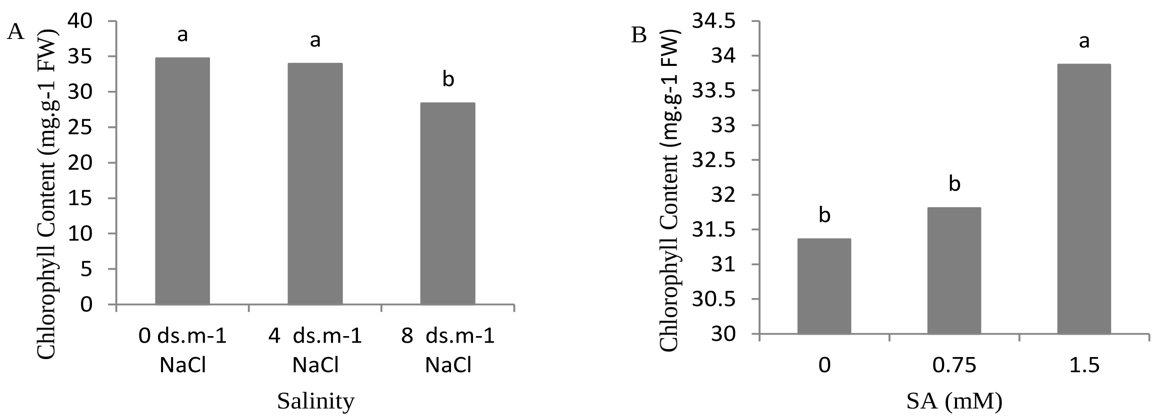

Figure 2. Effect of Salinity (A) and salicylic acid (B) on chlorophyll content

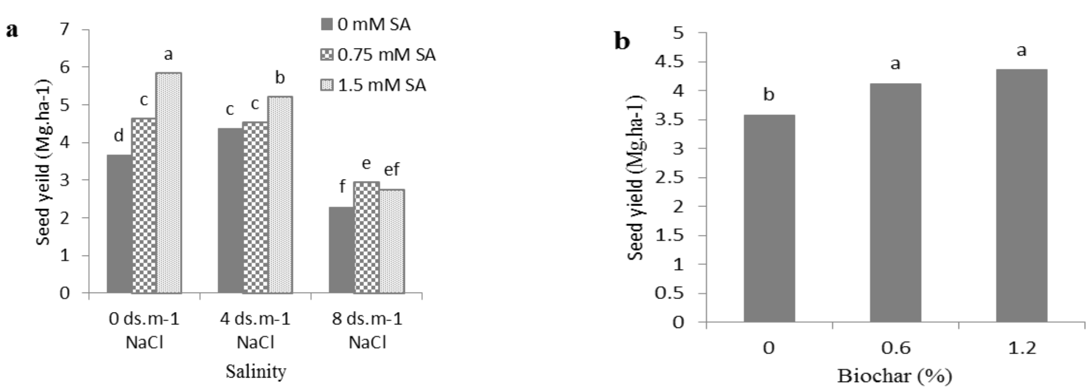

Figure 3. Effect of Salinity × SA (a) and biochar (b) on seed yield

The peroxidase enzymes in the plant were considerably more active when it was exposed to salt conditions. Using biochar within the soil and applying salicylic acid topically both enhanced the plant's peroxidase enzyme activity. For the treatment with 8 dS/m-1 NaCl, plus 1.5 mM SA, plus 1.2% Biochar, the maximum peroxidase activity of the enzyme was noted. In comparison to the control therapy (no salinity, plus without salicylic acid, plus no biochar), the peroxidase enzyme activity improved by 84.3% in this therapy. The addition of biochar and salicylic acid considerably raised the plant's peroxidase enzyme activity overall under salinity conditions (Fig. 4). The catalase enzyme activity of the maize plant was markedly enhanced by applying varying salt levels. The salinity stressing treatment involving 8 dS/m-1 NaCl, plus 0% biochar, plus 0 mM salicylic acid showed the maximum catalase enzyme activity. In comparison to the control at every salinity level, the application of salicylic acid & biochar to the plant under saline conditions decreased the catalase enzyme's activity (Figure. 5).

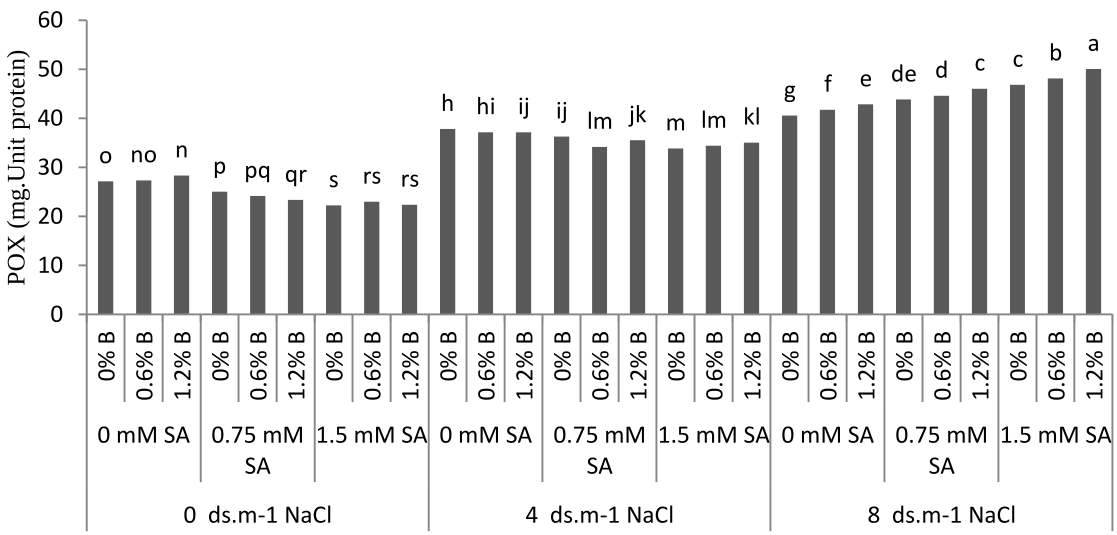

Figure 4. Effect of different concentrations of salicylic acid and biochar on POX activity under saline conditions

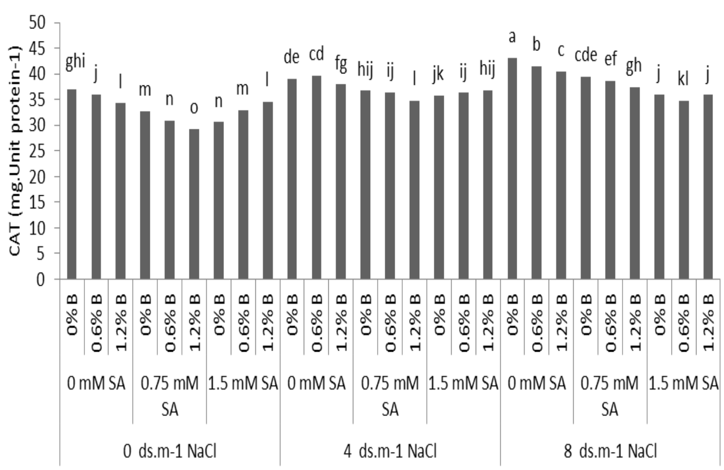

Figure 5. Effect of different concentrations of salicylic acid and biochar on POX activity under saline conditions

Discussion

Soil salinity stands as a major barrier to achieving high crop productivity, affecting not only yield but also various quality attributes of plants (Ekinci et al., 2022; Li et al., 2010). In this investigation, exposure to NaCl-induced salinity stress in maize resulted in significant declines in several growth parameters—namely plant height, both the dry and fresh weights of the aerial parts, leaf chlorophyll content, and ultimately grain yield—when compared to non-saline conditions (Rasheed et al., 2022).

Previous research supports these findings. Several studies have documented reductions in plant height under saline conditions (Cicek and Cakirlar, 2002; Kamkar et al., 2004; Lacerda et al., 2003), and it is also widely known that shoot biomass is declining (Cicek and Cakirlar, 2002). Shoots are often more negatively impacted by salt stress, even if roots were the first to experience it (Munns, 1993). Reduced internode elongation, faster beginning of leaf senescence, and reduced initiation of leaf growth and development are all possible explanations for the observed decrease in the total dry weight for the aerial components in our study (Agami, 2013).

Similarly, root biomass suffered under high salinity, likely due to impaired root development and lower root density (Noroozi et al., 2022). Additionally, salinity may reduce the plant's sink strength by lowering invertase enzyme activity, which in turn hampers grain filling and diminishes yield (Hutsch et al., 2014). These yield-reducing effects under saline conditions have been reported not only in maize (Kaya et al., 2013; Katerji et al., 1996; Cicek & Cakirlar, 2002) but also in other crop species (Steppuhn et al., 2001; Zhang et al., 2001).

However, our findings also suggest that adding biochar to the soil might mitigate some of these adverse effects. According to (Akhtar et al., 2015), biochar seems to improve photosynthetic efficiency and plant development in salt stress situations (de Lacerda et al., 2003). It is possible that improved nutritional availability, along with a stimulatory impact on plant hormones, including gibberellins with indole acetic acid, or IAA, are responsible for the improvements in morphological features, such as increasing height, larger biomass of shoots and roots, and higher grain production. Biochar's ability to improve nutrient and water retention probably encourages stronger root growth as well (Mohamed et al., 2016). A number of studies have verified the positive benefits of biochar under salinity stress, including those conducted on maize (Jabborova et al., 2021; Bogusz & Oleszczuk, 2018; Kocsis et al., 2022; Hussein et al., 2022) and even tomatoes (Usman et al., 2016).

Additionally, the use of biochar was linked to lower levels of antioxidant enzymes, including superoxide dismutase and catalase, suggesting a decreased requirement for an improved oxidative defence response in the presence of salt stress. This observation is consistent with the results of (Kocsis et al., 2022), who also noted decreased enzyme activity and drops in proline, sucrose, & malondialdehyde levels.

Conclusion

In conclusion, salt stress significantly reduced important physiological and morphological indicators, which had a detrimental effect on maize growth. Nevertheless, the use of charcoal and salicylic acid greatly increased the plant's resistance to these circumstances. Interestingly, mixing 1.2% biochar along with 1.5 millimetres of salicylic acid produced noticeable increases in growth parameters at a moderate saline level of 4 dS/m. These treatments significantly improved growth index maintenance compared to untreated plants, even under intense stress (8 dS/m). These findings demonstrate the potential of salicylic acid and biochar as practical methods to lessen the negative effects of salt on maize.

Author Contributions

All Authors contributed equally.

Conflict of Interest

The authors declared that no conflict of interest.

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