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Mitigation of Salinity Stress and Lead Toxicity in Maize by Exogenous Application of the Sorghum Water Extract

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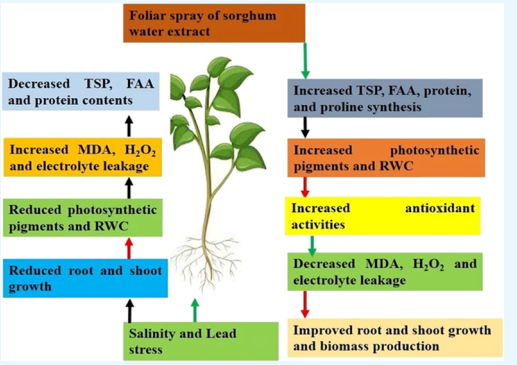
ABSTRACT: The increased concentration of lead (Pb) in soils is a serious threat to human beings and plants all over the world. Salinity stress is also a major issue across the globe, which limits crop productivity. The use of allelochemicals has become an effective strategy to mitigate the toxic effects of abiotic stresses. Sorghum is an important crop grown across the globe, and it also possesses an appreciably allelopathic potential. Therefore, this study was planned to determine the impacts of the sorghum water extract (SWE) on improving maize growth under Pb and salinity stress. The experiment included different treatments; control, SWE (3%), and different levels of Pb and salinity stress; T_1: control, T_2: 50 mM NaCl, T_3: 100 mM NaCl, T_4: 250 μM Pb, and T_5: 500 μM Pb. Lead and salinity stress reduced the maize growth by the genesis of reactive oxygen species (ROS), as evidenced by higher production of malondialdehyde (MDA: 39.1 and 32.28%) and hydrogen peroxide (H_2O_2: 20.62 and 17.81%). Spraying plants with SWE improved the maize growth by increasing antioxidant activities (ascorbate peroxidase: APX, catalase: CAT, peroxidase: POD and superoxide dismutase: SOD), photosynthetic pigments, relative water contents (RWC), osmolyte accumulation (proline, total soluble proteins: TSP, free amino acids: FAA), potassium accumulation, and decreasing MDA, H_2O_2, sodium, chloride, and Pb accumulation. In conclusion, the application of SWE mitigates adverse impacts of Pb and salinity stresses by improving chlorophyll synthesis and osmolyte accumulation, activating the antioxidant defense system, and preventing the entry of toxic ions.



1. INTRODUCTION

Heavy metal (HM) pollution is a serious concern across the globe that negatively affects crop productivity, ecosystem health, and biodiversity.[1,2] The concentration of HMs is continuously increasing owing to rapid industrial and economic growth, which is a serious issue and needs proper measures to tackle this problem to ensure global food security and environmental quality.[3] Lead (Pb) is a serious and dangerous HM that exists in the environment. It has been widely used since ancient times owing to its appreciable properties like weak conductors, elasticity, corrosion resistance, pliability, and ductility.[4−6] However, it is nondegradable and persistent in soil; therefore, it can pose negative effects to plants and human beings.[7−10]

Lead stress negatively affects plant functioning ranging from seed germination to growth, physiological, biochemical, and molecular processes.[11−13] It negatively affects root growth, root number, and reduces water and nutrient uptake, therefore resulting in a substantial reduction in plant growth.[14−16] Further, it also causes inflated, abnormal, undersized, short roots with a higher number of secondary roots.[17] High doses of Pb stress also decrease the photosynthetic rate, chlorophyll, plastoquinone, and carotenoid synthesis and damage the photosynthetic apparatus.[18−21] Besides this, Pb stress also stimulates the reactive oxygen species (ROS) production that damages the lipids, proteins, and membranes.[22−24]

Soil salinity is the main abiotic stress that negatively affects crop productivity. Globally, more than 833 million hectares are salt-affected, and this extent is continuously increasing due to anthropogenic activities.[25] Salinity is a major problem for crops across the globe, particularly in irrigated fields in semiarid and arid climates.[26,27] Soil salinity inhibits plant growth, disturbing plant physiological, biochemical, and molecular processes.[28−32] Soil salinity also reduces leaf water status and chlorophyll synthesis, and it damages the photosynthetic apparatus leading

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to a significant decrease in photosynthesis.[33,34] Moreover, salinity stress also increases ROS that damages membranes, proteins, and lipids, and it also causes osmotic and ionic toxicity, which negatively affects plant growth.[35] Allelopathy is a natural process in which different organisms positively and negatively affect the functions of another organism by releasing secondary metabolites.[36] Different allelochemicals have been discovered that can improve plant performance under normal and stress conditions.[37,38] The sorghum water extract (SWE), also named sorgaab, is a rich source of phenolic and antioxidants, and it can regulate plant growth under adverse conditions.[39] Allelochemicals can improve the tolerance against stress conditions by increasing seed germination, seedling growth, chlorophyll synthesis, photosynthetic, transpiration, and plant growth.[37] For instance, under drought and heat stress, a significant improvement in membrane stability, stay green character, proline, and phenolic synthesis were seen with seed priming with all chemicals.[37] Further, in another study, it was seen that allelochemicals increased salt tolerance by increasing phenolic, soluble sugars, protein synthesis, and chlorophyll and improving photosynthetic efficiency.[40,41]

Recently, the potential of SWE to enhance abiotic stresses has been tested in different crops like maize, wheat, sunflower maize,[42] wheat,[43] and sunflower.[44] Nonetheless, there is no information available about the role of SWE in enhancing the performance of maize against combined Pb and salinity stress. We hypothesized that SWE would increase the growth performance of maize under Pb and salinity stress by improving physiological functioning, photosynthetic efficiency, and osmolyte synthesis. Therefore, the goal of this study was to estimate the effect of foliar spraying of SWE on photosynthetic pigments, antioxidant activities, and osmolyte synthesis in maize growing under Pb and salinity stress conditions.

2. MATERIALS AND METHODS

2.1. Experimental Details. A pot experiment was conducted to check the impact of the sorghum water extract (SWE) in improving Pb and salinity tolerance in maize. The study was conducted at The Islamia University of Bahawalpur, Bahawalnagar campus (30.012764°N, 73.278286°E), Punjab, Pakistan. The pots having a capacity of 5 kg of soil were selected and filled with soil. The soil for filling of pots was taken from the experiment area, and it was loamy soil with pH 8.2, organic matter 0.59%, total N 0.07%, available phosphorus 9 mg kg^{-1}, available K 193 mg kg^{-1}, and an electrical conductivity of 2.1 ds m^{-1}. The soil physiochemical analysis was carried out by set protocols of the Association of Official Analytical Chemists set protocols. Afterward, ten seeds of maize variety Gohar-19 were sown in each pot. The experiment consisted of the following treatments: control, sorghum water extract (SWE 3%) and different levels of lead and salinity stress: (T_1: control, T_2: 50 mM NaCl, T_3: 100 mM NaCl, T_4: 250 μM $PbCl_2$ and T_5: 500 μM $PbCl_2$). Sodium chloride was used as a source to induce salinity stress, while lead chloride was applied through soil spiking to induce Pb stress. The mature plants of sorghum were harvested and collected, shade-dried for a few days, and then chopped into pieces. Thereafter, this material was soaked in water for a period of 24 h at room temperature in a 1:10 (W/V) ratio. Thereafter, the extract was filtered, and it was considered a 100% stock solution of SWE and it was diluted to 3% after adding the water. Sorghum water was applied to the experimental group at a rate of 3% as foliar spray after 7 days of applying Pb and salinity stress. All other agronomic practices were kept constant, and data on growth, physiological, and biochemical traits were collected 30 days after applying SWE.

2.2. Observations. *2.2.1. Growth Parameters.* Five plants were chosen from each experimental pot, and they were harvested from the base. The length of harvested plants were measured to determine the shoot length, and then they were weighed to determine the fresh weight and later dried (70 °C) until constant weight to determine the dry weight (DW). The roots of these plants were carefully pulled and washed to remove the soil, and then their lengths were measured and weighed to determine fresh weight (FW) and over dried for determining dry weight. Moreover, the leaves and numbers of roots from five randomly selected plants were counted, and the average was taken.

2.2.2. Photosynthetic Pigments and the Leaf Water Status. The fresh leaves of maize plants were taken to determine the concentration of chlorophyll and carotenoids.[45] For this, fresh leaves (0.5 g) were ground with 80% methanol to obtain the extract. After that, the extract was centrifuged and filtered, and absorbance was taken at different wavelengths (645, 480, and 663 nm) for determination of chlorophyll a, b, and carotenoid concentrations. For determination of leaf relative water contents (RWC), fresh leaves were taken and weighed to take fresh weight (FW), and then they were placed in water for 24 h. Then, samples from water were taken out, and extra water was removed and weighed to take the turgid weight (TW); then, these samples were oven-dried (70 °C), dry weight (DW) was taken, and RWC was assessed with the following formula: RWC (%) = FW-DR/TW-DR × 100. In the case of electrolyte leakage, 0.5 g of fresh maize leaves was dipped in water for 30 min, and the first EC1 was taken; afterward, these samples were placed in a water bath for 90 min and then taken out and the second EC2 was taken, and EL was calculated with the following formula: EL = EC1/EC2 × 100.

2.2.3. Osmolytes and Oxidative Stress Markers. The concentration of total soluble proteins (TSP) in maize leaves was determined by the protocols of Bradford et al.[46] Then, 0.5 g of fresh maize leaves was chopped and ground with phosphate buffer (5 mL) and then centrifuged for 15 min at 14,000 rpm. Thereafter, we took the Bradford mixture and added 2 mL of this mixture to the extract; then, absorbance (595 nm) was noted. To determine free amino acids (FAA), 0.5 g of leaves was ground and the extract was obtained; after that, 1 mL of the extract was added in test tubes containing 1 mL of pyridine and 1 mL of ninhydrin. Thereafter, test tubes were placed for 30 min in a water batch having 90 °C temperature and then rapidly cooled, and absorbance was noted at 570 nm.[47] In the case of H_2O_2, 0.5 g of leaves was ground in 5 mL of trichloroacetic acid, and the supernatant was obtained; then, potassium iodide (KI: 1 mL) and potassium phosphate buffer (PPB: 1 mL) were added and the mixture was allowed to stand for 30 min, and absorbance was taken at 390 nm to determine H_2O_2 concentration. Again, 0.5 g of fresh leaves was ground with 5 mL of 2,4,6-trichloroanisole (TCA) and placed for 15 min at 12,000 rpm, and then the supernatant was taken and boiled (100 °C) for 30 min after adding thiobarbituric acid (TBA) and cooled rapidly, and absorbance (532) was taken to determine malondialdehyde (MDA) activity. We took 0.5 g of fresh maize leaves and ground them with 10 mL of sulfosalicylic acid (3%) and then placed them at 10,000 rpm for 30 min. Then, these samples

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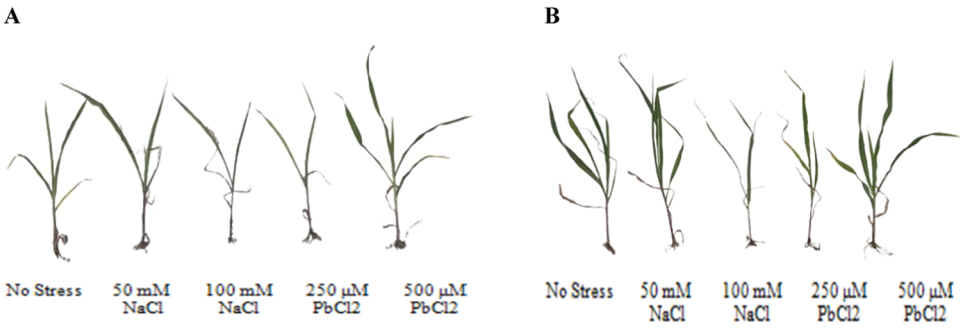

Figure 1. Pictorial view of the effect of no SWE (A) and SWE (B) application on maize plants.

were taken out, ninhydrin acid was added to the supernatant and placed for 90 min in a water bath (90 °C), and absorbance was recorded (520 nm) to measure proline concentration.

2.2.4. Antioxidants. Catalase (CAT) activity was measured with the methods of Aebi.[48] Next, 0.5 g of maize leaves was taken and ground with PPB (5 mL) and centrifuged at 1000 rpm for 15 min, and absorbance (240 nm) was taken for determination of CAT activity. For measuring ascorbate peroxidase (APX) activity, we ground 0.5 g of maize leaves by using 5 mL of PPB and centrifuged at 10,000 rpm for 15 min at 10,000 rpm, and absorbance (290 nm) was taken to determine APX activity.[49] In the case of peroxidase (POD) activity, fresh maize leaves (0.5 g) were homogenized in 5 mL of PPB and centrifuged at 10,000 rpm for 15 min, and absorbance (470 nm) was noted.[50] For SOD activity, a reaction mixture containing 400 μL of H_2O_2, 25 L of buffer, 100 μL of Triton, 50 μL of NBT, 50 μL of the sample, and 50 μL of riboflavin was prepared, and absorbance (560 nm) was measured.[50]

2.2.5. Ionic Concentration. Maize samples were collected, dried (65 °C), and ground into a powder. We digested 0.5 g of ground samples in two acids (HCl and HNO_3, 1:2), filtered them, and diluted them with distilled water. After that, the concentration of Na^+ and K^+ in digested samples was measured by a flame photometer, and the concentration of Cl^- was measured with a Cl analyzer. For determination of Pb concentration, maize samples were digested using nitric acid (67%) and hydrogen peroxide and then diluted by adding distilled water, and the concentration of Pb was determined by using atomic absorption spectrophotometry.

2.2.6. Statistical Analysis. The experiment was performed in a completely randomized layout design in a factorial arrangement with three replications. All of the collected data were analyzed by analysis of variance techniques using Co-stat software. The differences between the treatments were sorted out by using the least significant difference test ($P < 0.05$).

3. RESULTS

3.1. Growth Parameters. Lead and salinity stress induced a significant ($P < 0.05$) reduction in the growth and morphological traits of maize plants (Figures 1 and 2). The maximum shoot length (SL), root length (RL), and number of leaves (LPP) and roots were recorded in the control treatment with SWE (3%) application. However, minimum SL and RL were noted from plants under 100 mM salinity without a foliar spray of SWE (Figure 1). The maximum shoot fresh and dry weight (25.23 and 8.13 g) was recorded in control plants receiving the SWE, whereas the lowest shoot fresh and dry weight (7.43 and 2.43 g) was recorded in saline conditions without a foliar spray of SWE (Figure 3).

3.2. Photosynthetic Pigments and Leaf Water Relations. The synthesis of photosynthetic pigments was significantly ($P < 0.05$) decreased under both Pb and salinity stress (Table 1). The four photosynthetic pigments (chl a, chl b, total chl and carotenoid) showed a marked reduction under saline conditions (100 mM NaCl), followed by 50 mL salt stress. Lead stress also reduced the concentration of the aforementioned photosynthetic pigments; however, it showed less reduction as compared to salinity stress (Table 1). The foliar SWE spray appreciably increased the synthesis of photosynthetic pigments under both salinity and lead stress conditions (Table 1). The results showed that the RWC of maize plants was significantly decreased under Pb and saline stress conditions (Table 2). The maximum RWC (81.33%) was recorded in control conditions with SWE, following control conditions (80.33%) without SWE. Further, the application of SWE in stress conditions also increased the RWC, and the overall trend of SWE in increasing the RWC in different stress treatments was observed as control > 50 mM NaCl > 100 mM NaCl > 250 μM Pb > 500 μM Pb (Table 2).

3.3. Oxidative Stress Markers. Both stress conditions significantly increased the production of oxidative stress markers. Electrolyte leakage was significantly increased under 50 NaCl (152.50%), 100 mM NaCl (187.94%), 250 μM Pb (149.97%), and 500 μM Pb (191.96%), respectively, as compared to control conditions. The concentration of MDA and H_2O_2 was also considerably increased under Pb stress and saline conditions. Nonetheless, the foliar spray of the SWE extract appreciably reduced the synthesis of MDA, H_2O_2, and EL (Table 2).

3.4. Osmolyte Accumulation and Antioxidant Activities. The results indicated that plant application of SWE appreciably increased the synthesis of TSP, TFA, proteins, and proline (Table 3). The maximum TSP (1.61 mg/g FW) and TFA (19.63 mg/g FW) were recorded in no-stress plants, and the lowest TSP and FAA (0.91 and 16.70 mg/g FW) were recorded in 250 μM Pb stress (Table 3). The application of SWE significantly increased the synthesis of both TSP and TFA under Pb and salinity stress conditions (Table 3). Proline concentration also increased under stress conditions, and application of SWE considerably increased proline synthesis. The overall SWE in increasing proline synthesis was recorded as 50 mM NaCl > 100 mM NaCl > 250 μM Pb > 500 μM Pb (Table 3). The results indicate that Pb and salinity stress enhanced the activities of four antioxidants. The maximum SOD activity was recorded under Pb > 500 μM Pb with SWE, while maximum POD activity was recorded under saline

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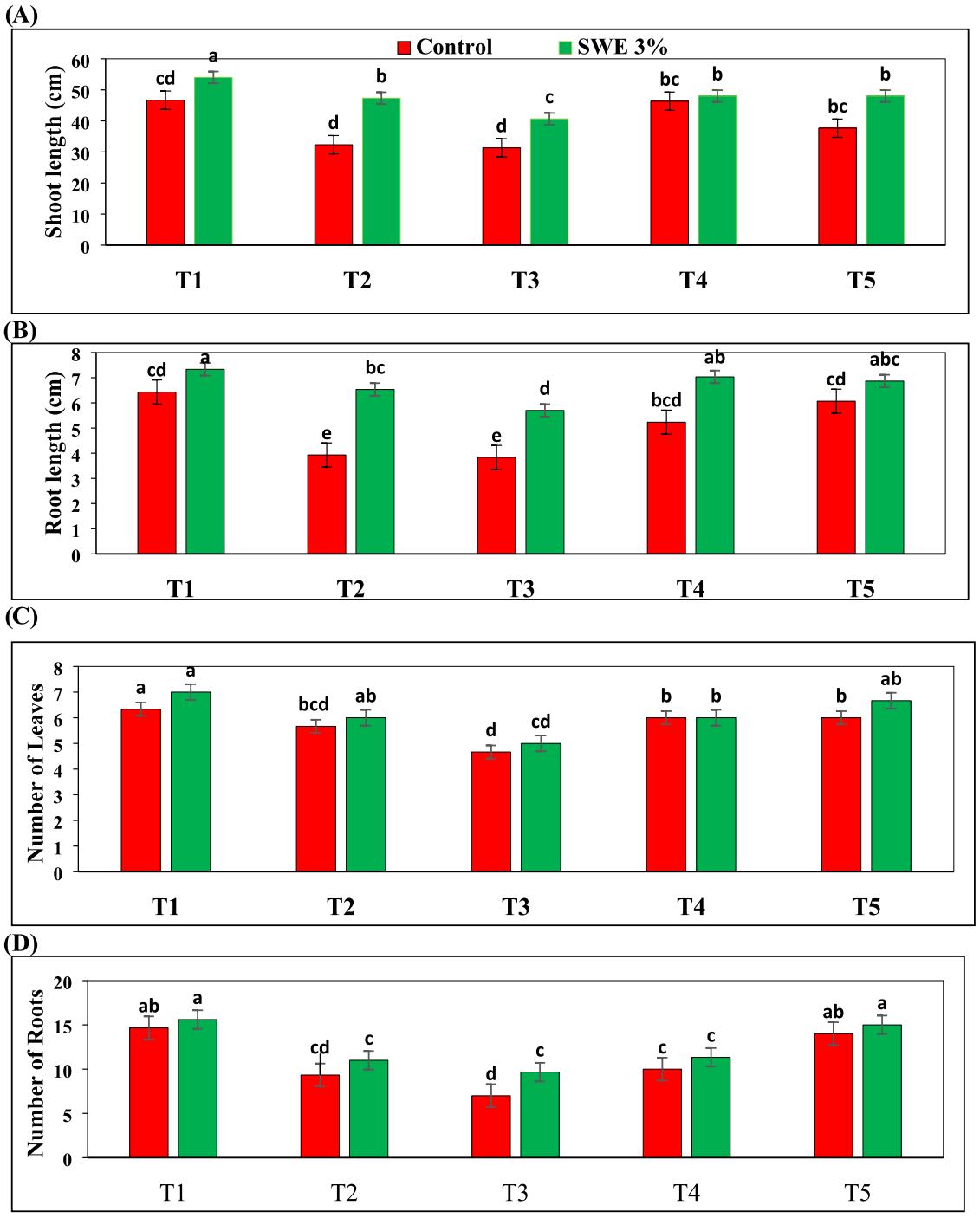

Figure 2. Effect of sorghum water extract application on the shoot length (A), root length (B), leaves (C), and number of roots (D) of the maize plant. Data are the mean value of three replications (n = 3) along with standard errors (SE). The lowercase letters on the bars indicate the significant differences among means (LSD test). T_1: control, T_2: 50 mM NaCl, T_3: 100 mM NaCl, T_4: 250 μM $PbCl_2$, and T_5: 500 μM $PbCl_2$.

conditions (100 mM) with the application of SWE (Table 4). The activity of CAT and APX also showed a substantial increase under Pb and saline conditions. Moreover, the foliar spray of SWE significantly increased the CAT and APX under both Pb and saline stress conditions (Table 4).

3.5. Ionic Concentration. The results showed that the maximum Pb concentration (15.23 mg kg^{-1} DW) was recorded in 500 μM Pb stress, followed by 250 μM Pb stress (12.13 mg kg^{-1} DW). The foliar spray of SWE reduced the Pb accumulation (Table 3). The maximum concentration of Na and Cl was observed in 100 mM salinity stress without a foliar spray of SWE and followed by 50 mM salinity stress, and the application of SWE reduced Na and Cl concentration (Figure 4). The potassium concentration showed an opposite trend as compared to Na and Cl. The concentration of potassium was significantly decreased under Pb and salinity stress; however, an exogenous spray of SWE appreciably increased the uptake and accumulation of K in saline and Pb stress (Figure 4).

4. DISCUSSION

Lead and salinity stress caused a marked decrease in maize growth. This reduction in growth was linked with increased

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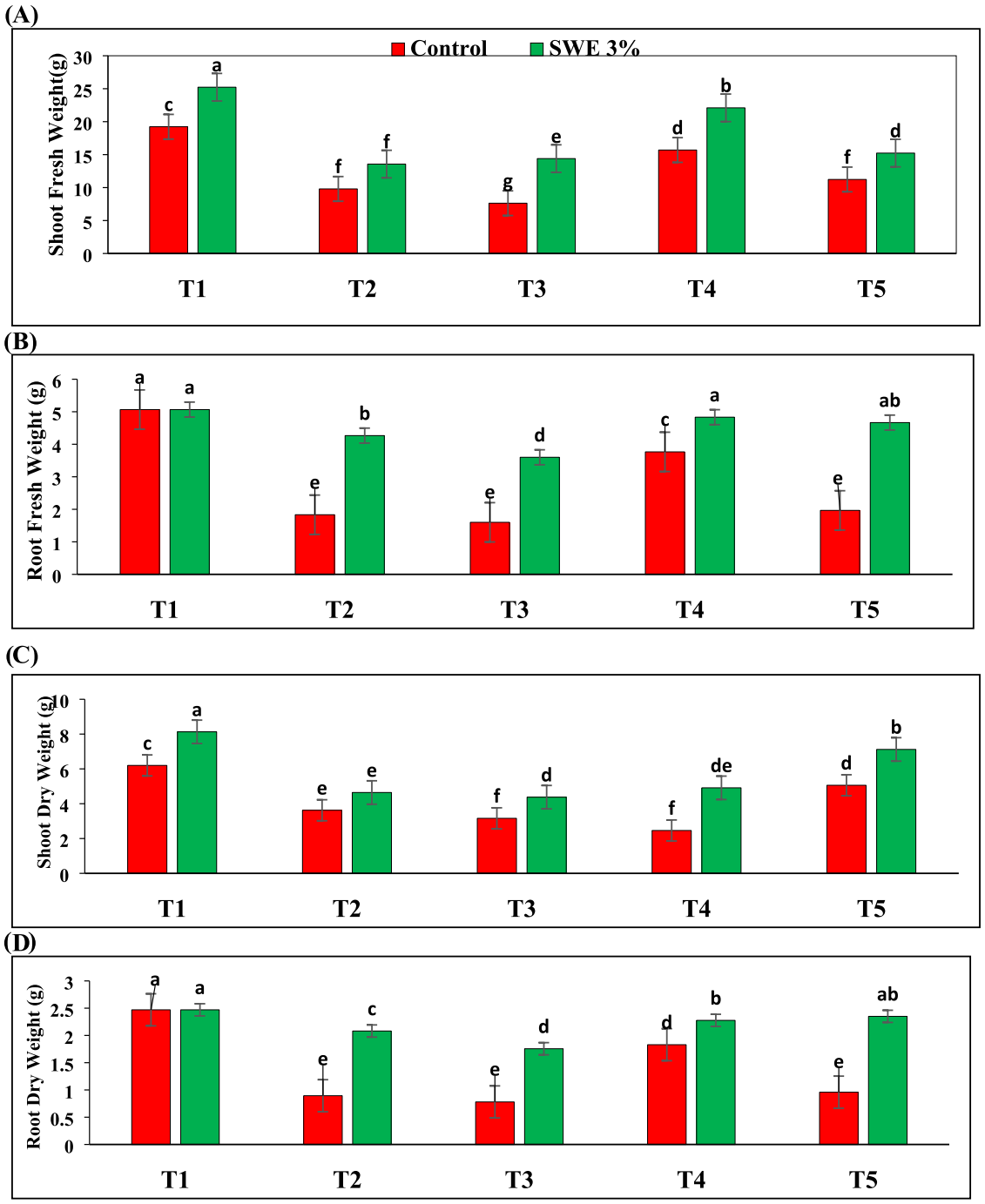

Figure 3. Effect of sorghum water extract application on the shoot fresh weight (A), root fresh weight (B), shoot dry weight (C), and root dry weight (D) of the maize plant. Data are the mean value of three replications (n = 3) along with standard errors (SE). The lowercase letters on the bars indicate the significant differences among means (LSD test). T_1: control, T_2: 50 mM NaCl, T_3: 100 mM NaCl, T_4: 250 μM $PbCl_2$, and T_5: 500 μM $PbCl_2$.

EL, MDA, and H_2O_2 production and disturbed nutrient homeostasis and antioxidant activities (Figures 1 and 2). Lead is a serious toxic that disturbs the soil microbial activity and plant functioning, resulting in a reduction in plant growth.[51,52] Salinity stress also induced ionic toxicity and increased ROS production (Table 2), which caused a marked reduction by decreasing nutrient and water uptake.[53] The foliar application of SWE appreciably improved the root and shoot growth of maize plants growing under Pb and salinity stress (Figures 1 and 2). The present increase in growth following SWE application could be related to the synthesis of proteins, enzymes, ATP, antioxidant activities, osmolyte accumulation, and nutrient uptake.[55] These outcomes are the same as the results of Ahmad et al.;[54] they also found that the application of SWE markedly improved the root and shoot growth and biomass production under stress conditions. Nonetheless, some authors like Mokhtari et al.[56] and Khaliq et al.[57] found that SWE can reduce germination and seedling growth owing to the presence of allelochemicals in SWE.

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Table 1. Effect of Sorghum Water Extract Application on Photosynthetic Pigments in the Leaf of the Maize Plant[a]

		photosynthetic pigments (mg/g FW)			
SWE application		Chl *a*	Chl *b*	total Chl	Car
SWE 0%	no stress	$0.178^{cde} \pm 0.01$	$0.119^{ab} \pm 0.01$	$0.325^{ab} \pm 0.03$	$1.65^{a} \pm 0.12$
	50 mM NaCl	$0.161^{de} \pm 0.01$	$0.168^{ab} \pm 0.03$	$0.409^{a} \pm 0.06$	$1.53^{a} \pm 0.28$
	100 mM NaCl	$0.145^{e} \pm 0.01$	$0.137^{ab} \pm 0.04$	$0.362^{ab} \pm 0.09$	$1.37^{a} \pm 0.03$
	250 μM $PbCl_2$	$0.253^{ab} \pm 0.05$	$0.199^{ab} \pm 0.03$	$0.478^{a} \pm 0.05$	1.77a ± 0.47
	500 μM $PbCl_2$	$0.223^{abc} \pm 0.01$	$0.125^{ab} \pm 0.01$	$0.330^{ab} \pm 0.02$	$1.46^{a} \pm 0.06$
SWE 3%	no stress	$0.206^{a-e} \pm 0.01$	$0.102^{b} \pm 0.01$	$0.252^{b} \pm 0.03$	$2.13^{a} \pm 0.23$
	50 mM NaCl	$0.239^{abc} \pm 0.03$	$0.138^{ab} \pm 0.01$	$0.432^{a} \pm 0.10$	$1.93^{a} \pm 0.55$
	100 mM NaCl	$0.222^{abcd} \pm 0.05$	$0.178^{ab} \pm 0.05$	$0.357^{ab} \pm 0.02$	$1.90^{a} \pm 0.38$
	250 μM $PbCl_2$	$0.206^{bcde} \pm 0.02$	$0.282^{a} \pm 0.20$	$0.487^{a} \pm 0.01$	$1.63^{a} \pm 0.03$
	500 μM $PbCl_2$	$0.279^{a} \pm 0.03$	$0.251^{ab} \pm 0.04$	$0.362^{ab} \pm 0.02$	$2.13^{a} \pm 0.43$

[a]Data are the mean values of three replications (n = 3) along with standard errors (SE). The lowercase letters indicate the significant differences among means (LSD test). Chl = chlorophyll, Car = carotenoids, and FW = fresh weight.

Table 2. Effect of Sorghum Water Extract Application on Relative Water Contents, Electrolyte Leakage, and Oxidative Stress Marker of Maize Plants[a]

		RWC	EL	MDA	H_2O_2
SWE application		(%)	(%)	(μmol/g FW)	(μmol/g FW)
SWE 0%	no stress	$80.33^{ab} \pm 1.69$	$16.67^{e} \pm 0.47$	$2.23^{bc} \pm 0.13$	$3.20^{ab} \pm 0.18$
	50 mM NaCl	$66.33^{d} \pm 1.24$	$41.33^{b} \pm 1.24$	$2.60^{bc} \pm 0.40$	3.54^{ab} +0.46
	100 mM NaCl	$66.00^{de} \pm 1.63$	$48.00^{a} \pm 2.16$	$2.95^{ab} \pm 0.43$	3.77^{ab} +0.56
	250 μM $PbCl_2$	$62.00^{ef} \pm 2.16$	$41.67^{b} \pm 3.29$	$2.47^{bc} \pm 0.27$	3.76^{ab} +0.57
	500 μM $PbCl_2$	$60.33^{f} \pm 2.05$	$46.67^{a} \pm 0.79$	$3.10^{a} \pm 0.07$	3.86^{a} + 0.49
SWE 3%	no stress	$81.33^{a} \pm 1.88$	$15.67^{e} \pm 2.62$	$1.97^{c} \pm 0.01$	$2.66^{b} \pm 0.01$
	50 mM NaCl	$76.33^{b} \pm 2.05$	$35.67^{d} \pm 0.82$	$2.86^{abc} \pm 0.49$	$3.40^{ab} \pm 0.07$
	100 mM NaCl	$71.66^{c} \pm 1.69$	$41.00^{bc} \pm 1.24$	$2.55^{bc} \pm 0.45$	$3.92^{a} \pm 0.45$
	250 μM $PbCl_2$	$69.00^{cd} \pm 3.55$	$37.33^{cd} \pm 1.86$	$2.62^{bc} \pm 0.41$	$3.28^{ab} \pm 0.18$
	500 μM $PbCl_2$	$59.67^{f} \pm 1.69$	$41.67^{b} \pm 1.96$	$2.73^{abc} \pm 0.57$	$3.72^{ab} \pm 0.55$

[a]Data are the mean values of three replications (n = 3) along with standard errors (SE). The lowercase letters indicate the significant differences among means (LSD test). RWC = relative water content, EL = electrolyte leakage, MDA = malondialdehyde, H_2O_2 = hydrogen peroxide, and FW = fresh weight.

Table 3. Effect of Sorghum Water Extract Application on Osmolyte Accumulation of the Maize Plant[a]

		TSP	TFA	protein content	proline content
SWE application		(mg/g FW)	(mg/g FW)	(mg/g FW)	(μg/g FW)
SWE 0%	no stress	$1.61^{a} \pm 0.47$	$19.63^{a} \pm 1.54$	5.54^{bcd} +0.35	$200.17^{a} \pm 19.06$
	50 mM NaCl	$1.32^{a} \pm 0.43$	$19.44^{a} \pm 0.94$	7.00^{ab} +0.77	$188.00^{a} \pm 11.64$
	100 mM NaCl	$0.98^{a} \pm 0.21$	$16.43^{b} \pm 0.31$	7.34^{a} + 0.58	$170.13^{a} \pm 5.24$
	250 μM $PbCl_2$	$0.96^{a} \pm 0.02$	$17.38^{b} \pm 0.03$	4.59^{d} + 0.13	$178.31^{a} \pm 0.40$
	500 μM $PbCl_2$	$0.91^{a} \pm 0.14$	$16.70^{b} \pm 0.10$	5.31^{cd} +0.05	$165.86^{a} \pm 0.38$
SWE 3%	no stress	$1.71^{a} \pm 0.44$	$20.83^{a} \pm 0.34$	$5.87^{abcd} \pm 0.68$	$218.73^{a} \pm 10.74$
	50 mM NaCl	$1.41^{a} \pm 0.01$	$18.33^{ab} \pm 1.44$	$7.13^{ab} \pm 0.83$	$178.03^{a} \pm 2.62$
	100 mM NaCl	$1.39^{a} \pm 0.45$	$17.81^{ab} \pm 0.83$	$6.56^{abc} \pm 0.88$	$199.77^{a} \pm 35.12$
	250 μM $PbCl_2$	$1.48^{a} \pm 0.56$	$20.53^{a} \pm 0.65$	$5.98^{abc} \pm 0.64$	$199.07^{a} \pm 9.22$
	500 μM $PbCl_2$	$1.41^{a} \pm 0.45$	$17.21^{b} \pm 0.49$	$8.07^{a} \pm 0.20$	$172.47^{a} \pm 4.56$

[a]Data are the mean values of three replications (n = 3) along with standard errors (SE). The lowercase letters indicate the significant differences among means (LSD test). TSS = total soluble sugars, TFA = total free amino acids, MDA = malondialdehyde, and FW = fresh weight.

The results indicate that Pb stress and salinity stress significantly reduced the photosynthesis of pigments. Lead stress induces phytotoxicity in leaves by degrading chlorophyll contents, and it also damages the photosynthetic apparatus, therefore leading to a reduction in chlorophyll synthesis.[58] Salinity stress also reduced the synthesis of photosynthetic pigments, which could be attributed to increased ROS production (Table 2) that damages the photosynthetic apparatus and degrades chlorophyll, resulting in a reduction in chlorophyll synthesis.[53,59] The exogenous supply of SWE improved the synthesis of photosynthetic pigments under both stresses, which is consistent with the findings of Tahira et al.[60] They found that the application of allelochemicals improves chlorophyll synthesis by reducing ROS production and decreasing the activity of chlorophyll degrading enzymes.

Lead and salinity stress increased EL due to an increase in the levels of MDA and H_2O_2 production. Abiotic stresses induce oxidative stress and result in increases in EL owing to an increase in MDA and H_2O_2 production.[53] Nonetheless, foliar-applied SWE ameliorated Pb and salinity-induced

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Table 4. Effect of Sorghum Water Extract Application on Antioxidant Activity and Pb Concentration in the Maize Plant[a]

SWE application		SOD (min^{-1} g^{-1} FW)	POD (min^{-1} g^{-1} FW)	CAT (min^{-1} g^{-1} FW)	APX (min^{-1} g^{-1} FW)	Pb (mg kg^{-1} DW)
SWE 0%	no stress	$59.27^{abc} \pm 6.17$	$33.67^{b} \pm 0.15$	$49.25^{b} \pm 1.37$	$1.17^{a} \pm 0.06$	0
	50 mM NaCl	$57.14^{abc} \pm 3.65$	$40.47^{ab} \pm 5.39$	$53.31^{ab} \pm 4.14$	$1.24^{a} \pm 0.14$	0
	100 mM NaCl	$64.27^{ab} \pm 5.41$	$43.87^{a} \pm 4.80$	$54.52^{ab} \pm 4.53$	$1.33^{a} \pm 0.10$	0
	250 μM $PbCl_2$	$53.93^{bc} \pm 1.32$	$36.87^{ab} \pm 2.77$	$51.44^{ab} \pm 5.03$	$1.13^{a} \pm 0.01$	$10.36^{c} \pm$
	500 μM $PbCl_2$	$56.63^{abc} \pm 3.34$	$38.63^{ab} \pm 2.71$	$52.26^{ab} \pm 5.84$	$1.34^{a} \pm 0.01$	$15.23^{a} \pm$
SWE 3%	no stress	$49.57^{c} \pm 0.20$	$35.63^{ab} \pm 1.18$	$48.93^{b} \pm 1.32$	$1.08^{a} \pm 0.10$	0
	50 mM NaCl	$57.71^{abc} \pm 3.37$	$40.62^{ab} \pm 5.48$	$54.50^{ab} \pm 4.45$	$1.30^{a} \pm 0.13$	0
	100 mM NaCl	$57.95^{abc} \pm 5.20$	$43.47^{ab} \pm 4.94$	$59.21^{a} \pm 0.32$	$1.40^{a} \pm 0.10$	0
	250 μM $PbCl_2$	$55.48^{bc} \pm 0.12$	$40.83^{ab} \pm 5.46$	$49.93^{b} \pm 4.13$	$1.08^{a} \pm 0.06$	$8.9^{d} \pm$
	500 μM $PbCl_2$	$65.10^{a} \pm 5.88$	$40.95^{ab} \pm 5.74$	$52.46^{ab} \pm 4.98$	$1.31^{a} \pm 0.08$	$12.13^{b} \pm$

[a]Data are the mean values of three replications (n = 3) along with standard errors (SE). The lowercase letters indicate the significant differences among means (LSD test). SOD = superoxide dismutase, POD = peroxidase, CAT = catalase, APX = ascorbate peroxidase, and FW = fresh weight.

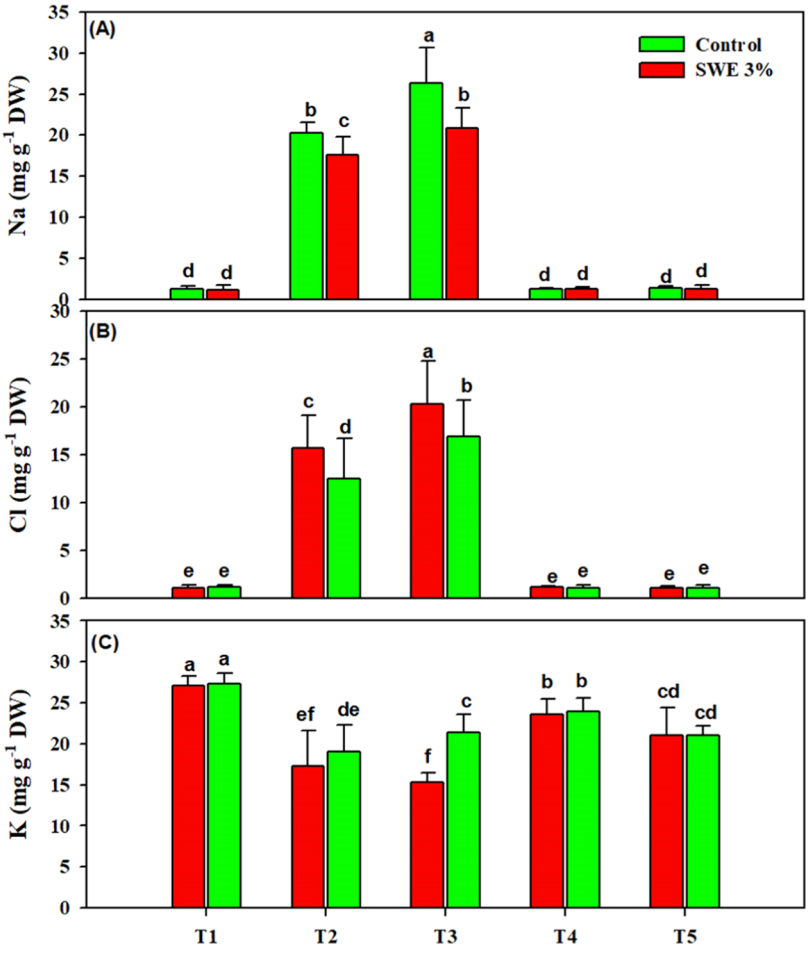

Figure 4. Effect of sorghum water extract application on Na^+ (A), Cl^- (B), and K^+ (C) concentrations of the maize plant. Data are the mean value of three replications (n = 3) along with standard errors (SE). The lowercase letters on the bars indicate the significant differences among means (LSD test). T_1: control, T_2: 50 mM NaCl, T_3: 100 mM NaCl, T_4: 250 μM $PbCl_2$, and T_5: 500 μM $PbCl_2$.

oxidative damages and reduced the EL by decreasing MDA and H_2O_2 production.[60] The increase in osmolyte synthesis and antioxidant activities is an important mechanism used by plants to mitigate the toxic impacts of abiotic Pb and salinity stress.[61−63] The osmolyte synthesis and antioxidant activities were increased in response to Pb and salinity stress. The foliar spray of SWE also caused a marked increase in osmolyte synthesis and antioxidant activities. Similarly, Mokhtari et al.[56] also found that antioxidant activities except CAT were significantly increased under saline conditions with SWE, while Qian et al.[64] found a decrease in POD and SOD in wheat with the application of allelochemicals.

The excessive concentration of Na^+ negatively affects plant metabolic and physiological functioning; therefore, Na^+/K^+ homeostasis is essential for better growth.[65] In the present study, application of SWE appreciably reduced the Na^+ and Cl^- accumulation and increased the accumulation of K, which is consistent with the findings of Rathod et al.[66] The foliar

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spray of SWE significantly improved the uptake of K under both Pb and salinity stress, which can be due to a reduction in the K^+ efflux.[41,67] However, the mechanism through which SWE reduced the uptake of Na^+ and Cl^- and increased the uptake of K^+ must be explored in future studies. The accumulation of Pb in maize plants was increased under Pb stress; however, foliar application of SWE appreciably reduced the Pb accumulation and resulted in a marked improvement in plant growth.

5. CONCLUSIONS

Lead and salinity stress reduced maize growth by causing ionic imbalance and increasing reactive oxygen species production, oxidative stress markers, and uptake of toxic ions. The application of the sorghum water extract effectively enhanced the growth of maize plants under lead and salinity stress by increasing photosynthetic pigments, antioxidant activities, and nutrient homeostasis and reducing the uptake of toxic ions. Hence, the use of the sorghum water extract as foliar application could be an effective strategy for mitigating the harmful impacts of lead and salinity stress. More field studies are direly needed before making recommendations for the farming community. Besides this, metabolomics, proteomics, and transcriptomics investigations are needed to explore the mechanisms of improved photosynthesis, antioxidant activities, and nutrient homeostasis with the sorghum water extract under lead and salinity stress.

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S.R. contributed to conceptualization; S.S. contributed to methodology; S.R. contributed to software and writing—original draft preparation; S.R., F.R., S.S., A.N.S., M.N., and M.U.H. contributed to data analysis, visualization, methodology, and writing—review and editing; and H.A.S.A., W.L., A.G., M.A., A.A.R., S.E., R.S.A., and S.H.Q. All authors have read and agreed to the published version of the manuscript.

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