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Research Article

Organic amendments mitigate salinity induced toxic effects in maize by modulating antioxidant defense system, photosynthetic pigments and ionic homeostasis

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Abstract

Salinity stress (SS) is a major and increasing abiotic stress adversely affecting plant growth and productivity across the globe. The application of organic amendments (OA) is considered to be an important practice to mitigate the adverse impacts of SS. Therefore, this study was performed to assess the impact of different OA on growth, physiology and anti-oxidant activities of maize plants grown under SS. The study was comprised of diverse SS levels; 0, 6 and 12 dS m^{-1}, and different OA; control, cow manure (CM: 5%), sugarcane pressmud (SPM: 5%) and combination of CM (2.5%) + SPM (2.5%). The study was conducted in a completely randomized design with factorial arrangement having three replications. The results indicated that SS reduced the growth and biomass production, relative water contents (RWC), chlorophyll contents, free amino acids (FAA), total soluble proteins (TSP) and increased the electrolyte leakage (EL: 40.92%), hydrogen peroxide (H_2O_2: 68.49%), malondialdehyde (MDA: 42.13%), and catalase (CAT: 34.24%) and ascorbate peroxide (APX: 25.70%). The application of OA significantly improved the maize growth under SS. However, the application of SPM (5%) significantly increased growth and biomass production by decreasing EL, MDA and

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H_2O_2 accumulation and increasing RWC, chlorophyll, CAT, POD, and anthocyanin, TSP and FAA. These findings suggest that application of SPM improved the growth and biomass production of maize by improving anti-oxidant activities, photosynthetic pigments, TSP, FAA reducing lipid peroxidation and EL.

Keywords: antioxidants; growth maize; organic amendments; photosynthetic pigments

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Introduction

Salinity stress is a major abiotic stress that is posing a serious threat to agricultural productivity and sustainability globally (Sultan *et al.*, 2021). The total area of salt affected soils is increasing at the rate of 8-10 million every year which is a serious concern for global crop production and food security (Machado and Serralheiro, 2017; Ivushkin *et al.*, 2019). Salinity stress is a major abiotic stress which also imposes undesirable effects on soil properties (Machado and Serralheiro, 2017; Shahid *et al.*, 2018; Seleiman *et al.*, 2022). However, this effect could be increased owing to climate change linked with increasing temperature and a decrease in rainfall (Corwin, 2021). Besides this, the use of poor quality water and poor fertilizer management further intensifies this problem (Corwin, 2021). Globally, 20% of irrigated soils producing one third of the world's food are salt affected which is serious threat to crop production (Machado and Serralheiro, 2017; Shahid *et al.*, 2018).

Salinity stress disturbs nutrient uptake and transportation which effectuate various physiological processes and leading to significant reduction in growth and yield (Alsaeedi *et al.*, 2019; Taher *et al.*, 2018). Soil salinity also induce osmotic stress (Sarker and Oba, 2020) and oxidative damages and resultantly reduce the crop growth and productivity (Sarker and Oba, 2018, 2019). Salinity induced osmotic and oxidative stress reduced the photosynthetic efficiency (Sarker *et al.*, 2018) and cause damages to plant proteins, membranes and DNA (Sarker *et al.*, 2018; Hassan *et al.*, 2019; Hassan *et al.,* 2020; Batool *et al.*, 2022a; Batool *et al.*, 2022b; Qari *et al.*, 2022). Despite this SS also damage the structure of chloroplast and reduce the plant photosynthetic efficiency and leading to serious reduction crop production (Hurtado *et al.*, 2019). Moreover, SS also reduce the root and shoot growth owing to reduction in nutrient and water uptake (Ramadan *et al.*, 2019; Taha *et al.*, 2020). The high concentration of salts in soil cause deficiency of nutrients like potassium (K^+) by increasing K^+ exclusion from the cells and decreasing the K^+ uptake (Ma *et al.*, 2016). However, plants use different approaches including reduced Na^+ uptake, and increasing osmolytes accumulation and anti-oxidant activities to reduce the salinity induce damages (Siddiqui *et al.*, 2017). Therefore, appropriate measures must be used to reduce the effects of SS for ensuring better crop productivity.

The application of organic amendments (OA) is considered to be an important approach to reduce the negative effects of SS owing to their beneficial impacts on soil physio-chemical and biological properties (Leogrande and Vitti, 2019). Globally, different OA including sugarcane press (SPM), cow manure (CM), sewage sludge and farmyard manure (FYM) are used to reclaim the salt affected soils (Hassan *et al.*, 2021; Khan *et al.*, 2022). SPM is by-product of the sugarcane industry and field application of SPM has shown positive effects on soil health and nutrient uptake (Chattha *et al.*, 2019; Chattha *et al.*, 2021; Sher *et al.*, 2022). The application of SPM improves the uptake of nutrients, microbes' activity and concentration of soil organic matter (SOM) (Chattha *et al.*, 2019; Nawaz *et al.*, 2017) thereby improve crop production in saline soils. The field application of SPM improves salinity tolerance by increasing membrane integrity, leaf water status, plant photosynthetic efficiency, and osmolytes accumulation (Sheoran *et al.*, 2021a). Moreover, SPM also mobilizes the soil $CaCO_3$ to maintain the optimum Ca^{2+} availability which ensures the leaching of Na^+ and improves the soil structure stability (Muhammad and Khattak, 2009). Likewise, FYM including CM is also considered a safe way to improve crop productivity, soil fertility and resistance against SS (Baddour *et al.*, 2017). The application of CM improves soil physio-chemical properties and ensures better plant functioning and leading to a significant increase in salinity stress (Baddour *et al.*, 2017).

Maize (*Zea mays*) is an important crop cultivated globally for food, feed and bio-energy purposes (Zahra *et al.*, 2020). It is also used to prepare different by-products including corn oil, glucose and starch which have a big use in our daily life (Zahra *et al.*, 2020). Moreover, maize is considered to be sensitive to SS and limited studies are available about the effect of SPM and CM on the performance of maize crop grown under SS. It is mandatory to explore the mechanism lying behind SPM and CM mediated salinity tolerance in maize crop. We hypothesized that SPM and CM application could alleviate the toxic effects of SS in maize crop. Thus, this study was performed to determine the impact of SPM and CM on growth, physiological traits, antioxidant activities, oxidative stress markers, and accumulation of TSP and FAA under SS.

Materials and Methods

Experimental details

A pot study was performed at the wire house Faculty of Agriculture to explore the effect of various OA on maize crop grown under SS. The completely randomized design (CRD) having two-factor factorial arrangements with three replications were used to perform study. A synthetic variety of maize (Malika) was used as a test crop and five seeds were sown in each pot. The maize variety was salt sensitive and it was collected from Ayub Agricultural Research Institute, Faisalabad. The study was comprised of different SS levels, control, 6 and 12 dSm^{-1} and OA; control, CM (5%), SPM (5%) and CM (2.5%) + SPM (2.5%).

Soil collection and imposition of salinity stress

The soil was collected with a spade from a depth of 1-10 cm and stored after that soil and silt were mixed in 3:1 ratio to fill the pots. The soil was sandy loam with pH 7.81, organic matter 0.82%, total nitrogen (N), available phosphorus (P) and potassium (K) 0.041%, 6.51 ppm and 159 ppm, respectively. The quantity of salts to achieve desired SS levels was calculated by following formula:

$$NaCl\ required\ \left(\frac{g}{kg}\right) = \frac{TSS \times 58.5 \times Saturation(\%)}{100 \times 1000}$$

To determine the soil saturation; a soil paste was made by adding distilled water into the soil and mixed it well with spatula and leave it for two hours to attain equilibrium. Afterward mixture was filtered to obtain the extract and later on soil sample was oven dried and soil saturation was measured with the following formula:

$$Saturation\ (\%) = \frac{Loss\ in\ soil\ weight\ on\ drying}{Weight\ of\ soil\ after\ drying} \times 100$$

Moreover, TSS was determined by given below formula. In given below formula EC_1 is refereeing to electrical conductivity (EC) of original soil and EC_2 is the EC that has to be attained as per treatments.

TSS (total soluble salts) = $EC_2 - EC_1 \times 10$

To achieve the respective levels of salinity NaCl and Na_2SO_4 salts were used. At the control level, no salt was added, whereas at 6 dS m^{-1} SS; NaCl and Na_2SO_4 were added at the rate of 1.8 g/kg 0.32 g/kg respectively. Moreover, in the case of 12 dS m^{-1} SS; NaCl and Na_2SO_4 were added at the rates of 2.34 g/kg and 0.64 g/kg. After adding salts pots were covered with a plastic sheet and allowed for three days to reach equilibrate.

Collection and application of OA and crop husbandry

After three days of addition of salt into the soil, OA (CM, SPM and CM+SPM) were added to pots as per treatments. We collected the CM from Agronomy Farm of University of Agriculture Faisalabad whereas SPM was collected from Madina sugar mills Ltd. Faisalabad. To achieve the respective levels of OA (5% CM and 5% SPM); 500g of CM and SPM was added in 10 kg soil, whereas in combined application of CM+SPM; we used CM and SPM at the rate of 250 g in each pot. The fertilizers di-ammonium phosphate and sulphate of potash was used at the rate of 5.50 and 1.82 g respectively. The pots were regulated visited and irrigation was applied with hand sprayer.

Collection of growth traits

We selected and harvested three plants from each experimental pot and shoot length and stem diameter was measured. After that those plants were separated into roots and shoots and weighed for determination of fresh weights afterward these plant parts were oven dried for determination of dry weights. The plants were harvested at a seedling stage after twenty-five days of application of OA.

Relative water contents and electrolyte leakage

Fresh leaves of maize were plucked weighed for determination of fresh weight (FW). After those leaves were dipped into the distal water for 24 hours. After that these leaves were removed from the water and wiped out the excess water and the leaf turgid weight was recorded (TW). Then these leaves were sun dried and packed in small paper bags, placed in a heating oven at 70 °C for three hours for determination of dry weight (DW) and RWC was determined with the given below formula:

$$RWC(\%) = \frac{(FW - DW)}{(TW - DW)} \times 100$$

Fresh leaves were plucked from three plants of each pot randomly to determine the electrical conductivity (EL) by following the method of Yan *et al.* (1996). Fresh leaves of 0.3 g were chopped into small pieces, dipped in a test tube and added with 25 ml of distal water after two hours EC_1 was obtained using EC meter. Then the test tubes were left for 24 hours, after that these were heated and EC_2 was obtained using the EC meter and EL was measured by subsequent formula: $EC\% = (EC1 \div EC2\ ) \ \times 100.$

Photosynthetic pigments

To determine chlorophyll and carotenoid contents Arnon (1949) method was used. Fresh leaves (0.25 g) were chopped into small pieces and dipped into 2.5 ml of 80% acetone overnight. Then the 200 µl of extract from each sample was loaded into Elisa plate and noted the absorbance at 663, 645 and 480 nm on a spectrophotometer to determine chlorophyll and carotenoid contents.

MDA and H_2O_2 contents

H_2O_2 concentration in plant samples was determined by the methods of Velikova *et al.* (2000). A fresh plant sample (0.5 g) was homogenized in 5 ml of 5% tri-chloric acid in a pestle and mortar under and centrifuged for 15 minutes. Afterward we took 1 ml of supernatant and added this with 1 ml of 1 M potassium iodide (KI) and 100 µl of potassium phosphate. Afterward we allowed the mixture at room absorbance was noted at 390 nm. To determine MDA; we took plant samples (0.5 g) and ground them with 5 ml of TCA and centrifuged for 15 minutes at 12000 rpm. Later on mixture having 1 ml plant extract and 1 ml TCA was heated at 100 °C and then cooled in an ice bath and absorbance was taken to determine MDA contents (Rao and Sresty, 2000).

Antioxidant activities

The activity of APX was determined by the methods of Asada (1987). Fresh leaves (0.5 g) of maize plants were finely grounded in a pestle and mortar under cold conditions. Then centrifuged the crud sample at 15,000 rpm for 15 minutes at 4 °C and supernatant was collected and stored at -20 °C. To access the APX activity 700 µl of potassium phosphate buffer (50 mM) was added in a test tube and treated with 100 µl of ascorbic acid and 6.1 mM of H_2O_2. Then the 100 µl of plant sample was added with mixture absorbance was taken at 290 nm.

To determine CAT the technique of Chance (1955) was used. Fresh plant material (0.5 g) was homogenized using for 15 minutes at 15,000 rpm using 5 ml of potassium phosphate buffer (50 mM) having pH 7.0 and the supernatant was stocked at -20 °C for determination of enzymatic activity. To access the activity of CAT 2.5 ml of potassium phosphate buffer was added in a test tube and 100 µl of (5.1 mM) H_2O_2 and 100 µl of plant sample were added in a test tube rapidly checked the absorbance at 290 nm using a spectrophotometer.

Total soluble proteins and free amino acids

To access the TSP Bradfords (1976) method was used. Fresh maize plant leaves (0.5 g) were grounded in a pestle and mortar by adding 5 ml of potassium phosphate buffer (pH: 7, 50 mM) and centrifuged at 15,000 rpm for 15 minutes at 4 °C. After that we took supernatant in test tubes and Bradford mixture (2 ml) was added to the supernatant and then absorbance was recorded at 595 nm. Moore and Stein (1954) technique was followed for the determination of FAA of maize plants. Fresh leaves (0.5 g) were homogenized using a pestle and mortar and placed in an ice bath by adding 5 ml of potassium phosphate buffer (50 mM). Then the homogenate was centrifuged at 15,000 rpm for 15 minutes at 4 °C. The plant extract was treated with 1 ml of 2% ninhydrin and 10% pyridine solution in a test tube. These test tubes containing a mixture was covered with aluminum foil and heated in a water bath at 90 °C for 30 minutes. After heating, the volume of the mixture was brought up to 20 ml by adding distilled water and absorbance was recorded at 570 nm.

Ionic analysis

The plant samples were taken and oven dried afterward they were ground to make powder. Later on, 0.5 g maize plant samples were taken on and digested on hot plant (180 °C) by adding a mixture of acids (HCl and HNO_3; 1:2). Afterward, concentration of Ca^{2+}, Na^+ and K^+ were measured by a flame photo-meter whereas the concentration of Cl- was measured by a chloride analyzer.

Statistical analysis

The data on diverse collects traits analyzed by analysis of variance technique and least significance difference (LSD) test was used to assess the significance among means at 5% probability level (Steel *et al.*, 1997).

Results

Growth attributes

Salinity stress (SS) significantly decreased the growth traits of maize plants as compared to control (Table 1). The results showed that plant height (PH: 13.24% and 35.82%), shoot fresh weight (SFW: 24.83% and 77.24%), shoot dry weight (SDW: 33.72% and 80.68%), root fresh weight (RFW: 24.37% and 55.35%), root dry weight (RDW: 28.14% and 58.12%) and stem diameter (SD: 19.56% and 34.37%) was decreased under 6 dS m^{-1} and 12 dS m^{-1} salinity respectively. The application of all the OA appreciably improved the growth traits under SS and control conditions, however, SPM (5%) remained the top performer in this regard (Table 1). Under 6 dS m^{-1} SS the application of SPM increased the plant height, SFW, SDW, RFW, RDW and SD by 43.42%, 95.16%, 96.40%, 97.47%, 99.68% and 66.18%, respectively (Table 1). Similarly, application of 5% SMP improved PH (40.66%), SFW (95.31%), SDW (93.84%), RFW (96.87%), RDW (99.69%) and SD (75.56%) under 12 dSm^{-1} SS as compared to control (Table 1).

Table 1. Impact of various organic amendments of the growth attributes of maize crop under salinity stress

Salinity Stress	OA	SL (cm)	SD (cm)	SFW (g)	RFW (g)	SDW (g)	RDW (g)
Control	Control	36.73± 0.99^{g}	2.06± 0.07^{g}	12.68±0.37^{i}	4.38±0.14^{i}	1.76±0.61^{gh}	0.81±0.03^{i}
	CM	65.79 ± 0.74^{cd}	4.70± 0.05^{b}	49.15±1.05^{e}	41.66±0.53^{f}	16.09±0.45^{d}	17.61e±0.23^{e}
	SPM	87.08 ± 0.88^{a}	5.66±0.07^{a}	124.16±1.06^{a}	115.40±0.31^{a}	30.94±0.59^{a}	50.78±0.47^{a}
	CM+SPM	71.46 ± 0.70^{bc}	4.87±0.03^{b}	60.72±1.21^{c}	67.62±0.32^{c}	19.47±0.59^{c}	25.89±0.39^{c}
6 dS m^{-1}	Control	31.83±0.79^{g}	1.60±0.05^{h}	4.62±0.23^{j}	2.38 ±0.23^{ij}	0.86±0.04^{h}	0.11±0.04^{i}
	CM	56.52±0.73^{e}	3.70±0.05^{d}	29.42±0.77^{g}	30.35±0.40^{g}	7.05±0.72^{f}	14.45±0.10^{f}
	SPM	73.31±0.80^{b}	4.73±0.07^{b}	95.37±1.17^{b}	93.90±0.47^{h}	24.04±0.69^{b}	35.21±0.77^{b}
	CM+SPM	64.86±0.88^{d}	3.89±0.04^{d}	56.06±1.57^{d}	46.63±0.41^{e}	13.28±0.56^{e}	18.30±0.39^{e}
12 dS m^{-1}	Control	24.44 ±0.79^{h}	1.02±0.02^{i}	1.61±0.03^{j}	1.74±0.04^{j}	0.45±0.04^{h}	0.07±0.00^{j}
	CM	35.72±0.62^{g}	2.70±0.05^{f}	4.00 ±0.08^{j}	16.44±0.17^{h}	2.09±0.04^{gh}	6.92±0.02^{h}
	SPM	60.10±0.99^{de}	4.20±0.05^{c}	34.27±1.83^{f}	55.48±0.47^{d}	7.31±1.25^{f}	23.53±0.15^{d}
	CM+SPM	47.31±0.76^{f}	3.43±0.16^{e}	16.30±0.27^{h}	28.63±0.18^{g}	3.33±0.20^{g}	9.30±0.12^{g}

The data is the mean of three replicates with S.E. (±) and diverse letters indicating significance at 0.05 P level. OA: organic amendments, CM: cow manure, SPM: sugarcane press-mud. SL: shoot length, SD: stem diameter, SFW: shoot fresh weight, RFW: root fresh weight, SDW: shoot dry weight, RDW: root dry weight.

Physiological attributes

The results indicated that SS significantly decreased the relative water contents (RWC) and increased the electrolyte leakage (EL) from the leaves of maize plants (Table 2). The RWC was decreased by 13.80% and 56.10%, respectively at 6 dS m^{-1} and 12 dS m^{-1} SS as compared to control (Table 2). On the other hand, EL was significantly increased by 17.76% and 40.92% at both SS levels (Table 2). The use of OA appreciably increased RWC while they decreased the EL from maize plants (Table 2). Among all the OA treatments application of 5% SPM showed the best results as compared to the application of CM and combined application of CM and SPM (Table 2).

Table 2. Impact of various organic amendments of the electrical conductivity, relative water contents and photosynthetic pigments of maize crop grown under salinity stress

Salinity Stress	OA	EC %	RWC %	Chl a (mg/g FW)	Chl b (mg/g FW	Car. (mg/g FW)
Control	Control	48.48± 0.36^{d}	45.14± 0.39^{c}	2.74 e ± 0.01^{e}	1.06 ± 0.06^{g}	0.49 ± 0.017^{h}
	CM	36.6 ± 0.44^{f}	51.09± 0.40^{b}	3.13 c ± 0.03^{c}	3.03± 0.03^{c}	0.85± 0.014^{e}
	SPM	19.25± 0.42^{g}	57.40± 0.33^{a}	3.62 a ± 0.05^{a}	3.93± 0.05^{a}	1.31± 0.014^{a}
	CM+SPM	33.80f± 0.48^{f}	54.82± 0.25^{a}	3.33 b ± 0.07^{b}	3.53± 0.03^{b}	1.16± 0.120^{b}
6 dS m^{-1}	Control	53.45± 0.44^{c}	34.26± 0.42^{e}	2.07 h ± 0.01^{h}	0.56± 0.00^{h}	0.32± 0.09^{j}
	CM	40.71± 0.48^{e}	44.51± 0.46^{c}	2.35 g ± 0.01^{g}	2.59± 0.04^{d}	0.79 ± 0.071^{f}
	SPM	35.37± 0.40^{ef}	54.10± 0.43^{a}	2.99 d ± 0.02^{d}	3.45± 0.04^{b}	1.16± 0.017^{b}
	CM+SPM	38.42± 0.39^{e}	46.88± 0.35^{c}	2.51 f ± 0.00^{f}	2.95± 0.06^{c}	1.97± 0.011^{c}
12 dS m^{-1}	Control	75.32± 0.41^{a}	9.59± 0.38^{h}	0.53 l ± 0.01^{l}	0.46± 0.05^{h}	0.22± 0.002^{k}
	CM	58.62± 0.36^{b}	15.0 ± 0.29^{g}	0.95 k ± 0.01^{k}	2.42± 0.04^{ef}	0.41± 0.018^{i}
	SPM	47.55± 0.42^{d}	38.77± 0.42^{d}	1.55 I ± 0.01^{i}	2.55± 0.01^{de}	0.89± 0.007^{d}
	CM+SPM	52.31± 0.48^{c}	28.12± 0.21^{f}	1.30 j ± 0.01^{j}	2.37± 0.00^{f}	0.61± 0.008^{g}

The data is the mean of three replicates with S.E. (±) and diverse letters indicating significance at 0.05 P level. OA: organic amendments, EC = electrical conductivity, RWC = relative water content, Chl = Chlorophyll, Car. = Carotenoids.

The results showed that SS caused a significant reduction in chlorophyll and carotenoid contents (Table 2). Under the 6 dS m^{-1} and 12 dS m^{-1} chlorophyll a (22.58% and 66.11%), chlorophyll b (17.27% and 32.45%) and carotenoid (14.73% and 43.8%) content significantly decreased as compared to their respective control. All OA significantly improved all the photosynthetic pigments under saline and non-saline conditions. Under the 6 dS m^{-1} application of SPM enhanced the chlorophyll-a, chlorophyll-b and carotenoid concentrations by 30.97%, 83.78% and 71.74% as compared to control (Table 2). Likewise, under 12 dS m^{-1} SS; the application of SPM caused an increase of 65.58%, 81.74% and 75.02% in chlorophyll-a, chlorophyll-b and carotenoid as compared to control (Table 2).

MDA and H_2O_2 contents

The results indicated that SS increased the concentration of H_2O_2 by 50.49% and 68.49% in maize seedlings subjected to both SS levels (Figure 1). The application of OA showed positive impacts on maize plants and significantly reduced H_2O_2 production (Figure 1). The application of SPM decreased the H_2O_2 contents by 42.42% and 40.74% at 6 dS m^{-1} and 12 dS m^{-1} as compared to control (Figure 1). SS also induced a significant increased MDA accumulation as compared to control. However, application of all OA reduced the MDA accumulation and however, SPM (5%) significantly reduced MDA contents as compared to other OA (Figure 1).

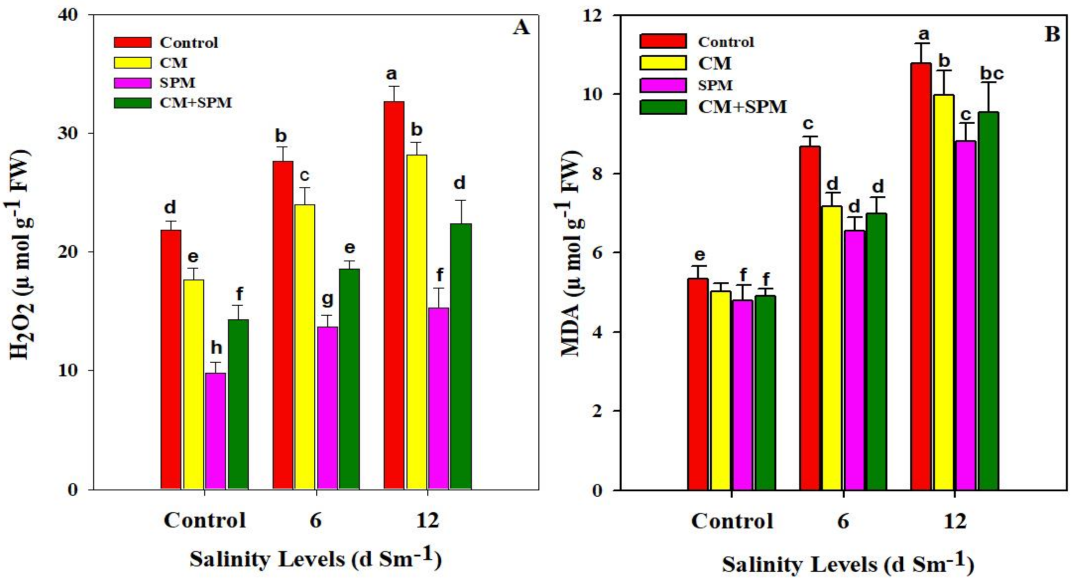

Figure 1. Impact of diverse organic amendments on H_2O_2 (A) MDA (B) contents of maize crop grown under salinity stress

The date indicating the means of three replicates (3n) with S.E. (±) and diverse letter indicating significance at 0.05 P level.

Antioxidant enzyme activity

SS gradually increased antioxidant enzyme activity (Table 3). The activity of CAT was increased by 17.61% and 26.70% whereas the activity of APX was increased by 19.43% and 34.24%, respectively at both levels of SS compared to control (Table 3). The antioxidant enzyme activity was enhanced by applying the different OA treatments (Table 3). Nonetheless, application of SPM significantly increased the CAT activity (30.72% and 44.65%) and APX activity (33.92% and 25.53%) as compared to control (Table 3). SS also showed a marked reduction in anthocyanin contents however, application of various OA appreciably improved the anthocyanin contents under both control and SS contents (Table 3). The application of SPM considerably increased the anthocyanin contents as compared application of other OA (Table 3).

Table 3. Impact of various organic amendments of the anti-oxidant activities and anthocyanin contents of maize plant under different levels of SS

Salinity Stress	Organic amendments	Catalase (U/mg protein)	Ascorbate peroxides (U/mg protein)	Anthocyanin (mg/g FW)
Control	Control	1.37± 0.02^{j}	24.49± 0.26^{f}	24.02± 0.01^{d}
	CM	1.50± 0.00^{i}	27.28± 0.08^{ed}	27.56± 0.07^{c}
	SPM	1.88± 0.00^{de}	34.55± 0.26^{d}	32.82± 0.16^{a}
	CM+SPM	1.63± 0.01^{g}	30.39± 0.22^{e}	28.16± 0.06^{b}
6 dS m^{-1}	Control	1.26± 0.00^{k}	29.29± 0.39^{e}	17.65± 0.12^{h}
	CM	1.86± 0.01^{e}	33.30± 0.23^{d}	18.53± 0.07^{g}
	SPM	2.68± 0.01^{b}	44.33± 0.24^{b}	22.39± 0.17^{e}
	CM+SPM	1.92± 0.01^{d}	37.94± 0.43^{cd}	21.86± 0.12^{f}
12 dS m^{-1}	Control	1.44± 0.01^{c}	38.16± 0.06^{c}	9.84 ± 0.10^{l}
	CM	1.55± 0.00^{h}	41.89± 0.79^{c}	13.15± 0.12^{k}
	SPM	2.81± 0.00^{a}	51.24± 0.17^{a}	15.32 ± 0.16^{i}
	CM+SPM	1.76± 0.00^{f}	46.19± 0.26^{b}	14.27± 0.12^{j}

The data is the mean of three replicates with S.E. (±) and diverse letters indicating significance at 0.05 P level.

Total soluble proteins and free amino acids

SS caused a marked reduction in both FAA and TSP contents (Figure 2). TSP was decreased by 4.55% and 21.84% at both levels of SS as compared to control (Figure 2). This decrease in the TSP was recovered by applying diverse OA (Figure 2). All OA performed well but SPM significantly increased the TSP by 34.56% and 34.45% at both SS levels as compared to control (Figure 2). Likewise, SS also significantly decreased the FAA content of maize plant as compared to control (Figure 2). However, OA significantly increased the accumulation of FAA as compared to control and in this regard application of SPM remained the top performer (Figure 2).

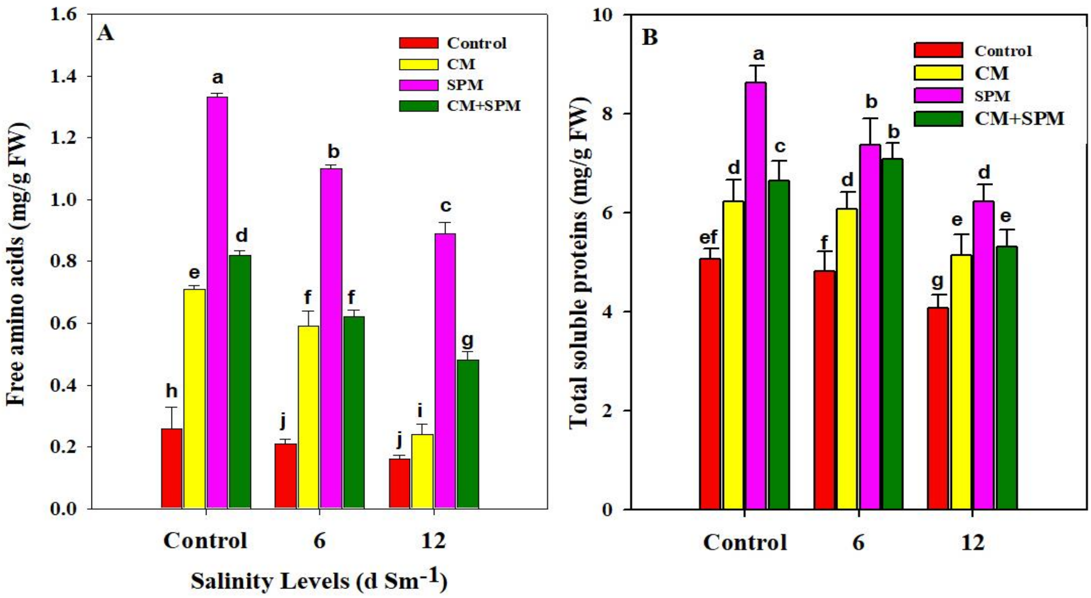

Figure 2. Impact of diverse organic amendments on FAA (A) TSP (B) contents of maize crop grown under salinity stress

The date indicating the means of three replicates (3n) with S.E. (±) and diverse letter indicating significance at 0.05 P level.

Ionic concentration

The results indicated that SS significantly increased the accumulation of Na^+ and Cl^-, however, maximum concentration of Na^+ and Cl^- was recorded under stronger SS (Figure 3). The application of OA significantly reduced the accumulation of both Na^+ and Cl^- and application of SPM significantly reduced their accumulation as compared to application of CM and combined application of CM+SPM (Figure 3). SS considerably reduced the K^+ and Ca^{2+} accumulation and a significant reduction in K^+ and Ca^{2+} accumulation was recorded with increasing SS in growing medium (Figure 3). The application of OA maintained higher K^+ and Ca^{2+} and in this regard the application of SPM remained the top performer as compared to other OA (Figure 3).

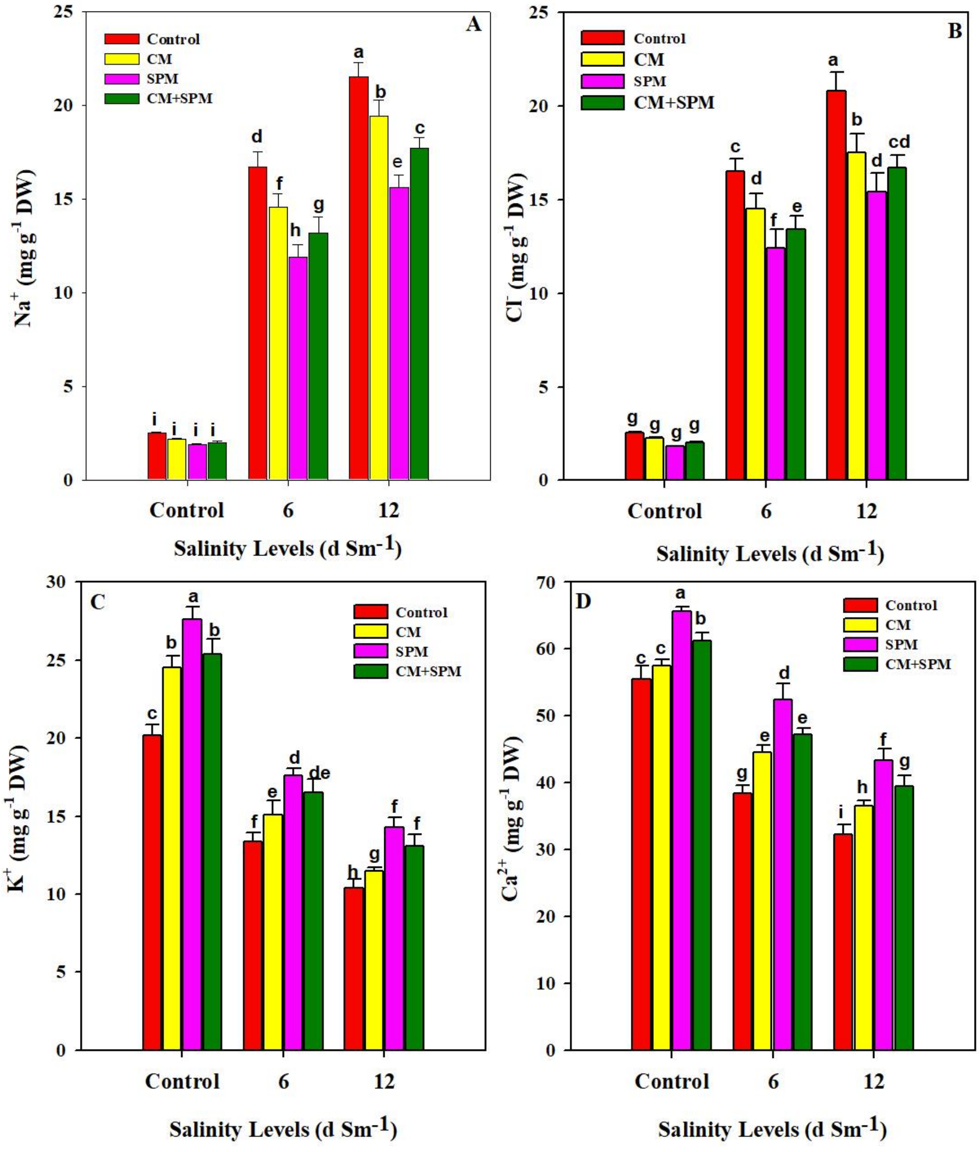

Figure 3. Impact of diverse organic amendments on Na^+ (A), Cl- (B), K^+ (C) and Ca^{2+} concentration of maize crop grown under salinity stress

Principal component analysis

The date on different traits was subjected to PCA to determine the impacts of OA on relationships between different traits under SS (Figure 4). The two components (Dim-1 and Dim-2) showed a total variance of 90.70% in which Dim-1 contributed 79% whereas Dim-2 has a share of 11.70% (Figure 4). The results of

PCA showed a negative linking between Na, Cl, MDA, APX, EC, CAT and H_2O_2 whereas RDW, RFW, TSP, Chl-a, Chl-b, SD, SFW, SDW, RWC, Ca, Anthocyanin and K has positive relationships with each other (Figure 4).

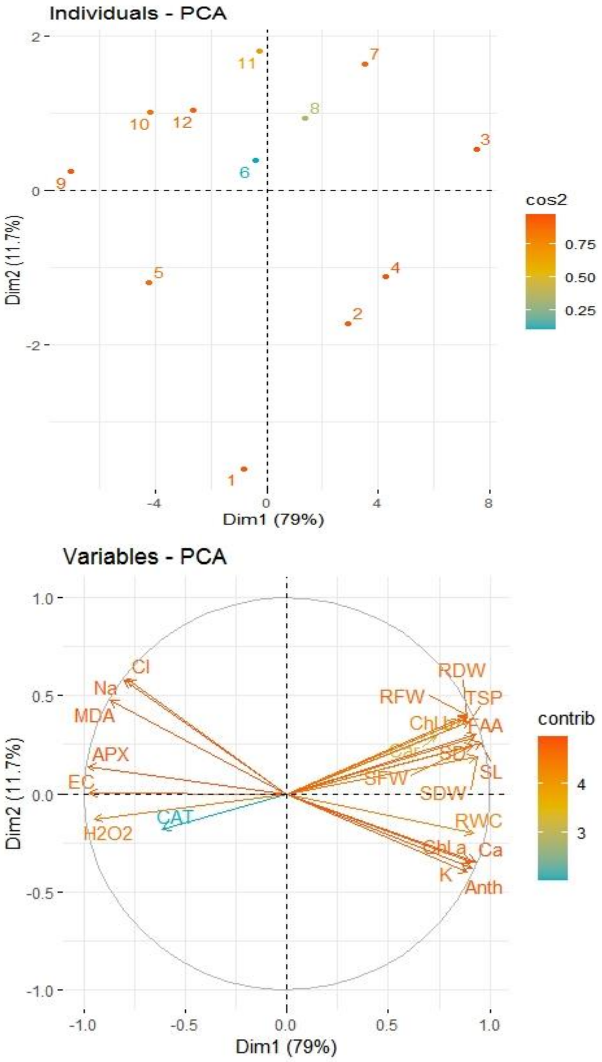

Figure 4. The loading plots of PCA on diverse studied traits of maize plants under SS

Cl: chloride, Na: sodium, H_2O_2: hydrogen peroxide, APX: ascorbate peroxidase, EC: electrical conductivity, RDW: root dry weights, RFW: root fresh weight, TSP: total soluble proteins, SD: stem diameter, Ca: calcium, K: potassium, RCW: relative water contents, Anth: anthocyanin contents.

Discussion

Salt stress is a major limiting factor for crop production across the globe therefore, it is mandatory to improve SS tolerance in plants to ensure better crop production. The results indicated that SS caused a marked reduction in the growth and biomass productivity of maize seedlings (Table 1). SS disturbs the hormonal and nutritional balance and induces the ROS production (Figure 1) thereby reduce plant growth and biomass production (Sarker and Oba, 2019, 2020).

SS also caused a significant increase in accretion of toxic ions (Na^+ and Cl-) around the plant roots which reduce the water uptake owing to increase in osmotic potential of soil solution which is also major reasons of reduction in growth and biomass production under saline conditions (Kosová *et al.*, 2011). Nonetheless, OA markedly improved seedling growth and biomass yield (Table 1). The field application of OA improves SOM which increases soil water holding capacity, nutrient availability, bulk density and hydraulic conductivity that ensures better plant performance (Kosová *et al.*, 2011). Moreover, addition of OA ensures the better leaching of Na^+ (Figure 3) which reduces SS toxicity and ensures better growth and biomass production under SS (Urbaniak *et al.*, 2017; Dineshkumar *et al.*, 2020).

SS caused a significant reduction in RWC of maize plants (Table 2). Plants exposed to SS face osmotic stress which reduce the water uptake, besides this ABA-induced closing of stomata that reduce the water uptake therefore, entails low leaf RWC in plants grown under SS (Evelin *et al.*, 2019). However, application of OA significantly improved the RWC which could be ascribed to better soil conditions created by OA that increased the water uptake and maintained higher RWC under SS (Soni *et al.*, 2016). In present study, SS caused a significant increase in MDA and H_2O_2 accumulation (Figure 1). The increase in H_2O_2 accumulation disturbs the normal functioning of plant by inducing oxidative stress which in turn causes huge growth and yield losses (Hussain *et al.*, 2016). Membrane damage is considered to be direct effect of SS and it can be measured in terms of MDA accumulation (Fatima *et al.*, 2021). Moreover, in present study, SS also significantly increased the EL owing to increase in membrane damage (Figure 1). SS increases production of ROS which damages the cellular membranes and cause significant increase in EL (Sultan *et al.*, 2021).

However, application of OA significantly reduced the EL from maize plants which was linked with lower MDA and H_2O_2 (Figure 1) and improved anti-oxidant activities (Table 3) (Sheoran *et al.*, 2021b). The application of OA reduced the MDA accumulation and impose favorable impacts on plants by reducing the salinity induced oxidative damages and MDA accumulation (Kusvuran *et al.*, 2021). The results indicated that SS significantly reduced the chlorophyll contents of maize plants (Table 2). SS induced ROS production which cause chlorosis, photo-reduction and damage the PS-I and PS-II and reduce the chlorophyll formation which cause reduction in growth under SS (Singh *et al.*, 2018). The increase in accumulation of Na^+ owing to SS (Figure 3) also denatures enzymes required for chlorophyll synthesis therefore, cause substantial decrease in chlorophyll contents under SS (Alzahib *et al.*, 2021). However, application of OA significantly improved the chlorophyll and carotenoid contents of maize plants grown under SS (Table 2). The increase in chlorophyll contents following application of OA could be attributed to improved soil properties, better uptake of NPK and Mg owing to fact Mg is considered as building block involved in chlorophyll formation (Darini, 2017; El-Saidi, 1997; Dineshkumar *et al.*, 2020).

The results indicated that activities of APX and CAT were significantly increased under SS (Table 3) which was further increased by the application of OA (Table 3). The increase in anti-oxidant activities scavenge the ROS and protects the plants from salinity induced oxidative damage (Parveen *et al.*, 2019; Mahmood *et al.*, 2021). The current improvement in antioxidant activity with OA could be attributed to better soils and improvement in nutrient uptake. Nonetheless, the mechanisms lying behind OA medicated increase in antioxidant activities is unexplored. Thus, in future, it is required to explore the mechanisms lying behind increase in antioxidant activities following the addition of OA. SS markedly decreased the FAA and TSP contents in maize plants (Figure 4). The higher accumulation of FAA produces an osmotic gradient which ensures better inward movement of water and protects the plants from the effects of SS (El-Saidi, 1997). Moreover, SS also decreased the TSP however, OA appreciably increased the TSP under control SS. The increase in TSP following OA application could be attributed to an increase in N uptake which increased the protein synthesis owing N play an integral part in protein synthesis. The increase in accumulation TSP regulates the plant processes and improves antioxidant activities (Table 3) which in turn increased salt tolerance in plants (Fahad and Bano, 2012).

The results showed that SS significantly increased the accretion of toxic ions (Na^+ and Cl^-) and reduced the accumulation of K^+ (Figure 3). The reduction in K^+ contents under SS causes oxidative damage and reduce the photosynthetic rate resulting cause huge yield losses (Gong *et al.*, 2011). Na^+ is a very toxic ion and it reduce water and potassium uptake therefore, substantially reduced the plant growth (Sumer *et al.*, 2004). Moreover, higher Na^+ concentration also disturbs the Ca^{2+} levels and impairs the Ca^{2+} availability to plant leaves (Hu *et al.*, 2007). A particular amount of Ca^{2+} is required for the maintenance of membrane integrity (Hu *et al.*, 2007) thus salinity induced reduction in Ca^{2+} uptake reduces the membrane integrity. Nonetheless, OA marked reduction accretion of both Na^+ and Cl- and increased the accumulation of both Ca^{2+} and K^+ (Figure 3). OA maintains better availability of Ca^{2+} in soil by mobilizing $CaCO_3$ and increasing the leaching of Na^+ therefore, prevent the salinity induced toxic effects (Prapagar *et al.*, 2012; Sheoran *et al.*, 2021b). Moreover, the application of OA also ensures better uptake of K^+ which reduces the uptake of Na^+ and Cl^- and therefore reduces the toxic effects of SS (Kumar *et al.*, 2017).

Conclusions

In present study, salinity stress caused a considerable decrease in seedling growth and biomass yield. However, these effects were reversed by application of OA. The increase in growth and biomass production following application of OA was linked with reduction in EL, MDA and H_2O_2 accumulation and increase in RWC, chlorophyll, CAT, POD, and anthocyanin, TSP and FAA. However, SPM (5%) markedly increased seedling growth and biomass yield as compared to other OA. Therefore, these findings suggested that application of SPM improve the growth, biomass, anti-oxidant activities, photosynthetic pigments and accumulation of TSP and FAA and alleviates salinity induced toxic effects on maize plants. These findings recommended that farmers can use FYM and PM to mitigate the salinity induced toxic effects. However, it is direly needed to optimize the rate of these OA keeping in mind the crop, soil and climatic conditions.

Authors' Contributions

Conceptualization: IK and MUC; Data collection: UA; Writing original draft: IK, MUC, MUH; Writing review and editing: RM, MBC, AN, MH, SA, HASA, SH, MAB, MUH and SHQ. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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