Pak. J. Bot., 54(2): 371-378, 2022. DOI: http://dx.doi.org/10.30848/PJB2022-2(13)

EXOGENOUS APPLICATION OF SALICYLIC ACID ENHANCES SALT STRESS TOLERANCE IN LEMONGRASS (*CYMBOPOGON FLEXUOSUS* STEUD. WATS.)

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Abstract

Salt stress is one of the key factors causing multifarious adverse effects on the growth, yield, and quality of crops worldwide. The present study was carried out to investigate the adverse effect of salinity (100 mM) in Lemongrass (*Cymbopogon flexuosus* L.) and to evaluate the ameliorative effect of salicylic acid (SA: 0.1, 0.5 and 1.0 mM) against salinity stress under field conditions. Various morphological and biochemical parameters were analysed after 60 days of salt and salicylic acid treatment to pot-grown plants. It was observed that when Lemongrass plants were irrigated with salt (NaCl) it drastically affected all the tested parameters, however various SA concentrations significantly minimized the negative effects of salinity when applied exogenously in the form of foliar spray on leaves. Particularly by 0.5 mM salicylic acid, this increases shoot/root length by 17.40 and 3.43 cm as compared to control i.e., 14.02 and 2.60 cm, respectively under saline stress. Salicylic acid possibly reduced the damage caused by salt toxicity and ROS by increasing POD, CAT and SOD activities. This study hints at a possibility that SA (0.5 mM) can be effectively used for the mitigation of salt stress in lemongrass, however, further experimentation under field condition is necessary to evaluate and harness the potential benefit of SA for normal plant growth and yield.

Key words: Antioxidant enzymes, Flavonoids, Phenolics, Soil salinity.

Introduction

Around the globe, various abiotic stresses like cold, heat, drought, flooding, heavy metals and soil salinity are the major reasons of growth reduction, plant development and crops productivity (Gontia-Mishra *et al*., 2014). Moreover, salinity of soil is considered as an environmental threat to world in food production and agriculture (Abbasi *et al*., 2015). Excess Na^+ imparts flush of cytosolic Ca^{2+} and K^+, which results in their cellular homeostasis imbalance, deficiency of nutrient, inhibition of growth, oxidative stress, and even cell death (Ahanger & Agarwal, 2017).

Salinity is causing loss of over 27 billion dollars per year to agriculture sector hence; salt resistance is an important issue for food production (Jayakannan *et al*., 2015). It was investigated that salinity affected arid and semiarid land from 2,000 to 4,000 ha/year worldwide and it is increasing day by day (Qadir *et al*., 2014). In Pakistan, about 6.323 million ha out of total land area has been adversely affected by salinity stress. This correlate with around 64% yield adversities and 14% of flooded lands on which salt influenced (Afzal *et al*., 2005). Furthermore, since crop productivity is seriously harnessed, saline soil has become a pertinent issue now-a-days (Ravindran *et al*., 2007). Salinity thoroughly stunned the plant growth and development rate which depends on some necessary events i.e., cell separation, cell amplification, cell division, along with morphological, physiological, biochemical, hereditary, environmental factors and their interlinkage (Islam *et al*., 2015).

Lemongrass (*Cymbopogon flexuosus* Steud. Wats.) along with various grasses, are being used for extraction of essential oils in Asia such as Pakistan, China and India. These oils are the part and parcel of aromatherapy, insect repellent, aromas, artificial violet scents and pharmaceutical industries. Generally, salt sensitivity is directly linked with lemongrass because it cannot grow well in salt rich soils (Loake & Grant, 2007).

In spite of using salt tolerant plant species, different techniques are being practiced by researchers and specialists in different plants to improve the adverse impacts of salt stress. Number of compounds have also been studied for alleviating the adverse effects of salt such as proline (Hanson *et al*., 1977), nicotinamide (Berglund, 1994), calcium (Tavallali *et al*., 2008), KNO_3 (Fayez & Salih, 2013), glycinebetaine (Hasanuzzaman *et al*., 2014), salicylic acid (SA) and ascorbic acid (Gul *et al*., 2015). Utilization of SA is considered as exceptionally valuable tool to up-regulate the various defence mechanisms against NaCl stress in various plants. SA has always been a key to mitigate an abiotic and biotic stresses for plants like banana in low temperature (Kang, 2003), wheat in water stress (Singh & Usha, 2003), Kentucky bluegrass in high temperature (He & Cramer, 2005), tomato in drought (Hayat *et al*., 2008), maize in heavy metal (Krantev *et al*., 2008), potato in salt (Sajid & Aftab, 2012). SA improvements to salt stress impacts has also been reported in various crops i.e. barley (El-tayeb, 2005) carrot (Eraslan *et al*., 2007), tomato (Stevens & Senaratna, 2006), and wheat (Elkelish *et al*., 2019). SA has been used for reduction of drastic influences of salinity stress by up-regulating the antioxidants and necessary photosynthetic pigments (Idrees *et al*., 2011). Moreover, SA is directly linked with the signal transduction, which triggers the movement of special enzymes. These are for the proline metabolic pathway to activate the barrier compounds like compatible solutes, proline and glycinebetaine due to salt (Misra & Saxena, 2009). Exogenously applied SA may take part in certain

reactions of plants, might be playing role in up-regulating and transporting of ions, and by closing the stomata (Gunes *et al*., 2005), growth, membranes permeability and photosynthesis (Barkosky & Einhellig, 1993). According to our knowledge, so far literature is scanty about studies regarding ameliorative effect of non-enzymatic antioxidant salicylic acid on lemongrass under salt stress conditions. The goal of present study is to investigate the deleterious effects of sodium chloride stress on morphological and biochemical attributes of lemongrass and to evaluate the potential benefit of this non-enzymatic antioxidant in improving salt stress tolerance with special reference to antioxidant enzymes.

Materials and Methods

Experimental layout and treatment plan for salt and salicylic acid: Lemongrass seedlings (2-months old) were obtained from the Punjab University Seed Centre, Lahore, Pakistan. Plastic pots (9×15 cm) were filled with exactly 3 kg dried and well mixed soil having 4.4 $mScm^{-1}$ electrical conductivity, 8.2 pH, 0.93% organic matter, 2.8 mg/kg available phosphorus, 176.5 mg/kg available potassium, 40% saturation and loamy surface (from "Soil Fertility Research Institute" Irrigation Colony, Lahore). Healthy plants of uniform size were planted manually (one plant/pot). At the start, plants were manually irrigated with tap water for 30-days for their well establishment. Plants were weekly watered having 613.5 $uScm^{-1}$ Electrical conductivity, 2.35 Residual Sodium Carbonate (RSC), 2.45 Ca^{++} & Mg^{++}, 3.65 Na^{+}, 4.8 HCO^{3-}, 1.15 Cl^{-}, and 3.25 Sodium Absorption Ratio (SAR: Soil Fertility Research Institute, Irrigation Colony, Lahore, Pakistan). To see the alleviation potential of salt stress in lemongrass a factorial (4×2; salt and SA) experiment was set up by selecting the suitable concentrations. The various treatments of salt and SA were as follows: T_1 Control (distilled water); T_2 100 mM NaCl; T_3 0.1 M of SA; T_4 0.5 M of SA; T_5 1.0 M of SA; T_6 0.1 M SA + 100 mM of NaCl; T_7 0.5 M SA + 100 mM of NaCl; T_8 1.0 M SA + 100 mM of NaCl. The salinity treatments were given as root drenching to 3-months-old plants. The distilled water was provided to control plants rather than solution of NaCl. To save from abrupt osmotic stress, NaCl was increased gradually to reach the required concentration of salt treatment. After every 7 days interval the plants were given 300 mL of 100 mM NaCl solution. Overall 3 concentrations (0.1, 0.5 and 1.0 mM) of SA were applied by foliar spray (100 ml / plant). At day 60^{th} of treatment, plants were up rooted for data recording (morphological and biochemical attributes) and leaves were collected from every treatment for biochemical assay.

Data collection for morphological parameters: Plants were harvested along with intact roots from the pot and with the help of measuring scale; average shoot and root length was recorded. Average number of leaves and tillers were counted manually. Diameter was determined by using Vernier calliper. Fresh weight of leaves was determined by using measuring balance (Scientech 5220). Afterward, a hot-air oven (70°C for 72 hrs) was used for estimating dry weight.

Biochemical assays: Plant material (1g fresh leaves) was crushed in sterilized ice-cold pestle/ mortar with 2-3 drops of 0.6% (v/v) Triton X-100 (Unilever, Karachi, Pakistan) and 0.1 g Polyvinyl Poly-pyrrolidone (PVP; Sigma-Aldrich) in a fine powder. This grinded sample was then thoroughly mixed in 2 mL of phosphate buffer (0.1 M: pH 7.8). The proportion for buffer to plant material was kept fixed i.e., 2:1 (v/w). Afterwards centrifugation was carried out by using Sorval RC-5B refrigerated centrifuge at 20000 rpm for 20 mints at 4°C. The crude extract (supernatant) was preserved at 4°C in an ependrof for estimation of enzyme activities.

Peroxidase (POX: EC 1.11.1.6) was quantitatively measured by using spectrophotometer as performed by the Guaicol H_2O_2 method of Racuson & Foote (1965). Two test tubes were prepared, one taken as control and other as an experimental. Experimental tube was consisted of 0.2 mL 1% Guaicol, 2.5 mL of 0.1M Tris HCl (pH 7.8) and 10 µL of crude extract. In the control sample distilled water was used instead of enzyme extract. Both the test tubes were left for 30 mints to complete the reaction at 25 ± 2°C. Then 0.2 mL of 0.3% Hydrogen peroxide was added drop-wise in both control and experimental samples. A reddish brown colour appeared indicating the completion of reaction. Following 3 minutes, absorbance was read at 470 nm in the spectrophotometer (UV-9900S).The enzyme specific activity for each sample was expressed in U/mL.

Catalase (E.C 1.11.1.5) was measured by using spectrophotometer, according to the method of Aebi (1983) with minor modifications. Briefly, 3 mL of reaction mixture in a test tube consisted of 100 mM sodium phosphate buffer (pH 7.8), 30 mM of H_2O_2 and 100 µL of crude extract. The decline in optical density was read at 240 nm resulting from the breakdown of H_2O_2. Total catalase activity was measured as the amount of enzyme that brought about a decline in absorbance A240 of 0.01 per mint and expressed as one unit (U).

The activity of superoxide dismutase (SOD: E.C 1.15.1.1) was analyzed by using the method of Kumar *et al.*, (2002). The reaction mixture (3.0 mL) containing 0.1 mM EDTA, phosphate buffer (50 mM: pH 7.8), 13 mM methionine, 75 µM NBT, 50 mM sodium carbonate, 10 µM riboflavin and 100 µL of supernatant (crude extract) in a experimental test tube. While control test tube containing reaction mixture with distilled water replaces crude extract. Samples were illuminated by putting the both test tubes (experimental and control) under two 15 W lamp (white fluorescent light) for 15 min at 25°C. The optical density was recorded at 560 nm to observe the decrease in absorbance by using a spectrophotometer. The amount of enzyme brought about 50% inhibition of photochemical reduction of NBT which was considered as one unit (U) of SOD activity.

Estimation of non enzymatic antioxidant activity: For Flavonoids estimation, colorimetric procedure of Chang *et al*., (2002) was employed with minor modifications. The crude extract (250 µL) of lemongrass leaves were well mixed with mixture of 75 µL of 5% $NaNO_2$ and 1.25 mL of distilled water. Afterward, 150 µL of 10% $AlCl_3$ was added and left for 5 mints at 25 ± 2°C. The reaction was started by

addition of 0.5 mL of 1M NaOH. Finely volume was raised up to 2.5 mL by addition of deionised water. Absorbance of reaction mixture was read at 510 nm.

Phenolic contents were measured by using Folin-Ciocalteu reagent method (Ghafoor *et al*., 2011). Briefly the reaction mixture was prepared by adding Folin-Ciocalteu (0.25 mL: Merk) reagent, 0.25 mL of the crude plant extract in dilute form and 3.5 mL of distilled water to raise volume up to 4.6 mL. After that added 1 mL of 20% Na_2CO_3 solution followed by vortex mixing and then samples were incubated at 25 ± 2°C for two hours. For the blank reading 0.25mL of 80% methanol was added instead of plant extract. Absorbance was recorded on a spectrophotometer (UV-9000S) at 765 nm.

Statistical analysis

The resultant data were analyzed by using statistical software SPSS-22.0.0 (SPSS Inc., Chicago city, IL, USA). Duncan Multiple Range Test (DMRT) was performed to the data for determining significance at $p<0.05$.

Results

Shoot and root length: As depicted in the Table 1, control sample showed plant shoot length 14.02 cm but shoot length was decreased to 12.31 cm by adding salt (100 mM). When only SA was applied exogenously, it increased shoot length maximum up to 16.64 cm at 1mM and minimum 15.49 cm at 0.1mM. While on applying SA i.e., 0.1, 0.5 and 1mM, to salt stressed (100 mM) plants it increased the shoot length and maximum shoot length (17.40 cm) was observed at 0.5 mM SA concentration.

The root length of control plants was 2.6 cm while salt treated plants showed the root length 2.1 cm at 100 mM concentration. On applying only SA in the form of spray to plants root length was 1.83 cm at 1mM, followed by maximum size of 2.6 cm at 0.1 mM SA treatment. But when SA was applied to salt treated plants, the maximum root length was 3.43 and 3.03 cm at 0.5 and 1.0 mM concentrations of SA and minimum (3.36 cm) at 0.1mM of SA.

Number of leaves and tillers: As shown in (Table 1) the control plants showed 15.94 leaves but this number of leaves was increased to 18.83 in 100 mM salt stress. Salinity stress increased the number of leaves with less intermodal distance as compared to control plants. When different treatments (0.1, 0.5 and 1mM) of SA was applied, the minimum number of plant leaves was13.73 at 1mM concentration of SA and maximum number of plant leaves was 16.99 at 0.5 mM of SA concentration. With increasing SA concentration, decrease in number of leaves was observed in salt treated plants. Highest number of leaves i.e., 12.63 was noted at 0.5 mM and minimum number of leaves i.e., 13.58 at 0.1 mM concentrations of SA.

The control plants (without any treatment) showed highest number of tillers i.e., 4.35 but salt treated plants showed 4.11 number of tillers. On applying only SA to plants maximum increase in number of tillers 4.52 were observed at 0.1 mM and minimum 3.38 at 1.0 mM concentration of SA. In case of both salt (100 mM) and SA application, the maximum number of tillers i.e., 3.15 was observed at 0.5 mM and minimum (2.68) at 0.1 mM concentration of salicylic acid.

Stem diameter: In case of stem diameter; control plants showed 1.55 cm but decrease (1.15 cm) in stem diameter was observed in salt treated plants. On applying only SA, stem diameter was decreased, which showed maximum value (1.08 cm) at 0.1 mM and minimum 0.87 cm at 1mM concentration of SA. On the other hand, SA sprayed plants when treated with 100 mM NaCl, stem diameter increased i.e., 1.57cm at 0.1mM and 1.79 cm at 0.5 mM concentration of SA.

Shoot fresh / dry weight: The control plants showed 21 g fresh weight but salt treated plants showed 19 g fresh weight (Table 2). On applying SA alone, the maximum shoot fresh weight was 25.24 g at 0.5mM concentration and minimum was 20.60 g at 1.0 mM concentration. However, the foliar spray of salicylic acid was significantly alleviated the salinity stress when applied to salt treated plants. Maximum shoot fresh weight 22.92 g was recorded at 0.5 mM and minimum (21.92 g) at 1.0 mM concentration of SA.

The control plants showed 1.94 g shoot dry weight but salt treated plants showed 1.04 g dry weight. On applying only SA, the maximum shoot dry weight was 2.20g at 0.5mM concentration and minimum was 1.80 g at 1.0 mM concentration of SA. However, on applying the foliar spray of SA to salt treated plants, the maximum shoot dry weight 3.70 g was observed at 0.5mM concentration and minimum 2.15g at 1.0 mM concentration of SA.

Root fresh/dry weight: As the Table 2 indicates the control plants showed 12.33g root fresh weight but salt treated plants showed 13.66 g fresh weight. On applying only SA, average fresh weight of root was lessened to 13 g at 0.5 mM and 10.73 g at 1.0 mM concentration of SA. However, salicylic acid spray significantly alleviated the salinity stress when applied to salt treated plants. The maximum root fresh weight was 15.33 g at 0.5 mM concentration and minimum was 12.33 g at 1.0 mM concentration of SA.

The control plants showed 2.28 g root dry weight and salt treated plants showed 2.05 g dry weight. On applying only SA, the maximum root dry weight was found 3.93 g at 0.5 mM concentration and minimum 2.63 g at 1.0mM concentration. However, on applying foliar spray to salt stressed plants, the maximum dry weight of root (4.86 g) was at 0.5 mM concentration and minimum (3.25 g) at 0. 1 mM concentration of SA.

Biochemical enzyme assays

Peroxidase activity: The peroxidase activity of control plants revealed 3.2 units/mL of tissue while salinity stress of 100 mM resulted in increased enzyme activity i.e., 3.8 units/mL of tissue. On applying SA alone, the maximum Peroxidase activity was 3.1 units/mL of tissue at 1.0 mM concentration and minimum (2.9 units/mL of tissue) was observed on 0.5 mM concentration. However, on the application of both SA and salt, the highest POD activity was 5.2 units/mL of tissue at 0.5 mM concentration and minimum was 4.5 units/mL of tissue at 0.1 mM foliar spray of SA under 100 mM concentration of NaCl.

Table 1. Comparative effect of various concentrations of salt and salicylic acid on morphological features of Lemongrass.

Treatments	Length of hoot (cm)	Length of root (cm)	Leaves No.	Tillers No.	Stem diameter (cm)
T1 (Without NaCl and SA)	14.02 ± 2.31^{c}	2.60 ± 0.20^{c}	15.94 ± 6.44^{c}	4.35 ± 2.40^{a}	1.55 ± 0.15^{b}
T2 (NaCl100 mM)	12.31 ± 2.01^{d}	2.13 ± 0.18^{bc}	18.83 ± 8.23^{a}	4.11 ± 2.22^{a}	1.15 ± 0.35^{c}
T3 (SA 0.1 mM)	15.49 ± 2.31^{b}	2.06 ± 0.08^{c}	14.74 ± 5.25^{c}	4.52 ± 3.26^{a}	1.08 ± 0.10^{c}
T4 (SA 0.5 mM)	16.38 ± 2.05^{a}	2.56 ± 0.47^{c}	16.99 ± 6.54^{b}	4.01 ± 2.50^{a}	1.18 ± 0.08^{c}
T5 (SA 1.0 mM)	16.64 ± 2.44^{a}	1.83 ± 0.44^{c}	13.73 ± 5.11^{d}	3.38 ± 1.83^{b}	0.93 ± 0.12^{b}
T6 (NaCl + SA 0.1 mM)	16.53 ± 2.25^{a}	3.36 ± 0.23^{a}	13.58 ± 5.46^{d}	2.68 ± 1.53^{h}	1.57 ± 0.13^{b}
T7 (NaCl + SA 0.5 mM)	17.40 ± 3.53^{a}	3.43 ± 0.97^{a}	12.63 ± 5.24^{e}	4.03 ± 1.52^{a}	1.79 ± 0.08^{a}
T8 (NaCl + SA 1.0 mM)	15.48 ± 2.68^{b}	3.03 ± 0.23^{b}	12.05 ± 1.83^{e}	3.15 ± 1.58^{b}	1.02 ± 0.09^{c}
Significance	NS	*	*	NS	*

Values having the same alphabets are not significantly different, according to DMRT at (p<0.05) non-significant (NS) & Significant (*) at 0.05 %

Values showing in table are means ± SE (n = 9)

Table 2. Comparative effect of different treatments of salt and salicylic acid on fresh/dry weight response of shoot and root of Lemongrass.

Treatments	Shoot		Root	
	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
T1 (Without NaCl and SA)	21.00 ± 0.57^{b}	1.94 ± 0.007^{c}	12.33 ± 1.76^{d}	2.28 ± 0.58^{c}
T2 (NaCl 100 mM)	19.00 ± 0.55^{d}	1.04 ± 0.005^{d}	13.66 ± 1.76^{c}	2.05 ± 0.76^{c}
T3 (SA 0.1 mM)	22.61 ± 0.005^{c}	2.10 ± 0.005^{b}	10.73 ± 0.17^{b}	3.24 ± 0.67^{b}
T4 (SA 0.5 mM)	25.24 ± 0.005^{a}	2.20 ± 0.057^{b}	14.00 ± 1.60^{b}	3.93 ± 0.41^{b}
T5 (SA 1.0 mM)	20.60 ± 0.07^{bc}	1.80 ± 0.057^{c}	12.33 ± 1.83^{d}	2.63 ± 0.56^{b}
T6 (NaCl + SA 0.1 mM)	21.93 ± 0.005^{c}	2.50 ± 0.025^{b}	13.33 ± 0.66^{c}	3.25 ± 1.00^{b}
T7 (NaCl + SA 0.5 mM)	22.92 ± 0.057^{bc}	3.70 ± 0.05^{a}	15.33 ± 1.20^{a}	4.86 ± 0.54^{a}
T8 (NaCl + SA 1.0 mM)	21.92 ± 0.05^{c}	2.15 ± 0.005^{b}	12.33 ± 2.94^{d}	3.75 ± 0.47^{b}
Significance	*	NS	*	NS

Values having the same alphabets are not significantly different according to DMRT at (p<0.05)

Non-significant (NS) & Significant (*) at 0.05 %

Values showing in table are means ± SE (n = 9)

Catalase activity: As evident from Fig. 1, the catalase activity of control plants was 2.20 units/mL; but plants showed 2.50 units/mL under salt stress. When only SA was applied, the maximum increase i.e., 2.32 units/mL was observed at 0.5mM concentration while minimum catalase activity was 2.25 units/mL at 1.0mM concentrations. On the application SA and salt, treated plants the maximum value of catalase activity 2.62 units/mL was at 0.1 mM SA and minimum catalase activity i.e., 2.59 units/Ml was at 1.0mM concentration of SA.

Superoxide dismutase activity: The SOD of control plants was 19 units/mg of protein while plants showed the increase in SOD activity i.e., 38.4 units/mg of protein under salt stress (100 mM). On applying salicylic acid exogenously, significant effect in ameliorating the drastic effects of NaCl on SOD activity was found. The maximum SOD activity was 39.8 units/mg of protein at 1.0 mM and minimum was 34 units/mg of protein at 0.1 mM. The SA showed its maximum increase 42.5 units/mg of protein at 0.1 mM and minimum 50.8 at 0.5 mM concentration with 100mM salt (Fig. 1).

Non enzymatic antioxidant activity

Flavonoid content: The control plants showed 11.25 mg/g of flavonoids but the plant under salinity stress showed enhanced flavonoids contents i.e., 23.5 mg/g (Fig. 1). On applying SA the maximum flavonoids were 16.4 mg/g at 1.0 mM concentration and minimum flavonoids 13.3 mg/g were found at 0.1 mM. The plants showed maximum flavonoids 30.1 mg/g at 0.1mM and minimum flavonoids i.e., 28.5 mg/g at 1.0 mM concentration of SA under 100 mM NaCl salt stress.

Phenolic contents: The control plants showed phenolic contents i.e., 1.21 mg/g but the plants under salinity stress of 100 mM NaCl showed enhanced phenolics contents up to 1.62 mg/g. On applying SA, the maximum phenolics 1.52 mg/g at 1.0mM concentration and minimum phenolics 1.1 mg/g at 0.1 mM concentration were observed. The plants showed maximum phenolics 1.93 mg/g at 1.0 mM and minimum phenolics 1.72 mg/g at 0.1 mM foliar spray of SA under 100 mM concentration of salt stress.

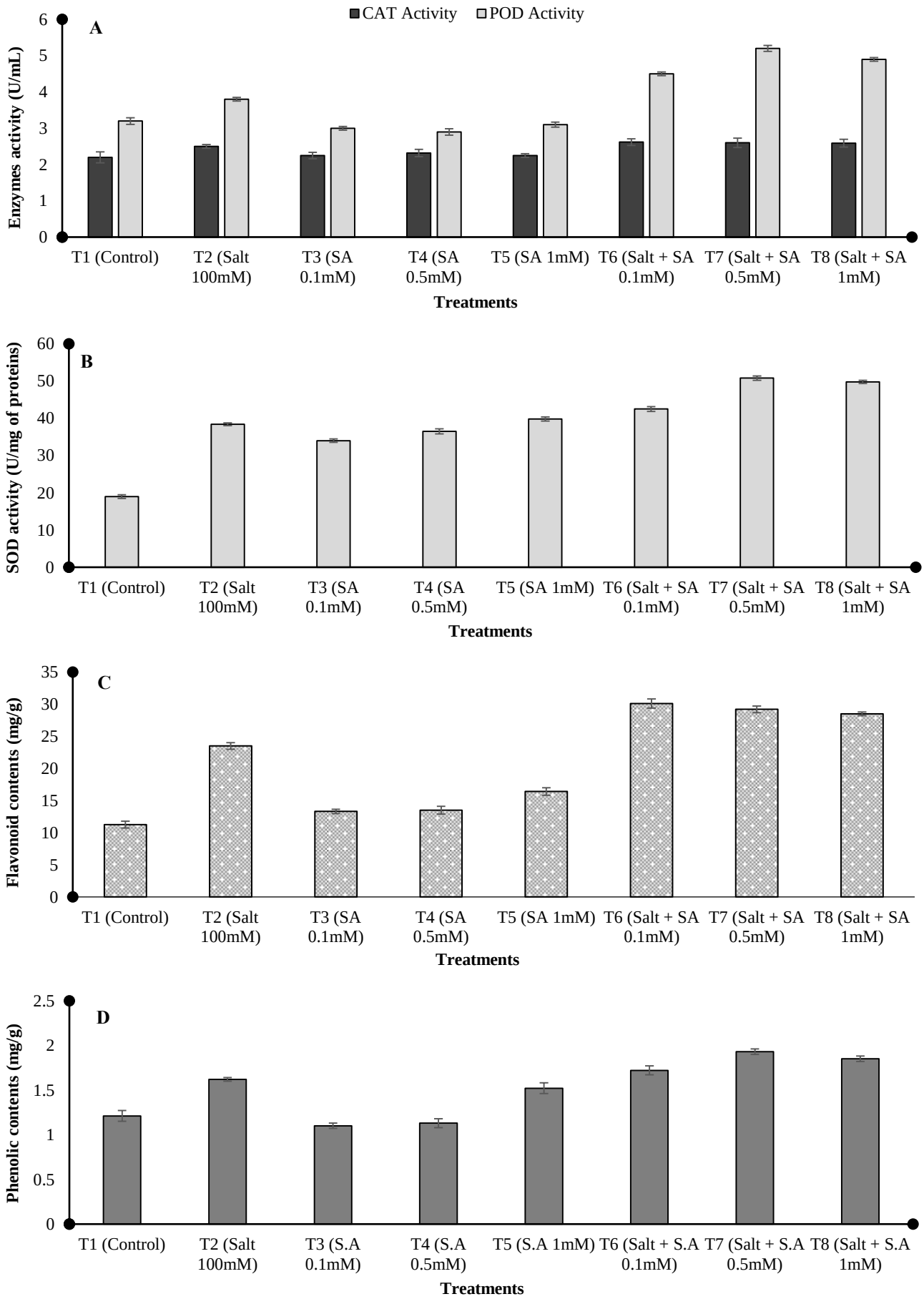

Fig. 1. Effect of SA on biochemical parameters of lemon grass under salt stress; (A) Catalase and POD Activity, (B) SOD Activity, (C) Flavonoids contents, (D) Phenolic contents.

Discussion

Salinity stress proves to be an inhibitor of not only certain plant growth parameters and development but yield as well. Excessive amounts of salt are found to create ionic imbalances and osmotic stresses in various plants spp. (Maggio *et al*., 2000). These effects thus create various types of abnormalities like severe oxidative damage that may ultimately end up in diminished growth of plants (Zhu, 2001). In this study, foliar spray of SA enhanced morphological attribute of lemongrass under salt stress conditions. The diminish in plant shoots/root lengths, number of root/tillers, leaves and fresh/dry weight and diameter of stem under salt stress might be a regular impact of the poisonous Na^+ and Cl^- ions accumulation in cells which extremely influence cell expansion and division as reported by Munns (1993). This declining growth under saline stress, a regular feature, has been observed in different plants by many authors (Alpaslan & Gunes, 2001; Kaya & Higgs, 2002; Sivritepe *et al*., 2003). Foliar sprays of salicylic acid overcome these constraints as compared with the non-sprayed salt stress plants. These comparative outcomes were studied by El-Tayeb (2005) in barley, Stevens & Senaratana (2006) in tomato, Gunes *et al*., (2007) in maize and Yildirim *et al*., (2008) in cucumber. They stated that foliar SA application improved the deleterious impacts of salinity stress on all the growth characteristics of plants. This positive outcome of salicylic acid could be ascribed to an enhanced CO_2 absorption, rate of photosynthesis and enhanced mineral up take by the plants treated with SA as compared to non-treated SA plants.

In literature, studies revealed that any type of stress enhance the production of Reactive Oxygen Species (ROS) in plants (Lalarukh & Shahbaz, 2020; Sharma *et al*., 2012). They reported that salt stress is thought to bring about production of ROS in plants causing oxidative damage. CAT (Catalases), POX (Peroxidases) and SOD (Superoxide dismutase) are antioxidant agents that shield cells from oxidative damage of highly reactive free radicals. To ameliorate and repair the damage done by ROS, plants build up a heavy framework of antioxidant agents. These antioxidant agents protecting framework can be found in various sub-cellular compartments as revealed by Gilani *et al*., (2020). Generally, salicylic acid work as an antioxidant agents and concentrated in the chloroplast, ensure the photosynthetic system intact and scavenging the free radicals which are actual reason behind damages. These impacts might be because of securing the endogenous antioxidant frameworks regularly related with enhanced protection from oxidative stress and additionally monitoring the free radicals level in tissues of plants, reported by Sreenivasulu *et al*., (2000). The up-regulation of antioxidant enzymes activities during this investigation in lemongrass by SA spray could be considered as a marker for developing a defensive mechanism and to diminish oxidative damage caused by salt stress. It has been observed that salt stress causes oxidative stress by limiting the carbon dioxide absorption, presenting chloroplasts to extreme excitation energy resulting into an increase the production of ROS from triplet chlorophyll (Gossett *et al*., 1994). Additionally, the production of antioxidant enzymes and defensive part of sheaths (membranes) by SA caused increment in resilience against the damage to plant (Turan & Aydin, 2005).

During this investigation, an increment in antioxidant enzymes activities with salt and increasing in salicylic acid concentration, turned out to be useful in mitigating the destructive impact of salt. The increase in POD and CAT activities might be due to increase in H_2O_2, a significant ROS created in response of various stresses. Present study revealed that scavenging of SOD increased under NaCl stress and this increasing pattern was also found by foliar spray of SA at various levels compared to non-sprayed control plants. These outcomes are comparative with Jaiswal *et al*., (2014) who reported that SOD was one of the major superoxide scavengers. It acts as first line of defence against the oxidative stress. They suggested that salt resistance in soybean might be due to enhanced activities of antioxidant enzymes. The activities of different antioxidative enzymes in turn re-established a specific oxidative balance and ultimately inhabit the cell damage caused by secondary oxidative stress. In addition to this, it is recommended that soybean being a moderately salt tolerant plant may have deteriorating ROS neutralizing mechanism, along with other resistance framework, to acclimatize stress. In present study, salinity enhanced superoxide dismutase movement (SOD) which in line with Fahad & Bano (2012) investigation on maize plants. According to their findings, saline condition brought about fundamentally higher SOD activity especially in leaves. During this investigation, SA enhanced the antioxidant system of lemongrass plants that reflected in better growth of plants under NaCl stress.

Conclusions

In conclusion, the significant reductions in all the studied morphological and biochemical parameters of lemongrass at concentration of 100 mM NaCl seems to indicate that it does not thrive well in high salt levels. Further, the salinity threshold level might be lower than 100 mM. Salicylic acid a potent non enzymatic antioxidant at lower concentration (0.5 mM) possibly reduced the damage caused by salt toxicity and ROS by increasing POD, CAT and SOD activities.

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(Received for publication 23 July 2020)