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SALICYLIC ACID ALLEVIATES THE ADVERSE EFFECTS OF SODIUM FLUORIDE STRESS BY IMPROVING GROWTH AND BIOCHEMICAL ATTRIBUTES IN *CAPSICUM ANNUUM* L.

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ABSTRACT: Although fluoride, the ion of fluorine, occurs in only a small amount in earth crust it is an important environmental pollutant. Soil contaminated by fluoride is one of the main problems in worldwide. *Capsicum annuum* L. (chili) is an edible crop used for human nutrition. It has antibacterial, anti-carcinogenic, anti-mutagenic, antioxidant, and immunosuppressive properties. The present study was done to investigate the effects of salicylic acid on plant growth, biomass production, and the photosynthetic attributes of *Capsicum* under sodium fluoride stress. Two varieties of *Capsicum annuum* L., Magma and Zenia, were studied by giving as a soil drench, twice a week to create stress, different concentrations of sodium fluoride, i.e., 50, 100, 150, 200, 250, and 300 ppm. The gradual accumulation of sodium fluoride created toxic effects on growth of plants. Significant reduction was observed in plant growth and biochemical parameters with increased concentrations of sodium fluoride in both varieties of *C. annuum*. The foliar applications of salicylic acid (100 ppm and 200 ppm) were also given to lessen the effects of the fluoride stress. It was observed that plant height and biomass production of *C. annuum* plant decreased under sodium fluoride stress while this stress was less in plants which were treated with NaF and SA. It is concluded that the foliar application of salicylic acid promotes salt tolerance in the two varieties of *C. annuum*.

Keywords: Antioxidants; *C. annuum*; Growth; Salicylic acid, Salt stress.

INTRODUCTION

Although fluoride, the ion of the element fluorine, occurs naturally in the earth's crust in only small amounts, it acts as an important pollutant in the environment.[1] Different types of soil have 20 to 1000 µg/g of soil fluoride content.[2] Compounds of fluorine and many fluorides are extremely poisonous compared to potassium and sodium. These are present in contaminated acidic soil along with other pollutants which come from different sources. The source of fluoride in irrigation water is the high use of phosphate fertilizers, deposition from ceramic industries, and pollution from brick kilns.[3] Soil and water, as well as vegetation, are being contaminated by the presence of fluoride. Effects of various fluoride sources have been reported on the biology of plants.[4]

Fluoride interferes with enzymatic activities as well as division and expansion of cells which causes slow growth of plants. It causes reduction in various growth parameters, such as percentage germination, plant height, leaf number and area, and biomass.[5] It also inhibits photosynthesis as well as other processes while moving through transpiration stream from roots or by stomata. It accumulates in plant parts especially in leaf margins, and therefore it is known as accumulative poison.[6] Other

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physical responses that are the result of fluoride stress are necrosis and abscission of both leaves and flowers and reduced seed production.[7]

Salicylic acid (SA) is a growth regulating hormone naturally produced in plants. It is a water soluble phenolic compound which is antioxidant in nature. It acts as a signaling molecule which helps in maintaining various physio-biochemical processes in plants.[8] It is reported that it helps in improving the salt tolerance by enhancing plant growth and biomass production when applied exogenously.[9] Scientist believe that it lowers the lethal effects of salinity by increasing relative water content and its ameliorative role in nutrient uptake.[10]

Capsicum annuum L., chili, is an edible plant used to nourish humans and belongs to the family Solanaceae. Its seeds are rich in protein and carbohydrates and these constitute 18–20% of the dry matter. It is grown annually as a fresh vegetable in the cool season. The main objective of the present study was to understand whether the foliar application of salicylic acid could increase the NaF stress tolerance in two varieties of chili plants, *Capsicum annuum* (Magma and Zenia), and to observe changes in various growth parameters and biochemical attributes.

MATERIALS AND METHODS

Two hybrid varieties of *Capsicum annuum* plant, named as Magma and Zenia, were raised using certified seeds purchased from Roshan Centre Zarai Markaz, Lahore, Pakistan. Healthy seeds, without damage or any fungal infection, were selected for sowing and stored in paper bags for future use. Analytical grade of chemicals; sodium fluoride (NaF) and salicylic acid (SA) were purchased from market. Botanical Garden of botany department in University of the Punjab, Lahore, was chosen as an experimental site to conduct pot experiment. Duration of experimental work was from March 2018 to June 2018. The earthen pots were used to carry out experiment. The holes present at the bottom of pots were partially closed with pebbles to inhibit water loss due to extreme drainage. Each pot was filled with 7 kg of soil and labelled properly. Soil was prepared by mixing sandy and loamy soil in 1:3 ratio along with farmyard and leaf manure. The randomized complete block design (RCBD) was used to design the experiment with three replicates of all treatments. Solutions of sodium fluoride (NaF) and salicylic acid (SA) were prepared in various concentrations by mixing the required amount in distilled water. Four seeds were sown in each pot but before sowing they were soaked for 24 hours in clean tap water. After one week of germination, an equal sized and healthy seedling was selected in each pot for further experiment, and the others were removed by manual thinning. Pots were carefully examined regularly to remove weeds and protect from pathogen attack. Prepared dilutions of sodium fluoride with different concentrations, i.e., 50, 100, 150, and 200 ppm were applied biweekly as a soil drench (150 mL in each pot) to create stress conditions. In addition, 6mL of salicylic acid with different concentrations (100, 200, and 300 ppm) was applied exogenously as a foliar spray by using a shower bottle. Control was provided with only tap water. Biomass estimation and different growth parameters were measured at three destructive harvests, at 44 days after sowing (DAS), 67 DAS, and 105 DAS.

Plants were carried in properly labeled bags to laboratory for measurement of growth parameters, i.e. shoot length (cm), root length (cm), number of leaves, and

number of branches. Then, treated plants were dried in electric oven (Gallenkamp, Model OV-455 England) at 70ºC for 72 hours after taking fresh weight of root, shoots and leaves using Sartorius GMBH, Type 1216MP 6E, Gottingen, Germany. The leaf area was calculated by the formula:

$$\text{Leaf area} = \text{length} \times \text{maximum width} \times 0.75 \text{ (correction factor)}.^{11}$$

Data collected from experiment was statistically analyzed. Costat (version 3.03) was used to calculate treatment mean, standard error, and Duncan's Multiple Range Test.[12]

RESULTS

The growth parameters of *C. annuum* L. were improved and enhanced in the presence of salicylic acid. By increasing the sodium fluoride concentration, reduction in growth was recorded. Maximum morphological growth was recorded in Magma variety. Reduction in morphological parameters e.g. root length, leaf number, and shoot length was studied in Zenia variety as compared to Magma.

Shoot length: During the present investigation 21 different treatments were used including:

Control, NaF-50, NaF-100, NaF-150, NaF-200, NaF-250, NaF-300, SA- 100 ppm, SA-200 ppm, NaF-50+SA-100, NaF-100+SA-100, NaF-150+SA-100, NaF-200+SA-100, NaF-250+SA-100, NaF-300+SA-100, NaF-50+SA-200, NaF-100+SA-200, NaF-150+SA-200, NaF-200+SA-200, NaF-250+SA-200, and NaF-300+SA-200.

Figure 1A represents the reduction in shoot length of Magma variety with increasing the concentration of sodium fluoride. Sodium fluoride at its higher level 300 ppm showed the maximum reduction percentage, 34.11%, as compared to 250, 200, 150, 100, and 50 ppm when the reductions were 26.61, 18.73, 12.27, 8.66, and 5.81%, respectively. However, shoot growth has been significantly improved by applying salicylic acid in foliar form. Plants showed maximum growth under salicylic acid application. SA in 100 ppm enhanced the growth of plant (–4.52%) while salicylic acid in 200 ppm has reducing effects similar to that of NaF.

Salicylic acid reduced the sodium fluoride stress when both applied in combination. Increase in shoot length was studied in the presence of NaF-50+SA-100 while growth was decreased in presence of NaF-50+SA-200 and NaF-50. High percentage reductions were recorded with NaF-50+SA-200, NaF-100+SA-200, NaF-150+SA-200, NaF-200+SA-200, NaF-250+SA-200, and NaF-300+SA-200 which were 11.37, 16.28, 21.96, 28.55, 38.76 and 46.51%, respectively, due to stress of both SA and NaF. However, NaF-50+SA-100, NaF-100+SA-100, NaF-150+SA-100, NaF-200+SA-100, NaF-250+SA-100, NaF-300+SA-100 showed lower reductions which were 4.39, 8.27, 11.37, 16.28, 21.96, and 28.55%, respectively.

According to the Figure 2C, shoot length of Zenia variety has maximum growth at NaF-50 ppm as compared to other sodium fluoride concentrations. At NaF-50 ppm plant shoot length showed higher growth and percentage reduction was 4.69% as compared to 100, 150, 200, 250, and 300 ppm during the whole experiment which had reductions of 6.69,12.99, 17.67, 27.04, and 32.26%, respectively. Application of

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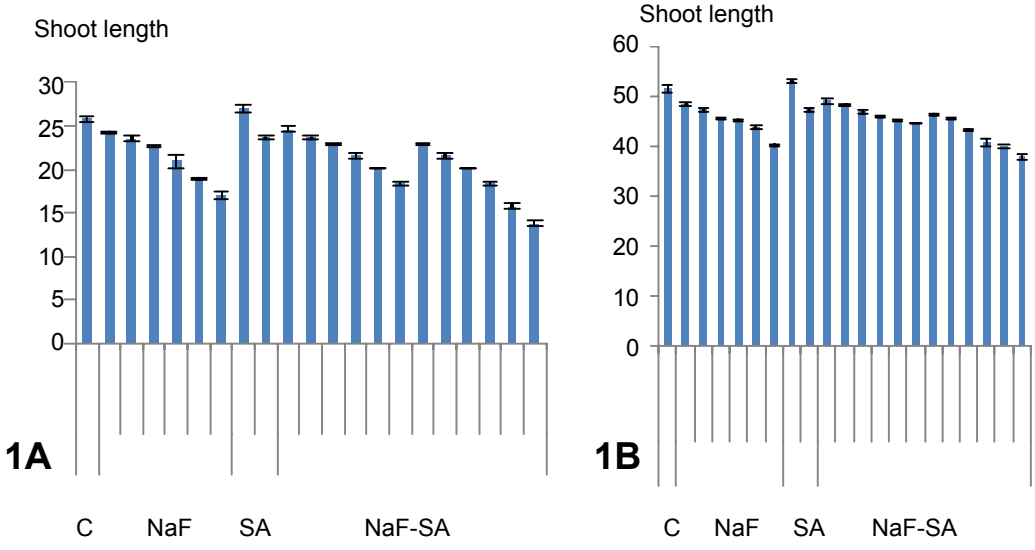
foliar salicylic acid with SA-100 ppm was more effective compared to SA-200 ppm. The combination of various concentrations of sodium fluoride 100, 150, 200, 250, and 300 ppm along with SA-200 had high percentage reductions of 8.43, 18.61, 27.04, 32.26, 33.47, and 41.10%, respectively, as compared to SA-100 ppm with reductions of 1.87, 7.10, 8.03, 8.84, 12.85, and 18.34%, respectively. Salicylic acid in SA-100 ppm showed a better growth effect on Capsicum growth in comparison to SA-200 ppm. The similar growth pattern of shoot length was recorded in both varieties (Magma and Zenia) on the growth stage at 103 DAS. The shoot length in both varieties at 103 DAS increased as compared to the 56 DAS. The maximum values for both varieties were recorded at 103 DAS at which date NaF has reducing and SA has enhancing effects.



Figures 1A–1B. Shoot length in *Capsicum* varieties. 1A: Magma at 56 days after sowing DAS; 1B: Magma at 103 DAS; under different stress concentration of sodium fluoride (NaF) with the ameliorative effect of salicylic acid (SA) during growth season 2018. C=control. The 21 treatments studied, numbering from the left, were:

1	Control	12	NaF-150 ppm + SA-100 ppm
2	NaF-50 ppm	13	NaF-200 ppm + SA-100 ppm
3	NaF-100 ppm	14	NaF-250 ppm + SA-100 ppm
4	NaF-150 ppm	15	NaF-300 ppm + SA-100 ppm
5	NaF-200 ppm	16	NaF-50 ppm + SA-100 ppm
6	NaF-250 ppm	17	NaF-100 ppm + SA-200 ppm
7	NaF-300 ppm	18	NaF-150 ppm + SA-200 ppm
8	SA-100 ppm	19	NaF-200 ppm + SA-200 ppm
9	SA-200 ppm	20	NaF-250 ppm + SA-200 ppm
10	NaF-50 ppm + SA-100 ppm	21	NaF-300 ppm + SA-200 ppm
11	NaF-100 ppm + SA-100 ppm		

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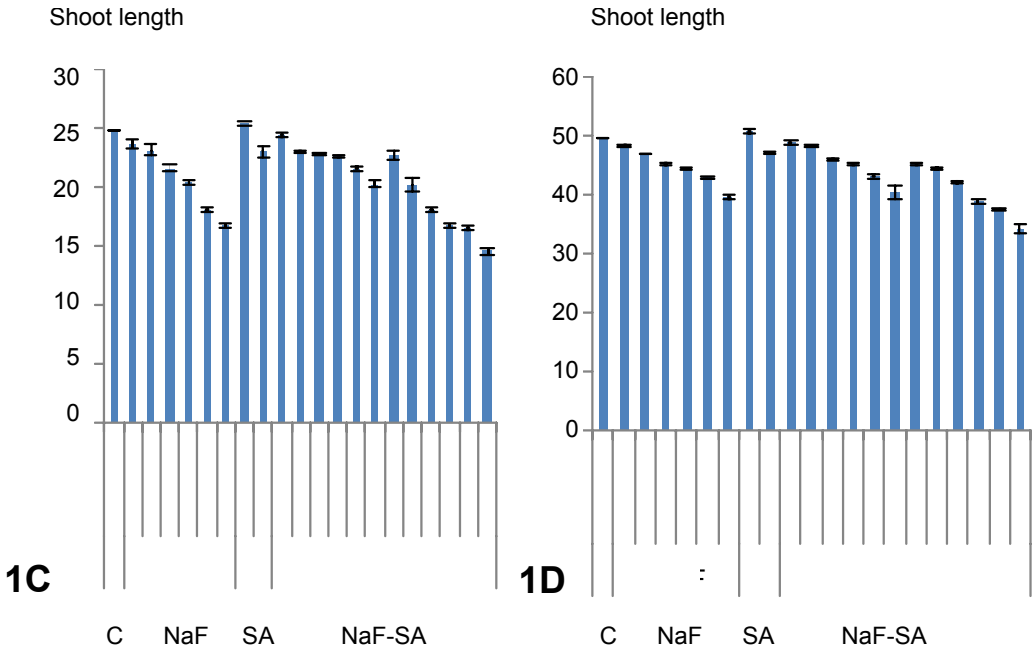

Figures 1C–1D. Shoot length in *Capsicum* varieties. 1C: Zenia at 56 days after sowing DAS; 1D: Zenia at 103 DAS; under different stress concentration of sodium fluoride (NaF) with the ameliorative effect of salicylic acid (SA) during growth season 2018. C=control. The 21 treatments studied, numbering from the left, were:

1	Control	12	NaF-150 ppm + SA-100 ppm
2	NaF-50 ppm	13	NaF-200 ppm + SA-100 ppm
3	NaF-100 ppm	14	NaF-250 ppm + SA-100 ppm
4	NaF-150 ppm	15	NaF-300 ppm + SA-100 ppm
5	NaF-200 ppm	16	NaF-50 ppm + SA-100 ppm
6	NaF-250 ppm	17	NaF-100 ppm + SA-200 ppm
7	NaF-300 ppm	18	NaF-150 ppm + SA-200 ppm
8	SA-100 ppm	19	NaF-200 ppm + SA-200 ppm
9	SA-200 ppm	20	NaF-250 ppm + SA-200 ppm
10	NaF-50 ppm + SA-100 ppm	21	NaF-300 ppm + SA-200 ppm
11	NaF-100 ppm + SA-100 ppm		

Root length: Figures 2A and 2B represents the root length of variety Magma for treated and non-treated plants at 56 and 103 DAS, respectively. It was found that sodium fluoride at NaF-300 ppm showed the maximum percentage reduction of root growth, 42.40%. With NaF-50, NaF-100, NaF-150, NaF-200, and NaF-250 the percentage reduction was lower at levels of 10.60, 20.51, 28.11, 36.41, and 38.48%, respectively.

Root growth was significantly enhanced in the presence of salicylic acid as a foliar application. SA-100 ppm showed the maximum root growth (–13.82%) as compared to SA-200 ppm (9.91%). The NaF-50+SA-100, NaF-100+SA-100, NaF-150+SA-100, NaF-200+SA-100, NaF-250+SA-100, and NaF-300+SA-100 showed reductions of 1.15, 8.76, 10.37, 27.65, 36.64, and 40.55%, respectively. NaF-50+SA-200, NaF-100+SA-200, NaF-150+SA-200, NaF-200+SA-200, NaF-250+SA-200, and NaF-

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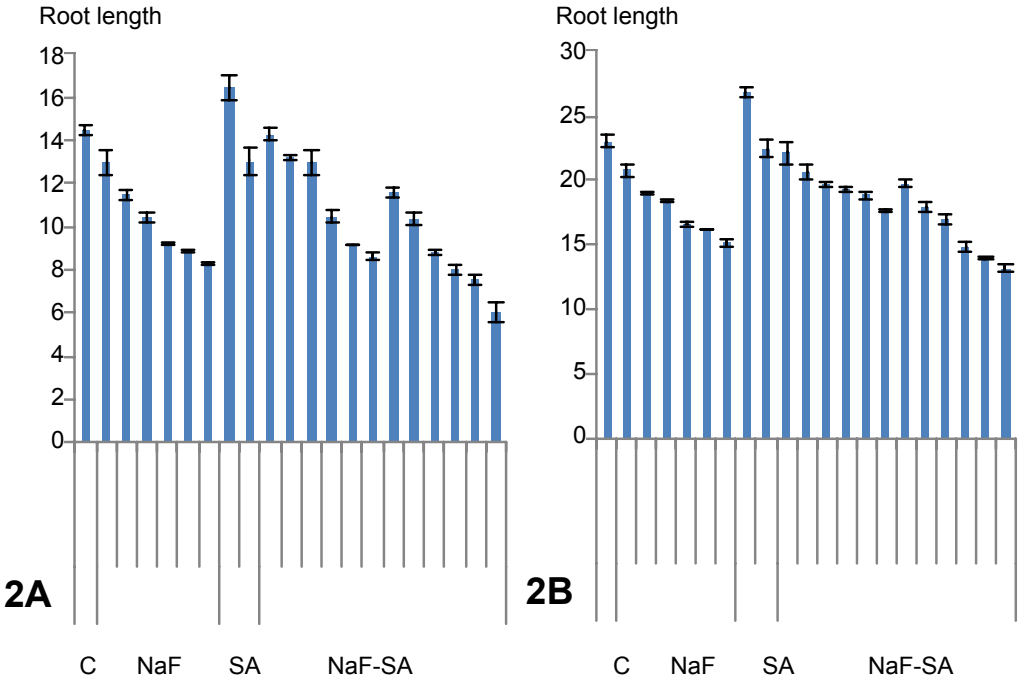
300+SA-200 showed high values of reduction of 20.05, 28.34, 39.17, 44.70, 47.70, and 58.53%, respectively, due to the combined stress of both salicylic acid and sodium fluoride.



Figures 2A–2B. Root length in *Capsicum* varieties. 2A: Magma at 56 days after sowing DAS; 2B: Magma at 103 DAS; under different stress concentration of sodium fluoride (NaF) with the ameliorative effect of salicylic acid (SA) during growth season 2018. C=control. The 21 treatments studied, numbering from the left, were:

1	Control	12	NaF-150 ppm + SA-100 ppm
2	NaF-50 ppm	13	NaF-200 ppm + SA-100 ppm
3	NaF-100 ppm	14	NaF-250 ppm + SA-100 ppm
4	NaF-150 ppm	15	NaF-300 ppm + SA-100 ppm
5	NaF-200 ppm	16	NaF-50 ppm + SA-100 ppm
6	NaF-250 ppm	17	NaF-100 ppm + SA-200 ppm
7	NaF-300 ppm	18	NaF-150 ppm + SA-200 ppm
8	SA-100 ppm	19	NaF-200 ppm + SA-200 ppm
9	SA-200 ppm	20	NaF-250 ppm + SA-200 ppm
10	NaF-50 ppm + SA-100 ppm	21	NaF-300 ppm + SA-200 ppm
11	NaF-100 ppm + SA-100 ppm		

Figures 2C and 2D represents root growth of Zenia variety recorded for treated and non-treated plants at 56 and 103 DAS, respectively. Sodium fluoride in NaF-50 and 100 ppm concentrations has a lesser effects on plant root growth with values of 11.14 and 21.55%, respectively, in comparison to 150, 200, 250, and 300 ppm which had values of 29.54, 38.26, 40.44, and 44.55%, respectively. Foliar application of salicylic acid SA-100 was enhanced the growth more efficiently (–11.38%) in

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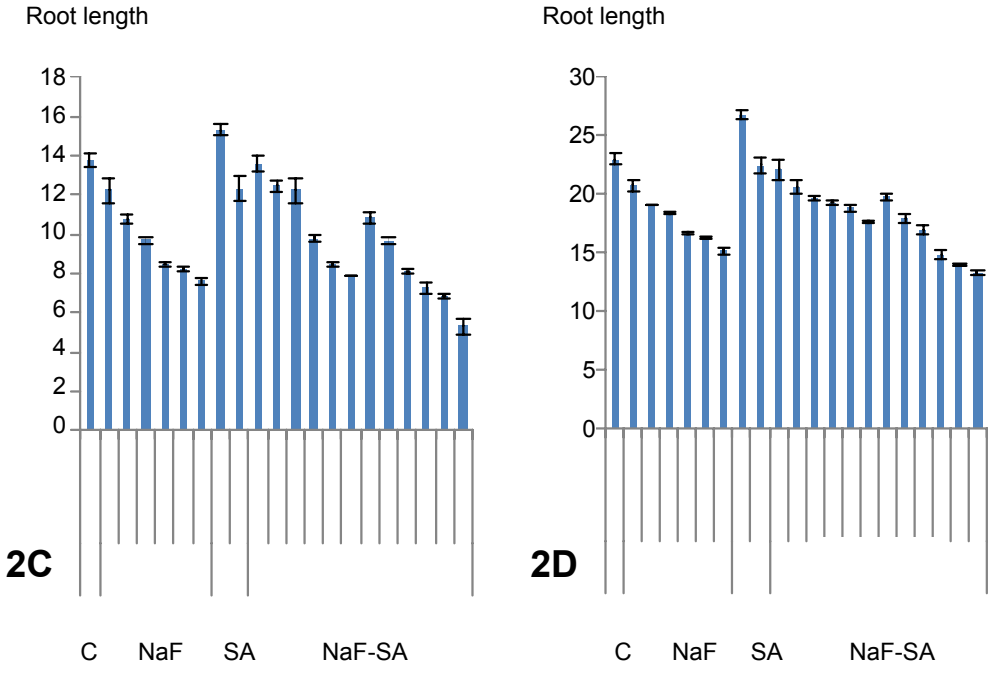
relation to SA-200 (10.41%). Capsicum showed maximum root growth at NaF-50+SA-100 as 1.21% compared to NaF-50 and NaF-50+SA-200 with values of 11.14 and 21.07%, respectively. Hence, root length in the presence of NaF-300+SA-200 and NaF-300 showed high reductions of 61.50% and 44.55% in contrast to NaF-300+ SA-100 (42.62%). Treatments of sodium fluoride with salicylic acid 100 and 200 showed the same trend of percentage reduction.

Root length recorded at 103 DAS for both Capsicum varieties (Magma and Zenia) showed the same pattern of growth as described above. The overall root length was increased at 103 DAS having maximum value as compared to 56 DAS.



Figures 2C–2D. Root length in *Capsicum* varieties. 2C: Zenia at 56 days after sowing DAS; 2D: Zenia at 103 DAS; under different stress concentration of sodium fluoride (NaF) with the ameliorative effect of salicylic acid (SA) during growth season 2018. C=control. The 21 treatments studied, numbering from the left, were:

1	Control	12	NaF-150 ppm + SA-100 ppm
2	NaF-50 ppm	13	NaF-200 ppm + SA-100 ppm
3	NaF-100 ppm	14	NaF-250 ppm + SA-100 ppm
4	NaF-150 ppm	15	NaF-300 ppm + SA-100 ppm
5	NaF-200 ppm	16	NaF-50 ppm + SA-100 ppm
6	NaF-250 ppm	17	NaF-100 ppm + SA-200 ppm
7	NaF-300 ppm	18	NaF-150 ppm + SA-200 ppm
8	SA-100 ppm	19	NaF-200 ppm + SA-200 ppm
9	SA-200 ppm	20	NaF-250 ppm + SA-200 ppm
10	NaF-50 ppm + SA-100 ppm	21	NaF-300 ppm + SA-200 ppm
11	NaF-100 ppm + SA-100 ppm		

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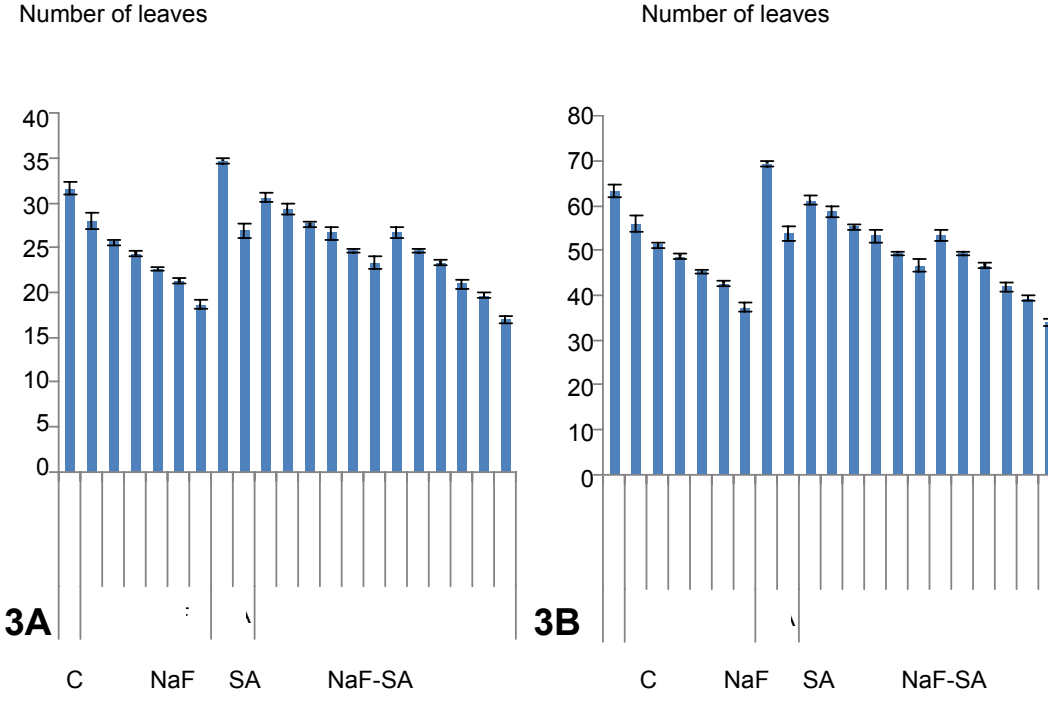
Number of leaves: Number of leaves recorded for variety Magma is presented in Figure 3A. Plants grown under control and SA-100 ppm have higher leaf numbers. Under NaF-50 and NaF-100 ppm a low reduction of the leaves number was found (11.58 and 18.95%, respectively) than the reductions found of 23.16, 28.42, 32.63, and 41.05% under treatment with 150, 200, 250, and 300 ppm NaF concentrations, respectively.



Figures 3A–3B. Number of leaves in *Capsicum* varieties. 2A: Magma at 56 days after sowing DAS; 2B: Magma at 103 DAS; under different stress concentration of sodium fluoride (NaF) with the ameliorative effect of salicylic acid (SA) during growth season 2018. C=control. The 21 treatments studied, numbering from the left, were:

1	Control	12	NaF-150 ppm + SA-100 ppm
2	NaF-50 ppm	13	NaF-200 ppm + SA-100 ppm
3	NaF-100 ppm	14	NaF-250 ppm + SA-100 ppm
4	NaF-150 ppm	15	NaF-300 ppm + SA-100 ppm
5	NaF-200 ppm	16	NaF-50 ppm + SA-100 ppm
6	NaF-250 ppm	17	NaF-100 ppm + SA-200 ppm
7	NaF-300 ppm	18	NaF-150 ppm + SA-200 ppm
8	SA-100 ppm	19	NaF-200 ppm + SA-200 ppm
9	SA-200 ppm	20	NaF-250 ppm + SA-200 ppm
10	NaF-50 ppm + SA-100 ppm	21	NaF-300 ppm + SA-200 ppm
11	NaF-100 ppm + SA-100 ppm		

Foliar application of SA-100 gave better results compared to SA-200. Higher leaf numbers were recorded under NaF-50+SA-100 as compared to NaF-50 and NaF-50+SA-200. SA-200 with the other sodium fluoride treatments, viz., 100, 150, 200, 250, and 300 ppm, also had lower leaf numbers as compared to SA-100 ppm. Percentage reduction values under NaF-50+SA-100, NaF-100+SA-100, NaF-

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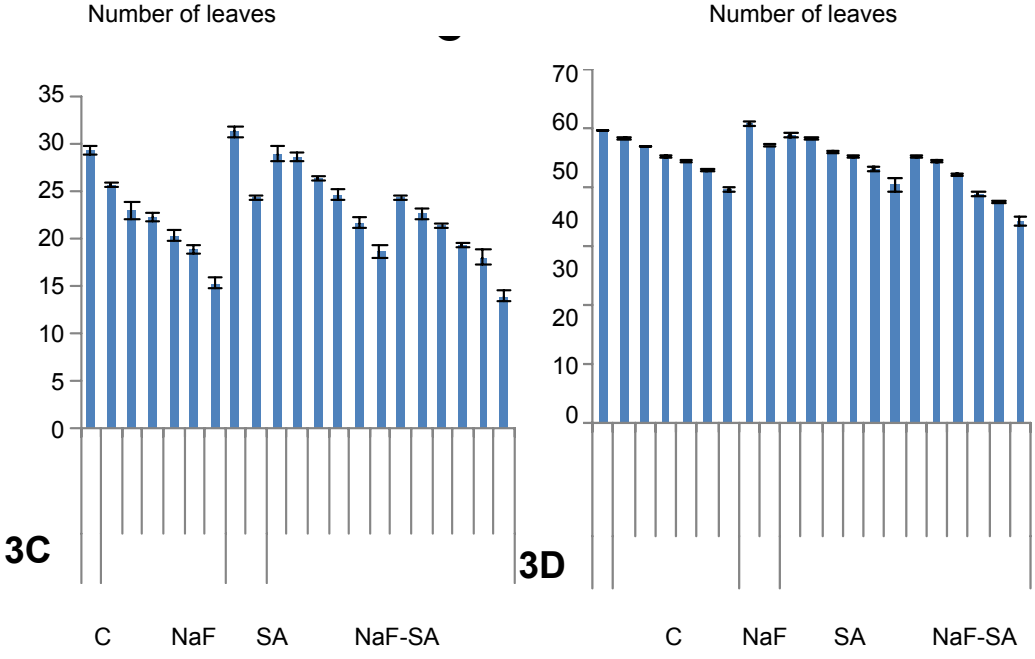
150+SA-100, NaF-200+SA-100, NaF-250+SA-100, and NaF-300+SA-100 were 3.16, 7.37, 12.63, 15.79, 22.11, and 26.32%, respectively, as compared 15.79, 22.11, 26.32, 33.68, 37.89, and 46.32% for NaF-50+SA-200, NaF-100+SA-200, NaF-150+SA-200, NaF-200+SA-200, NaF-250+SA-200, and NaF-300+SA-200 due to the combined reducing effects of sodium fluoride and salicylic acid.

Figure 3C showed that number of leaves in variety Zenia reduced by increasing the NaF concentrations.



Figures 3C–3D. Number of leaves in *Capsicum* varieties. 2C: Zenia at 56 days after sowing DAS; 2D: Zenia at 103 DAS; under different stress concentration of sodium fluoride (NaF) with the ameliorative effect of salicylic acid (SA) during growth season 2018. C=control. The 21 treatments studied, numbering from the left, were:

1	Control	12	NaF-150 ppm + SA-100 ppm
2	NaF-50 ppm	13	NaF-200 ppm + SA-100 ppm
3	NaF-100 ppm	14	NaF-250 ppm + SA-100 ppm
4	NaF-150 ppm	15	NaF-300 ppm + SA-100 ppm
5	NaF-200 ppm	16	NaF-50 ppm + SA-100 ppm
6	NaF-250 ppm	17	NaF-100 ppm + SA-200 ppm
7	NaF-300 ppm	18	NaF-150 ppm + SA-200 ppm
8	SA-100 ppm	19	NaF-200 ppm + SA-200 ppm
9	SA-200 ppm	20	NaF-250 ppm + SA-200 ppm
10	NaF-50 ppm + SA-100 ppm	21	NaF-300 ppm + SA-200 ppm
11	NaF-100 ppm + SA-100 ppm		

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Under the presence of NaF-300, maximum reduction was found, 47.73%, in comparison to 50, 100, 150, 200, and 250 ppm which had values of 12.50, 21.59, 23.86, 30.68, 35.23, and 47.73%, respectively. The leaves number was increased in the presence of foliar application of SA-100 ppm, while a reducing effect was noted under the SA-200. Significant increases in leaves number were recorded under NaF-50+SA-100, NaF-100+SA-100, NaF-150+SA-100, NaF-200+SA-100, NaF-250+SA-100, and NaF-300+SA-100 that were 1.14, 2.27, 10.23, 15.91, 26.14, and 36.36%, respectively. However, high reductions were found of 34,17.05, 22.73, 27.27, 34.09, 38.64, and 52.27% under the influence of NaF-50+SA-200, NaF-100+SA-200, NaF-150+SA-200, NaF-200+SA-200, NaF-250+SA-200, and NaF-300+SA-200, respectively. The values in similar growth pattern at 103 DAS were taken for both varieties (Magma and Zenia) of *Capsicum*. The higher values for leaves number were observed at 103 DAS.

Biomass assessment: Biomass assessment of Capsicum plant, harvested at 56 and 103 DAS during the present investigation, are shown in Tables 1 and 2 for variety Magma and in Tables 3 and 4 for variety Zenia.

Adverse effects of sodium fluoride were recorded on shoot and root fresh weight. By increasing the sodium fluoride concentrations, shoot and root fresh weight values were decreased considerably in Table 1 at 56 DAS.

Magma variety exhibited maximum root and shoot fresh weight values in control conditions. Percentage reduction values studied in variety Magma were 6.52, 12.01, 17.86, 21.11, 24.40, and 33.01% for shoot while in the case of root 5.48, 12.01, 17.23, 24.28, 29.50, and 38.12%, when grown in the vicinity of NaF, i.e., 50, 100, 150, 200, 250, and 300 ppm, respectively. Hence, plant stress was reduced in the presence of salicylic acid. Plants shoot and root fresh weights were effectively increased by using SA-100, with values of –4.70 and –17.49%, respectively, in comparison to SA-200 ppm, with values of 4.75 and 25.85%, respectively, showing reduction for shoot and root fresh weights respectively.

Table 3 shows the percentage reduction trend of shoot and root fresh weight at 56 DAS for variety Zenia. Plants grown under control conditions have maximum fresh weight values for shoot and root. Under NaF-50+SA-100 treatment, percentage reduction value of fresh weight was minimum as in case of shoot 2.65% and root 2.96% comparatively to NaF-50 and NaF-50+SA-200 (8.44 and 12.23%, respectively) for shoot and (5.38 and 8.60%, respectively) for root respectively. However, high percentage reductions of shoot and root fresh weight was recorded with NaF-300+SA-200, e.g., 43.51% and 44.09%, respectively, in contrast to NaF-300 and NaF-300+SA-100 (35.39 and 29.27%, respectively) for shoot and (39.52 and 36.02%, respectively) for root respectively. Same reduction trend was exhibited in plants with other treatments of NaF with SA-100 and SA-200 ppm. Plants of both varieties of Capsicum followed the same trend in shoot and root fresh weight and the highest values for fresh weights were recorded at 103 DAS.

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Table 1. Growth parameters of *Capsicum annuum* var. Magma harvested at 56 DAS by using different concentration of sodium fluoride and salicylic acid during growth season 2018. (Values are mean ± S.E; NaF: sodium fluoride; SA: salicylic acid; NaF-SA: sodium fluoride-salicylic acid)

Treatment	Growth parameter				
	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Leaf area (cm^2)
Control	34.16±0.67	6.38±0.08	10.66±0.59	3.57±0.33	17.08±0.88
NaF-50	31.93±0.52	6.03±0.07	10.21±0.57	3.33±0.33	15.53±0.77
NaF-100	30.06±0.60	5.62±0.06	9.12±0.76	3.03±0.35	15.03±0.56
NaF-150	28.06±0.56	5.28±0.10	8.12±0.76	2.79±0.34	14.92±0.55
NaF-200	26.95±0.56	4.83±0.10	8.11±0.57	2.69±0.34	12.99±0.75
NaF-250	25.83±0.65	4.50±0.12	6.74±0.75	2.41±0.33	12.57±0.57
NaF-300	22.89±0.76	3.95±0.09	6.39±066.	2.29±0.24	11.01±0.74
SA-100	35.76±0.94	7.50±0.07	11.95±0.74	3.97±0.40	20.78±0.47
SA-200	32.54±0.79	4.73±0.12	9.22±0.28	3.30±0.37	12.91±0.48
NaF-50+SA-100	32.78±0.56	6.15±0.07	10.35±0.56	3.53±0.43	16.63±0.38
NaF-100+SA-100	32.37±0.99	5.77±0.12	10.45±0.56	3.13±0.52	16.37±0.53
NaF-150+SA-100	29.84±0.95	5.53±0.10	9.65±0.52	3.02±0.52	15.47±0.57
NaF-200+SA-100	27.80±0.95	5.08±0.08	8.52±0.52	2.87±0.54	14.55±0.66
NaF-250+SA-100	26.72±0.91	4.73±0.12	8.29±0.52	2.77±0.50	13.14±0.41
NaF-300+SA-100	24.69±0.99	4.18±0.08	7.45±0.52	2.50±0.46	11.87±0.52
NaF-50+SA-200	29.72±0.98	5.83±0.10	8.55±0.37	2.77±0.33	14.74±0.36
NaF-100+SA-200	28.61±0.76	5.38±0.06	8.58±0.38	2.45±0.35	14.51±0.50
NaF-150+SA-200	24.87±0.97	4.97±0.03	7.3±0.33	2.27±0.28	14.26±0.22
NaF-200+SA-200	22.57±0.97	4.62±0.06	6.89±0.37	1.77±0.08	11.92±0.35
NaF-250+SA-200	21.65±1.00	4.08±0.18	6.50±0.22	1.72±0.13	11.28±0.44
NaF-300+SA-200	20.18±0.98	3.67±0.30	5.89±0.37	1.59±0.09	10.50±0.38

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Table 2. Growth parameters of *Capsicum annuum* var. Magma harvested at 103 DAS by using different concentration of sodium fluoride and salicylic acid during growth season 2018. (Values are mean ± S.E; NaF: sodium fluoride; SA: salicylic acid; NaF-SA: sodium fluoride-salicylic acid)

Treatment	Growth parameter				
	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Leaf area (cm^2)
Control	71.50±0.24	12.77±0.15	44.95±0.24	8.75±0.50	22.56±0.84
NaF-50	66.30±0.29	12.07±0.14	39.88±0.24	8.12±0.62	20.81±0.82
NaF-100	62.56±0.17	11.23±0.12	36.48±0.07	7.41±0.56	19.99±0.60
NaF-150	58.63±0.70	10.57±0.19	32.63±0.31	6.67±0.69	19.58±0.49
NaF-200	56.57±0.66	9.67±0.20	29.87±0.64	5.56±0.74	17.56±0.49
NaF-250	54.83±0.22	9.00±0.24	28.23±0.21	5.26±0.83	17.86±0.64
NaF-300	49.43±0.59	7.56±0.17	22.69±0.55	4.32±0.88	16.80±0.68
SA-100	75.47±0.61	15.00±0.14	49.41±0.22	10.72±0.35	25.68±0.80
SA-200	68.30±0.53	9.47±0.24	41.55±0.48	5.81±0.92	17.98±0.61
NaF-50+SA-100	69.50±0.62	12.30±0.14	43.45±0.24	8.26±0.59	21.67±0.79
NaF-100+SA-100	68.47±0.61	11.53±0.24	42.41±0.22	7.47±0.55	20.96±0.64
NaF-150+SA-100	63.97±0.45	11.07±0.19	37.11±0.27	7.01±0.56	20.71±0.88
NaF-200+SA-100	59.83±0.36	10.17±0.17	33.56±0.16	6.26±0.63	19.77±0.70
NaF-250+SA-100	57.17±0.36	9.47±0.24	30.67±0.35	5.52±0.68	18.09±0.60
NaF-300+SA-100	53.50±0.62	8.37±0.17	27.12±0.59	4.63±0.77	16.91±0.65
NaF-50+SA-200	63.73±0.31	11.67±0.20	37.13±0.31	7.53±0.45	19.30±0.48
NaF-100+SA-200	60.17±0.18	10.77±0.12	33.67±0.17	6.78±0.45	19.70±0.68
NaF-150+SA-200	53.93±0.19	9.93±0.05	27.36±0.19	6.01±0.53	18.95±0.61
NaF-200+SA-200	49.37±0.34	9.23±0.12	22.94±0.31	5.20±0.56	18.79±0.94
NaF-250+SA-200	47.43±0.59	8.17±0.36	20.69±0.55	4.10±0.70	17.82±0.78
NaF-300+SA-200	44.07±0.52	7.33±0.59	17.87±0.32	3.25±0.83	16.65±0.81

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Table 3. Growth parameters of *Capsicum annuum* var. Zenia harvested at 56 DAS by using different concentration of sodium fluoride and salicylic acid during growth season 2018 (Values are mean ± S.E; NaF: sodium fluoride; SA: salicylic acid; NaF-SA: sodium fluoride-salicylic acid)

Treatment	Growth parameter				
	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry Weight (g)	Leaf area (cm^2)
Control	30.80±0.12	6.20±0.09	9.77±0.38	2.92±0.48	16.59±0.65
NaF-50	28.20±0.18	5.87±0.07	8.95±0.46	2.63±0.36	15.61±0.30
NaF-100	26.43±0.12	5.42±0.04	8.25±0.64	2.47±0.23	14.49±0.40
NaF-150	24.48±0.25	5.07±0.10	7.50±0.59	2.36±0.28	14.29±0.53
NaF-200	23.48±0.25	4.60±0.11	7.42±0.63	2.16±0.28	13.64±0.33
NaF-250	22.82±0.05	4.27±0.12	6.19±0.60	1.74±0.38	12.23±0.37
NaF-300	19.56±0.19	3.75±0.09	6.13±0.67	1.62±0.38	10.75±0.50
SA-100	32.98±0.20	7.27±0.08	10.43±0.38	3.00±0.49	19.75±0.38
SA-200	29.08±0.25	4.53±0.12	9.03±0.59	2.60±0.37	12.61±0.36
NaF-50+SA-100	29.98±0.22	6.02±0.08	9.55±0.59	2.65±0.35	15.82±0.42
NaF-100+SA-100	29.35±0.31	5.58±0.15	9.23±0.59	2.57±0.38	15.72±0.31
NaF-150+SA-100	27.08±0.15	5.35±0.09	8.40±0.62	2.47±0.37	14.46±0.47
NaF-200+SA-100	25.00±0.21	4.88±0.08	8.02±0.22	2.31±0.34	13.65±0.53
NaF-250+SA-100	23.92±0.21	4.57±0.12	7.26±0.64	2.13±0.33	12.42±0.57
NaF-300+SA-100	21.78±0.34	3.97±0.07	6.38±0.36	1.93±0.33	11.23±0.35
NaF-50+SA-200	27.03±0.09	5.67±0.12	8.63±0.59	2.53±0.38	14.83±0.38
NaF-100+SA-200	24.95±0.13	5.17±0.07	8.03±0.63	2.43±0.38	13.46±0.57
NaF-150+SA-200	22.60±0.29	4.73±0.04	7.33±0.63	2.30±0.25	13.13±0.49
NaF-200+SA-200	19.75±0.20	4.42±0.06	7.22±0.66	2.01±0.34	12.66±0.38
NaF-250+SA-200	18.97±0.32	3.87±0.19	5.45±0.61	1.69±0.37	11.63±0.32
NaF-300+SA-200	17.40±0.18	3.47±0.30	5.43±0.63	1.53±0.27	9.80±0.71

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Table 4. Growth parameters of *Capsicum annuum* var. Zenia harvested at 103 DAS by using different concentration of sodium fluoride and salicylic acid during growth season 2018 (Values are mean ± S.E; NaF: sodium fluoride; SA: salicylic acid; NaF-SA: sodium fluoride-salicylic acid)

Treatment	Growth parameter				
	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Leaf area (cm^2)
Control	61.60±0.25	12.40±0.17	34.92±0.20	8.02±0.34	19.39±0.48
NaF-50	56.40±0.36	11.73±0.14	29.58±0.18	7.43±0.44	17.58±0.59
NaF-100	52.87±0.24	10.83±0.07	26.27±0.24	6.56±0.54	17.38±0.42
NaF-150	48.97±0.50	10.13±0.20	22.07±0.30	6.01±0.60	16.83±0.46
NaF-200	46.97±0.50	9.23±0.22	20.11±0.35	5.26±0.62	16.42±0.29
NaF-250	45.63±0.11	8.53±0.24	18.99±0.04	4.50±0.64	14.97±0.48
NaF-300	39.80±0.39	7.50±0.17	13.02±0.28	3.59±0.73	13.82±0.47
SA-100	65.97±0.40	14.53±0.17	39.24±0.34	9.89±0.18	21.57±0.62
SA-200	58.17±0.49	9.07±0.24	31.34±0.37	5.24±0.78	15.57±0.34
NaF-50+SA-100	59.97±0.45	12.03±0.15	33.11±0.27	7.71±0.52	18.34±0.47
NaF-100+SA-100	58.70±0.62	11.17±0.31	31.85±0.51	7.05±0.47	18.26±0.38
NaF-150+SA-100	54.17±0.31	10.70±0.17	27.61±0.31	6.51±0.55	17.74±0.37
NaF-200+SA-100	50.00±0.42	9.77±0.17	23.22±0.32	5.70±0.62	16.82±0.45
NaF-250+SA-100	47.83±0.43	9.13±0.24	20.99±0.26	5.17±0.63	15.70±0.31
NaF-300+SA-100	43.57±0.69	7.93±0.14	16.47±0.28	4.03±0.76	13.92±0.47
NaF-50+SA-200	54.07±0.18	11.33±0.24	27.37±0.06	7.13±0.45	17.42±0.44
NaF-100+SA-200	49.56±0.26	10.33±0.14	23.18±0.18	6.20±0.57	16.69±0.49
NaF-150+SA-200	45.20±0.57	9.47±0.07	18.42±0.50	5.38±0.68	16.57±0.34
NaF-200+SA-200	39.50±0.40	8.83±0.12	12.65±0.21	4.71±0.71	15.72±0.36
NaF-250+SA-200	37.93±0.63	7.73±0.38	11.19±0.59	3.92±0.71	14.63±0.32
NaF-300+SA-200	34.80±0.36	6.93±0.59	8.02±0.23	3.40±0.70	13.67±0.43

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It was observed that dry weight of shoot and root for variety Magma followed the same pattern as fresh weight complements of this variety with all the 21 treatments.

Percentage reductions of shoot dry weight of 4.19, 14.47, 23.85, 23.91, 36.76, and 40.02% and root dry weight of 6.54, 14.95, 21.87, 24.67, 32.34, and 35.70 were recorded for variety Magma under treatments of sodium fluoride at 50 to 300 ppm concentrations (56 DAS) as shown in Tables 1 and 2. Dry weights of shoot and root were high in control and the plants treated with 100 ppm SA with values of –12.14 and –11.21%, respectively, for shoots and roots. In comparison, the differences were minor with the other treatments.

Percentage reductions were also recorded for variety Zenia of 8.37, 15.48, 23.21, 24.07, 36.59, and 37.20% for shoots and 9.92, 15.62, 19.27, 26.11, 40.48, and 44.47% for roots, respectively, but these reductions in the dry weights of shoots and roots were more pronounced than with the Magma variety, as represented in Tables 3 and 4. Salicylic acid showed highest increases in root and shoot dry weights, after the control, of –2.62% in root and –6.83% in shoot.

At 103 DAS, the same trend of reduction in shoot and root dry weight for both Capsicum varieties was observed as described above. The maximum values were also recorded on 103 DAS in which reducing effects of NaF and enhancing effects of salicylic acid was seen.

Leaf area: The leaf area at 56 DAS for both Capsicum varieties (Magma and Zenia) are shown in Tables 1 and 3, respectively. Significant decrease in leaf area was observed with increasing NaF concentrations. In the case of Magma, percentage reduction for leaf area recorded was 9.07, 11.97, 12.62, 23.95, 26.39, and 35.55% and in case of Zenia 5.92, 12.65, 13.86, 17.76, 26.29, and 35.19% at 50, 100, 150, 200, 250, and 300 ppm NaF treatments, respectively. However, salicylic acid application on plants under sodium fluoride stress improved the leaf area. More effective results were noted by applying SA-100 ppm compared to SA-200 ppm. SA-100 ppm showed increase in leaf ares of –21.70% and –19.05% for the Magma and Zenia varieties, respectively.

Same pattern of leaf area growth was observed at 103 DAS for both Capsicum varieties (Magma and Zenia). At 103 DAS, the greatest values were found for leaf area.

Determination of plant pigments: The data given in Tables 5 and 6 show that the amount of photosynthetic pigments for both varieties of *Capsicum annuum* (Magma and Zenia). The maximum values of chlorophyll "a", chlorophyll "b", total chlorophyll, and carotenoids were observed in control and SA-100 ppm. By increasing sodium fluoride stress, plant pigment values were decreased. Reduction in chlorophyll "a"', chlorophyll "b" values were found to be lower at 50 ppm, whereas at 300 ppm NaF the maximum reduction was observed. Sodium fluoride treated plants had higher percentage reduction values compared to the control and salicylic acid 100 ppm groups and was also shown by the plants treated with the higher NaF levels being less green.

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Table 5. Plant pigments of *Capsicum annuum* var. Magma harvested at 56 DAS by using different concentration of sodium fluoride and salicylic acid during growth season 2018 (Values are mean ± S.E; NaF: sodium fluoride; SA: salicylic acid; NaF-SA: sodium fluoride-salicylic acid)

Treatment	Plant pigment			
	Chlorophyll "a" (ug/g fr. wt)	Chlorophyll "b" (ug/g fr. wt)	Total chlorophyll (ug/g fr. wt)	Carotenoids (ug/g fr.wt)
Control	9.02±0.01	16.51±0.24	16.52±0.11	626.65±0.39
NaF-50	7.54±0.05	15.46±0.31	15.01±0.02	488.76±0.53
NaF-100	7.33±0.04	13.10±0.02	14.13±0.35	475.49±0.51
NaF-150	7.12±0.04	12.92±0.15	13.12±0.03	448.39±0.60
NaF-200	6.66±0.06	12.73±0.11	12.51±0.03	428.42±0.48
NaF-250	6.15±0.04	12.29±0.06	12.24±0.24	333.96±0.47
NaF-300	6.13±0.04	11.09±0.02	10.97±0.29	221.44±0.44
SA-100	9.78±0.05	17.00±0.06	17.29±0.37	668.25±0.57
SA-200	8.06±0.05	15.30±0.17	14.38±0.08	533.95±0.47
NaF-50+SA-100	8.21±0.02	16.13±0.02	15.44±0.05	545.58±0.38
NaF-100+SA-100	8.09±0.02	15.79±0.05	15.08±0.03	485.59±0.35
NaF-150+SA-100	8.03±0.03	15.73±0.05	15.07±0.03	468.61±0.32
NaF-200+SA-100	7.96±0.03	15.13±0.03	14.68±0.04	440.79±0.95
NaF-250+SA-100	7.63±0.02	14.42±0.02	14.37±0.06	380.60±0.51
NaF-300+SA-100	7.63±0.04	13.59±0.01	13.85±0.04	331.38±0.98
NaF-50+SA-200	6.11±0.01	12.10±0.03	13.15±0.06	478.54±0.39
NaF-100+SA-200	5.91±0.03	10.81±0.03	12.17±0.07	466.81±0.43
NaF-150+SA-200	4.94±0.03	10.47±0.03	11.45±0.06	433.85±0.64
NaF-200+SA-200	4.73±0.02	9.11±0.03	10.56±0.02	392.34±0.43
NaF-250+SA-200	4.04±0.02	8.33±0.03	10.11±0.04	281.99±0.70
NaF-300+SA-200	3.27±0.08	7.78±0.04	9.17±0.35	197.36±0.54

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Table 6. Plant pigments of *Capsicum annuum* var. Zenia harvested at 56 DAS by using different concentration of sodium fluoride and salicylic acid during growth season 2018 (Values are mean ± S.E; NaF: sodium fluoride; SA: salicylic acid; NaF-SA: sodium fluoride-salicylic acid)

Treatment	Plant pigment			
	Chlorophyll "a" (ug/g fr. wt)	Chlorophyll"b" (ug/g fr. wt)	Total chlorophyll (ug/g fr. wt)	Carotenoids (ug/g fr.wt)
Control	7.89±0.02	15.40±0.08	14.66±0.04	502.55±0.49
NaF50	7.04±0.04	14.45±0.06	13.66±0.03	462.75±0.38
NaF-100	6.99±0.06	12.96±0.06	13.46±0.05	414.51±0.47
NaF-150	6.85±0.03	12.83±0.02	12.95±0.04	381.44±0.44
NaF-200	6.67±0.04	12.63±0.03	12.18±0.07	366.28±0.57
NaF-250	6.12±0.04	12.11±0.03	11.46±0.05	351.61±0.56
NaF-300	5.80±0.01	9.95±0.05	11.15±0.06	260.45±0.38
SA-100	8.32±0.04	15.71±0.02	15.15±0.03	556.64±0.58
SA-200	7.37±0.03	14.80±0.01	12.54±0.12	441.73±0.42
NaF-50+SA-100	7.80±0.01	14.98±0.01	14.47±0.04	473.60±0.44
NaF-100+SA-100	7.22±0.05	14.62±0.03	13.50±0.24	472.22±0.78
NaF-150+SA-100	7.15±0.04	14.59±0.04	13.12±0.44	391.69±0.69
NaF-200+SA-100	7.10±0.02	14.15±0.02	12.91±0.06	374.91±0.15
NaF-250+SA-100	6.56±0.03	13.87±0.04	12.65±0.04	374.14±0.61
NaF-300+SA-100	6.64±0.03	13.43±0.05	12.55±0.04	325.62±0.69
NaF-50+SA-200	6.01±0.05	12.02±0.05	12.58±0.46	429.58±0.40
NaF-100+SA-200	5.81±0.05	10.68±0.02	12.35±0.20	397.44±0.57
NaF-150+SA-200	4.83±0.03	10.28±0.05	11.19±0.25	372.56±0.61
NaF-200+SA-200	4.61±0.03	8.75±0.19	10.46±0.04	358.75±0.56
NaF-250+SA-200	3.85±0.04	8.20±0.00	10.44±0.31	338.61±0.35
NaF-300+SA-200	3.59±0.04	7.53±0.05	7.59±0.36	201.17±0.99

In Magma variety, control, and SA-100 ppm plants have higher values of chlorophyll "a", "b", carotenoids, and total chlorophyll. Percentage reduction values for chlorophyll "a" were 16.40, 18.73, 21.07, 26.16, 31.78, and 32.06% under NaF treatments of 50, 100, 150, 200, 250, and 300 ppm, respectively. In the case of chlorophyll "b", decreases of 6.39, 20.67, 21.73, 22.88, 25.59, and 32.81% were found. A similar trend of percentage reduction was recorded in carotenoids and total chlorophyll with the maximum values in the highest NaF concentration (300 ppm), of 64.66% and 33.62%, respectively.

In variety Zenia, for SA-100 ppm treatment, maximum values of total chlorophyll, chlorophyll "a", "b", and carotenoid recorded were –3.31, –5.43, –2.06, and –10.76%, respectively. Percentage reduction values recorded for chlorophyll "a" were 10.76, 11.34, 13.10, 15.39, 22.40, and 26.44% at sodium fluoride concentrations of 50, 100, 150, 200, 250, and 300 ppm, respectively. For chlorophyll "b", the values were 6.16, 15.83, 16.65, 18.01, 21.32, and 35.39%, respectively, at the same NaF concentrations. The percentage reduction values for total chlorophyll found were 6.84, 8.18, 11.69, 16.92, 21.86, and 23.93%, and for carotenoids they were 7.92, 17.52, 24.10, 27.12, 30.04, and 48.17%, for the 50–300 ppm NaF treatments, respectively.

Capsicum plants also treated with 100 and 200 ppm salicylic acid along with 50-300 ppm sodium fluoride concentrations. Significant growth was enhanced in combination of SA-100 and NaF compared to SA-200 and NaF. Salicylic acid at 100 ppm concentration suppressed the sodium fluoride effects but, in contrast, salicylic acid at 200 ppm also caused stress along with the sodium fluoride-induced stress.

Evaluation of total phenolic content: In the sodium fluoride stress conditions, plants produce phenolic compounds and accumulate in leaves. Excess amount of phenols is produced at higher salinity levels. NaF stress was reduced by applying SA exogenously and hence production of phenolics was also affected. In Magma variety, at maximum salinity level, viz, NaF-300 ppm, a maximum amount of phenol content was observed of 1.06 mg/g fresh weight (fr. wt.) in comparison to control conditions with a value of 0.75 mg/g fr. wt. NaF concentrations 50, 100, 150, 200, 250, and 300 ppm showed phenolic contents 0.80, 0.85, 0.91, 0.95, 1.02, and 1.06 mg/g fr. wt for variety Magma. In the combined treatments of SA and NaF, salicylic acid minimized the phenol production even at higher salinity levels as shown in Table 7.

Overall lower phenolic contents were observed in variety Zenia (Table 8) in comparison to variety Magma. Total phenol content was observed of 0.78, 0.81, 0.88, 0.92, 0.56, and 1.05 mg/g fr. wt in the 50 to 300 ppm NaF concentrations, respectively, in the case of Zenia. In control conditions, SA-100 and 200 treatments, a low phenolic content was observed of 0.72, 0.68, and 0.64 mg/g fr. wt, respectively.

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Table 7. Biochemical parameters of Capsicum annuum var. Magma harvested at 56 DAS by using different concentration of sodium fluoride and salicylic acid during growth season 2018 (Values are mean ± S.E; NaF: sodium fluoride; SA: salicylic acid; NaF-SA: sodium fluoride-salicylic acid)

Treatment	Biochemical parameter			
	Total phenol content (mg/g fr. wt)	Free proline content (mg/g fr. wt)	Ascorbic acid content (mg/g fr. wt)	Total soluble sugar content (mg/g fr. wt)
Control	0.75± 0.02	0.6 ± 0.02	19.82 ± 0.43	0.21±0.01
NaF-50	0.80 ± 0.04	0.69 ± 0.01	27.36 ± 0.15	0.24 ± 0.02
NaF-100	0.85±0.06	0.77±0.05	35.63±0.71	0.30±0.04
NaF-150	0.91 ±0.01	0.80 ±0.04	50.87 ±0.77	0.37 ±0.06
NaF-200	0.95 ±0.01	0.78 ±0.04	53.50 ±0.61	0.42 ±0.03
NaF-250	1.02 ±0.01	0.92 ±0.02	216.30 ±0.79	0.44 ±0.01
NaF-300	1.06±0.21	0.95±0.03	347.20±0.94	0.53±0.02
SA-100	0.70±0.02	0.49±0.04	11.78±0.14	0.18±0.01
SA-200	0.69±0.01	0.36±0.03	1.23±0.07	0.16±0.03
NaF-50+SA-100	0.68±0.01	0.5±0.03	18.83±0.72	0.23±0.02
NaF-100+SA-100	0.62±0.002	0.51±0.03	26.03±0.62	0.28±0.01
NaF-150+SA-100	0.55±0.01	0.57±0.01	35.72±0.57	0.37±0.02
NaF-200+SA-100	0.48±0.01	0.60±0.01	44.78±0.91	0.37±0.03
NaF-250+SA-100	0.41±0.01	0.63±0.03	68.02±0.62	0.42±0.05
NaF-300+SA-100	0.35±0.02	0.70±0.01	152.79±0.56	0.48±0.01
NaF-50+SA-200	0.66±0.01	0.46±0.02	17.63±0.26	0.18±0.01
NaF-100+SA-200	0.58±0.001	0.49±0.04	23.75±0.56	0.25±0.02
NaF-150+SA-200	0.51±0.005	0.55±0.02	30.42±0.50	0.30±0.04
NaF-200+SA-200	0.441±0.01	0.59±0.01	40.10±0.53	0.36±0.05
NaF-250+SA-200	0.38±0.01	0.66±0.02	45.00±0.58	0.42±0.07
NaF-300+SA-200	0.31±0.01	0.68±0.01	102.30±0.97	0.46±0.07

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Table 8. Biochemical parameters of Capsicum annuum var. Zenia harvested at 56 DAS by using different concentration of sodium fluoride and salicylic acid during growth season 2018 (Values are mean ± S.E; NaF: sodium fluoride; SA: salicylic acid; NaF-SA: sodium fluoride-salicylic acid)

Treatment	Biochemical parameter			
	Total phenol content (mg/g fr. wt)	Free proline content (mg/g fr. wt)	Ascorbic acid content (mg/g fr. wt)	Total soluble sugar content (mg/g fr. wt)
Control	0.72±0.03	0.59±0.005	5.60±0.29	0.17±0.01
NaF-50	0.78±0.01	0.60±0.005	9.13±0.34	0.25±0.01
NaF-100	0.81±0.02	0.65±0.02	11.53±0.34	0.27±0.04
NaF-150	0.88±0.01	0.67±0.02	29.35±0.49	0.31±0.02
NaF-200	0.92±0.01	0.72±0.02	39.37±0.15	0.32±0.02
NaF-250	0.56±0.05	0.82±0.02	50.10±0.78	0.43±0.01
NaF-300	1.05±0.20	0.87±0.05	116.37±0.47	0.48±0.01
SA-100	0.68±0.03	0.32±0.03	2.03±0.26	0.15±0.03
SA-200	0.64±0.02	0.30±0.03	1.27±0.10	0.14±0.02
NaF-50+SA-100	0.61±0.01	0.41±0.03	6.17±0.45	0.24±0.01
NaF-100+SA-100	0.54±0.01	0.50±0.04	10.83±0.07	0.24±0.02
NaF-150+SA-100	0.48±0.01	0.52±0.03	22.03±0.40	0.27±0.01
NaF-200+SA-100	0.44±0.001	0.55±0.02	26.83±0.87	0.31±0.03
NaF-250+SA-100	0.35±0.003	0.59±0.01	39.03±0.60	0.39±0.01
NaF-300+SA-100	0.27±0.02	0.62±0.01	53.47±0.61	0.45±0.01
NaF-50+SA-200	0.58±0.01	0.32±0.03	5.53±0.65	0.17±0.05
NaF-100+SA-200	0.51±0.01	0.40±0.04	6.80±0.29	0.22±0.02
NaF-150+SA-200	0.46±0.02	0.41±0.01	13.56±0.63	0.24±0.01
NaF-200+SA-200	0.41±0.01	0.46±0.02	20.83±0.87	0.29±0.02
NaF-250+SA-200	0.30±0.02	0.50±0.008	28.30±0.70	0.37±0.02
NaF-300+SA-200	0.25±0.02	0.55±0.01	42.87±0.77	0.42±0.03

Assessment of proline content: Stress conditions under sodium fluoride enhanced the production of proline content. SA in foliar application reduced the production of proline in both varieties (Magma and Zenia) as shown in Tables 7 and 8. Plants treated with NaF-300 have the maximum proline content 0.95 mg/g fr. wt in Magma and 0.87 mg/g fr. wt in Zenia. Gradual increase in proline production was observed of 0.62, 0.69, 0.77, 0.80, 0.78, 0.92, and 0.95 mg/g fr. wt and 0.59, 0.60, 0.65, 0.67, 0.72, 0.82, and 0.87 mg/g fr. wt under control, 50, 100, 150, 200, 250 and 300 ppm sodium fluoride concentrations, respectively, for both Magma and Zenia varieties. Salicylic acid in 100 and 200 ppm concentrations lowered the production of proline at 0.49, 0.36 mg/g fr. wt in case of Magma and 0.32, 0.30 mg/g fr. wt in case of Zenia, respectively.

Determination of ascorbic acid: Ascorbic acid as an antioxidant plays an important role in protection against physiological stress. Ascorbic acid content was higher with increasing NaF concentrations as shown in Tables 7 and 8 for both Magma and Zenia varieties. The highest ascorbic acid contents were measured in NaF-300 as 347.20 and 116.37 mg/g fr. wt for Magma and Zenia respectively. Sodium fluoride 50, 100, 150, 200, and 250 ppm showed a gradual increase (19.82, 27.36, 35.63, 50.87, 53.50, and 216.30 mg/g fr. wt) for var. Magma and (5.60, 9.13, 11.53, 29.35, 39.37, and 50.10 mg/g fr. wt) for var Zenia in ascorbic acid content. Salicylic acid in 200 ppm lowered the ascorbic acid production (1.23 and1.27 mg/g fr. wt) as compared to other treatments, for both the Magma & Zenia varieties, respectively.

Evaluation of total soluble sugars: Table 7 showed that the amount of total soluble sugar was increased with increase in sodium fluoride level in variety Magma. The highest value of soluble sugar was recorded at 300 ppm NaF. It was gradually increased by increasing the sodium fluoride concentrations. The amount of soluble sugar was lowest in the control and salicylic acid 100 ppm groups.

The highest value of total soluble sugar was recorded in 300 ppm NaF. The minimum total sugar was observed in the 100 ppm salicylic acid for var. Zenia represented in Table 8. The total soluble sugar also increased by increasing the NaF concentrations from 50 ppm to 300 ppm in Zenia.

DISCUSSION

Fluoride is well known mineral occurring in small amounts in the earth's crust. However, when present in soil in a large amount it causes damaging effects on growth and developmental processes. It is recognized that it non-essential as a plant element. An increased level of fluoride in acidic soil enhances the toxicity of the soil so plants face difficulty in surviving in such toxic conditions. Different kinds of fluorides are present in soil, air, and water and are a continuous source of environmental pollution.

Sodium fluoride causes harmful effects on chili (*Capsicum annuum* L.) growth. During the present research, we investigated the effect that sodium fluoride has on *Capsicum annuum* L., which is one of the most important crops grown in agricultural areas of Pakistan and is an essential source of nutrients and carotenoids. The factors that can affect its yield are pollutant stresses, like soil salinity and sodium fluoride.

There is need to develop such genotypes of *Capsicum* that have a better ability to tolerate salt stress and give high yields in order to provide better economic return.

The *Capsicum* growth, development, and the biochemical processes were adversely affected under different treatments. However, salicylic acid as a plant growth regulator increased the tolerance of *Capsicum* plants against toxicity of sodium fluoride. The exogenous foliar applications of 100 ppm salicylic acid reduced the NaF stress as compared to 200 ppm SA. The salicylic acid in 100 ppm was most effective for decreasing the NaF stress of *Capsicum*. It was also found that salicylic acid in low concentrations is very effective for plant growth while at a high level it reduced plant growth and caused a high level of stress in the plants.[13]

The results of the present investigation suggest that vegetative and reproductive growth of *Capsicum* was significantly reduced by increasing the sodium fluoride concentration. Fluoride is quickly absorbed through roots disturbing the plant root length. The *Capsicum* plants which were irrigated with 50, 100, and 150 ppm of sodium fluoride concentrations showed a lower reduction in root lengths compared to to the 200, 250, and 300 ppm treatments. An elevated level of fluoride in plant roots, due to its rapid accumulation from soil, was also observed.[14,15] This high amount of fluoride affected the root growth in *Capsicum*. It was found that the average length of shoot and root significantly decreased with increased an sodium fluoride level. It was observed that the length of shoots and roots was reduced, 27 and 23.7%, respectively, compared to the control at 5 µm.[15] It was also found that growth parameters of the plant, such as root length, shoot length and biomass, were reduced with an increased sodium fluoride level. At 30 mg NaF/L, the reductions compared to the control were 27% for the root length and 29% for the shoot length.[16]

During the experimental study a gradual reduction in the leaves number was also observed. The number of leaves showed an inverse relationship with sodium fluoride. Leaves were decreased with an increase in the sodium fluoride stress. The number of leaves also reduced with increased sodium fluoride concentrations in cabbage species[17] and beans (*Phaseolus vulgaris* L.).[18] The increased accumulation of sodium fluoride reduced the leaf area reported in *Vigna aconitifolia* L., commonly called moth bean plant.[19] The leaf area reduction was also observed in the leaves of *Beta vulgaris* L., sugar cane, studied under stress from 50 ppm, 100 ppm, and 150 ppm of sodium fluoride. The leaf area showed an inverse relation with increasing stress of NaF that was closely related to the current investigation.[20]

The present findings are also in accordance with the results of some other researchers who have worked on different plants treated with fluoride stress. The research showed that leaves of *Hordeum vulgare* var. RD 2683 showed the signs of chlorosis and necrosis due to the high concentration of fluoride in irrigation water.[21] The other growth and yield parameters also reduced with increasing fluoride concentration. During the current investigation leaf chlorotic and necrotic regions did not appear but other parameters were successively decreased due to the increased stress of fluoride in the vicinity of the plant.

The fluoride exerts a harmful effect and its role in plants' growth is very complex.[22] When fluoride enters the leaf mesophyll cells it then it disturbs the mineral metabolism and affects other physiological and morphological parameters of

the plant, including number of leaves, height of plant, biomass, yield, and productivity.[23] A reduction in all these parameters was observed during the present research with increasing sodium fluoride concentrations.

The fluoride stress significantly reduced the plant growth and developmental parameters.[21] The plant height, number of leaves, leaf area, shoot length, root length, root diameter, and flower number per plant were decreased with an increase in the fluoride concentration in soil. In our present study, the root length, shoot length and number of leaves show a high level of growth at a low level (50 ppm and 100 ppm) of fluoride.[22] Successive and progressive reductions were observed at the other treatments (150 ppm, 200 ppm, 250 ppm, and 300 ppm) of fluoride in contrast to control. These results showed close resemblance with the investigations of Mathur et al.[24]

The fresh weights of shoot and root also decreased with an increase in the sodium fluoride level. The maximum weight of shoot and root were recorded in the control plants and lower values were seen in the 300 ppm sodium fluoride treatment group. It was observed that *Capsicum* shows a significant reduction in root and shoot fresh weight during the growth season. The results of present study are consistent with other findings that the fresh and dry weights of plants decrease with an increase in sodium fluoride concentration.[25]

The current study proved that a high level of fluoride disturbed the biochemical parameters of *Capsicum*. Sodium fluoride reduced the photosynthetic pigments of plant, i.e., chlorophyll "a", chlorophyll "b", and carotenoids. The decreased amount of these pigments affected the photosynthesis process of plant, ultimately resulting in leaf maturity and death due to sequential chlorosis and necrosis. The amount of photosynthetic pigments showed a maximum reduction at 300 ppm sodium fluoride compared to the control plants. Our results are consistent with those of Gray who reported that leaf chlorophyll content reduced with chlorophyll breakdown due to the increased stress of fluoride.[26] Mustard and Renault also found that chlorophyll pigments decreased with increased fluoride stress.[27]

The present research showed that sodium fluoride stress reduced the photosynthetic activity of plant. But this stress is reduced by the foliar application of 100 ppm salicylic acid. Noreen et al. also found the same result that exogenously applied salicylic acid enhances the rate of photosynthesis in plants.[28] Noreen et al. also reported that a high level of fluoride reduces the photosynthetic rate by upsetting the leaf membrane and enzymes associated with CO_2 fixation.[29] Saini and Kahn found that salicylic acid increased the photosynthetic mechanism in barley plants grown under saline soil by stabilizing its membrane.[30]

Our results regarding to the reduction in chlorophyll "a", chlorophyll "b", and total chlorophyll agreed with those of Saini and Kahn that exposure of barley to zero, 120, and 240 ppm of sodium fluoride led to a decrease in chlorophyll "a", chlorophyll "b", and total chlorophyll.[31] In addition, studies on *Paspalum vaginatum* L. and *Centaurium erythrea* L. reported that chlorophyll "a", chlorophyll "b", and total chlorophyll decreases with an increase of the salt concentrations in soil.[32,33] Similar results were also found in studies on bean plant *Phaseolus vulgaris* L., on *Catharanthus roseus* L., and on cow pea *Vigna unguiculata* L., which demonstrated

that the salt stress of sodium fluoride caused a decrease in the total chlorophyll.[34,35,36]

Sodium fluoride stress successively decreased the carotene content during the present study. Increasing concentrations of sodium fluoride exerts a hindering effect on the carotene. Taffuou et al. found that the carotene content reduced significantly with an increased level of fluoride stress in *Cornus sericea* L., dog wood cultivars. [37]

The amount of total soluble sugar increased with increased sodium fluoride stress in soil during the present investigations in both varieties of *Capsicum*. Turan et al. found an increased level of total soluble sugar in *Oryza sativa* under stress conditions of sodium fluoride.[38] Tort et al. found that depositions of total soluble sugar play an essential role in membrane stabilization, enzymatic activities, membrane protection, and osmotic adjustments.[39] The results of present study were supported by those of Niu et al. who found that the level of phenolic compounds was increased under salinity stress in a lentil crop.[40] This increased phenolic content results in stimulation of secondary metabolites under fluoride stress. Furthermore, phenolic compounds are involved in the defense against oxidative stress developing due to fluoride stress. In the present study, phenolic concentration was enhanced with increased sodium fluoride concentration while foliar application of SA reduced the phenolic content.

CONCLUSIONS

During the present research sodium fluoride in different concentrations was given to the *Capsicum* varieties twice a week. The increased concentrations of sodium fluoride successively reduced the growth of *Capsicum* and some biochemical parameters. This is a matter of serious concern because Pakistan is an agricultural country and its agrarian lands are polluted with high load of sodium fluoride from different soil sources. However the foliar applications of salicylic acid reduced the sodium fluoride stress in the soil and proved to be effective in response of the plants to the NaF stress conditions. The exogenously applied salicylic acid increased the morphological and biochemical parameters in both varieties of *Capsicum annuum* by reducing the sodium fluoride stress. However, salicylic acid in a very high concentration also caused a high level of plant stress and reduced the *Capsicum* growth and hence salicylic acid at a very high concentration is less effective.

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