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SALICYLIC ACID MITIGATES FLUORIDE STRESS IN *ABELMOSCHUS ESCULENTUS* L. THROUGH ENHANCED GROWTH AND BIOCHEMICAL ATTRIBUTES

Shakil Ahmed,[a,*] Mehtab Qasim,[a] Rehana Sardar,[a] Madeeha Ansari,[a] Sundas Babar,[a] Shanila Bukhari,[b] M Nauman Ahmad,[c] M Amir Ismail,[d] M. Arif,[e] Afia Zia[c]

Lahore, Peshawar, and Perhawar, Pakistan

ABSTRACT: Fluoride is a non-essential electronegative element for plant growth. It enters the food chain through natural and anthropogenic processes. The aim of this research was to evaluate the therapeutic impact of Salicylic acid (100ppm, 200ppm) on *Abelmoschus esculentus* L. (Okra) under the stress of different sodium fluoride concentrations (50ppm, 100ppm, and 150ppm) in order to achieve the best growth and biochemical characteristics. The Okra variety "Sabz Pari" was studied during the spring growing season (February to April) of 2021 under the sodium fluoride stress. Sodium fluoride impede the growth attributes such as root and shoot length, number of leaves, and leaf area of Okra plant. Exogenous application of Salicylic acid improved chl *a*, chl *b* and carotenoid of Okra plants under sodium fluoride stress. Salicylic acid enhanced the production of protein and ascorbic acid that reduced the toxic effect of sodium fluoride. Salicylic acid has anti-oxidant and growth promoting properties that increase salt tolerance of Okra plants and perhaps ameliorates the deleterious impact of salt stress and enhances the growth and biomass of Okra plants

Key words: Chlorophyll content, Okra, Sodium fluoride, Salicylic acid

INTRODUCTION

Due to salt stress, 20% of total cultivable land is under threat in Pakistan[1]. It is estimated that by 2050, half of the cultivated land will be under salinity stress [2]. Fluoride is coming from several man-made sources, viz, brick industries, oil refineries and from excessive use of fluoride-based fertilizers [3]. In Rawalpindi the plant species near the brick kilns were eradicated out due to fluoride pollution [4]. F^- is the thirteenth most negatively charged element. The acidic pH of the soil is thought to be the source of fluoride in plants [5]. The presence of this mineral nutrient is not required for plant proliferation and growth [6].

With fluoride toxicity, all the plant's biochemical characteristics are altered [7], culminating in necrosis and leaf fall [8]. The lower epidermis and spongy mesophyll cells are the main target of fluoride attack while palisade mesophyll and upper epidermis are the last to show the symptoms [9]. The accumulation site of fluoride is completely unknown in the plants, but the symptoms of exposure are the leaf tip burn, yellowing of leaves and leaf blackening [10]. When crops are supplied with the fluoride contaminated water, there is a huge decline in their growth and rates of photosynthesis [11]. reactive oxygen species increase in case of fluoride toxicity with

[a]a Institute of Botany, University of the Punjab, Lahore 54590, Pakistan;
[b]Department of Botany, Kinnaird College for Women Lahore-Pakistan;
[c]Agricultural Chemistry Department, University of Agriculture, Peshawar-Pakistan;
[d]Department of Information Technology, Lahore Institute of Technical Education (LITE), Lahore-Pakistan;
[e]Directorate of Soil & Plant Nutrition, ARI Tarnab, Perhawar-Pakistan;
*Corresponding author:
Prof. Dr. Shakil Ahmed, Applied Environmental Biology & Environmental Biotechnology Research Lab, Institute of Botany, University of the Punjab, Lahore 54590, Pakistan
E-mail: shakil.botany@pu.edu.pk

decreased photosynthetic rates by damaging the enzymes and lipid membranes [12]. The main target of attack are the tips and margins of the leaves but in conifers it damages the base of leaves [13]. Calcium, magnesium, and manganese are the ions which are hindered by the fluoride toxicity affecting the seedlings more effectively than any other plant part [14].

Palm residues can be used to remediate the fluoride present in the ground water [15]. microbial remediation of fluoride is also highly effective [16]. The contents of fluorides in the protoplast can be reduced by the chelation of fluoride with calcium and magnesium ions. Fluoride can also be reduced by the compartmentalization in vacuoles by preventing the freely occurrence of fluoride ions in the cells [17]. The growth rate can be enhanced under the stress situation by application of various growth regulators, viz, ethylene, auxins, Salicylic acid [18]. Seed germination can be enhanced in the plants under fluoride stress by applications of Salicylic acids [19]. Salicylic acid regulates various physiochemical and biological phenomenon by translocating certain nutrients, stimulation of pigments and activation of antioxidant system [20]. Concentration of osmolytes and proline are regulated by foliar applications of Salicylic acid hence reducing the abiotic stress [21]. The phosphoryl transferase triggering event aids the organism in gaining control of F^- salts [22]. In general, two routes are involved in the production of Salicylic acid from Charismata [23]. The foliar spray of Salicylic acid influences ion entrance and transport [24].

In Pakistan, the vegetable *Abelmoschus esculentus* L. (Okra) belongs to the Malvaceae family of dicotyledonous plants. It belongs to the kingdom Plantae, which has 244 genera. *Abelmoschus esculentus* is farmed at a rate of 6 million tons per year over the world. “Bhindi” and “Ladyfinger” are two other names for Okra. Linoleic acid can be derived from *Abelmoschus esculentus* seeds. Vitamin C, antioxidants, folic acid, and fiber are all found in Okra [25]. Mucilage of Okra seems to be beneficial as it maintains the cholesterol levels in the body [26]. Yield of Okra at rate of 10 tons/ha is considered the ideal yield [26]. Okra is consumed in various ways like in form of salad, completely cooked/fried [27]. Also, its roasted seeds can also be used as the substitute of the coffee, free from caffeine [28].

The purpose of this study was to assess the benefits of Salicylic acid on *Abelmoschus esculentus* L. (Okra) under varied concentrations of sodium fluoride stress to achieve the maximum levels of the growth and biochemical attributes.

MATERIALS AND METHODS

Hybrid seeds of *Abelmoschus esculentus* L. variety “Sabz Pari” were purchased from Roshan seed center, Lahore. A pot experiment was performed during spring season from February-April 2021, in botanical garden, University of the Punjab Lahore. Soil was prepared using the mixture of sandy loamy soil in the ratio of 1:3. Farm manure, organic matter and DAP were the supplements for enhancing the growth of Okra. 48 Clay pots were arranged in 12 rows with 4 pots in each row containing 2 replicates for each treatment. All 4 pots in each row were having 1 treatment as total number of treatments were 12. Pots were 5 and 12 inches wide and long respectively. Each pot was filled with 7 kg of prepared soil by leaving upper one inch of the pot. Pots were arranged in Randomized Complete Block Design. Pots were tagged according to their respective treatments. Solutions of Sodium fluoride

(50ppm, 100ppm, and 150ppm) and Salicylic acids (100ppm and 200ppm) were prepared by weighing the required amount on weighing balance and then making solutions. After 37 days of sowing (DAS) first treatment was given on March 29, 2021. These solutions were applied twice a week throughout the whole experiment, using 150mL of each concentration of NaF per pot in soil drench and 6mL of foliar spray of Salicylic acid in each pot for fluoride stress. Two harvests vegetative and reproductive were taken on April 5 and April 30, 2021. Morphological growth parameters were measured after the vegetative and reproductive harvests, 45DAS and 70DAS, respectively.

Leaf Area = Length of leaf Þ width of leaf × 0.75 (correction factor) described by Carleton and Foote [29].

Biochemical parameters: The biochemical parameters including plant pigments, carotene content, protein content & ascorbic acid were assessed at 45 DAS (vegetative harvest).

Photosynthetic pigments were measured by Arnon [30], by crushing a leaf of 100mg weight along with 20mL of 80% acetone in pestle and mortar. Then the homogenate mixture was centrifuged for about 15 minutes at 3000 rpm. Supernatant was saved and pellet was again treated with 80% acetone (5mL). Extract was placed in test tube by covering it with aluminum foil. Now this supernatant was shifted to the cuvettes. Absorbance of supernatant was noted at 645nm and 663nm in spectrophotometer. Chlorophyll content was measured by using Arnon formula.

Estimation of Carotenoids: The same extract which was used for chlorophyll determination was observed at 480nm for estimation of carotenoids content by using Kirk and Allen method [31], by placing the extract in cuvettes.

Carotenoids (mg/g FW) = (1000OD 480 – 3.27) (chl a) – 104 (chl b) / 227

Evaluation of Protein Content: The protein content of each plant sample was determined using the Lowry et al. [32] method. The plant material (1 g) was crushed with 1N phosphate buffer in a pestle and mortar. For around 10 minutes, the samples were centrifuged at 10,000 rpm. After that, 2mL Folin mixture was added to 0.4mL of supernatant in a test tube. For around 15 minutes, test tubes were left at room temperature. After that, each test tube received 0.2mL of Folin Ciocalteus Phenol Reagent. The color development in these test tubes took 45 minutes. In a spectrophotometer, optical density was measured at 750nm, and the value of protein content was calculated by comparing it to a standard Bovine Serum Albumin curve.

Estimation of Ascorbic acid: 100 mL of distilled water, 0.75 grams of EDTA and 5 grams of oxalic Acid were taken to produce extracting solution. Leaves were homogenized with help of 20mL of this solution. Extract was then filtered and 1 mL of it was then centrifuged for 15 minutes at about 6000 rpm. Supernatant was Collected in the test tube. 5mL of 20µg/ml of 2,6-Dichlorophenol indophenol was added in it, the color of supernatant was changed to pink. Absorbance was noted at about 520nm (E_3). To bleach the pink color, a drop of ascorbic acid was added to the test tube with the modified pink colors. At 520nm, the absorbance was measured once more (Et). Then, at 520nm, the absorbance of 2,6-Dichlorophenol indophenol was measured (Eo). Keller and Schwager [33] method was used to find the absorbance of DCPIP.

Ascorbic Acid:

$$\textbf{Ascorbic Acid}(\text{mg/g}) = \frac{[\text{Eo}-(\text{E3}-\text{Et})] \times \text{Volume of supernatant and DCPIP}}{\text{Wt.of sample} \times \text{Volume of sample used} \times 1000}$$

Statistical analysis: Statistical analysis was performed using the software Costat version (3.03) described by Steel and Torrie [34] in laboratory.

RESULTS

Salicylic acid has beneficial effects on the development and metabolic characteristics of *Abelmoschus esculentus* L. under fluoride stress. A considerable drop in morphological characteristics was noted. with an increase in fluoride concentration at both harvests of 45DAS and 70DAS, respectively. However, all the parameters were enhanced by applications of Salicylic acid. Minimum growth rates were observed in Na-150. While maximum growth was noted in the SA-100.

a) Trend of shoot length: Figures 1A and 1B shows that the highest percentage reduction was observed in Na-150, i.e., 93.82% compared to other concentrations of NaF-50 and NaF-100 (86.36% and 92.36%), respectively. SA-100ppm showed the least percentage reduction of 43.75%. Maximum reduction in the case of a 200 ppm concentration of salicylic acid was noted, NaF-50+SA-200 (21.66%), NaF-100+SA-200 (55.22%), and NaF-150+SA-200 (84.8%). While the reduction was minimal in the cases of Na-F-50+SA-100 (6.1%), NaF-100+SA-100 (31.46%) and NaF-150+SA-100 (68.67%), Overall shoot length in both the harvests, 45DAS (Table 1) and 70DAS (Table 2), was higher in NaF-50 compared to 100ppm and 150ppm (86.36%, 92.32%, and 93.82%), respectively (45 DAS) and 55.84%, 63.6%, and 70.52%, respectively, at 70DAS. Salicylic acid at 100 ppm (43.75%) was found to improve growth more than that of 200 ppm (24.49%). At 70 DAS, the similar trend was found for shoot length.

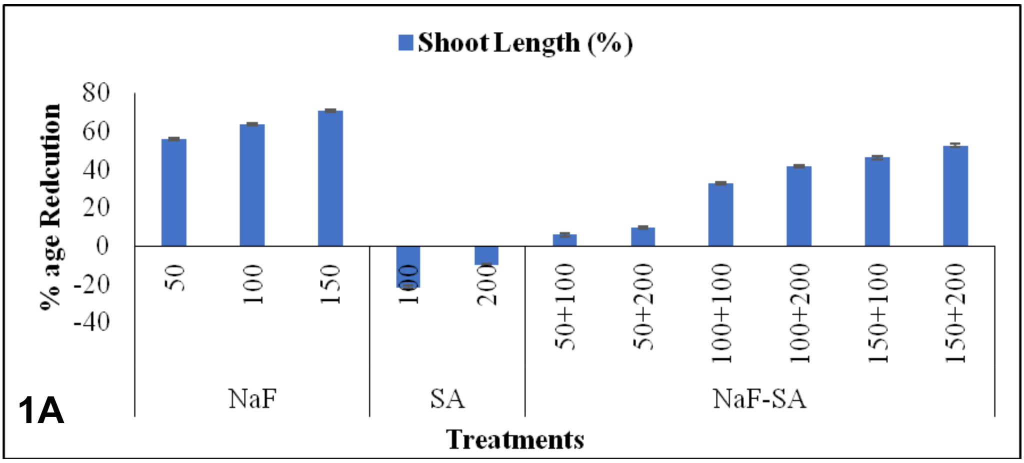

Figure 1A. Pattern of percentage reduction in Shoot Length in A. esculentus L. at 45 DAS (1A) under Sodium fluoride and Salicylic acid.

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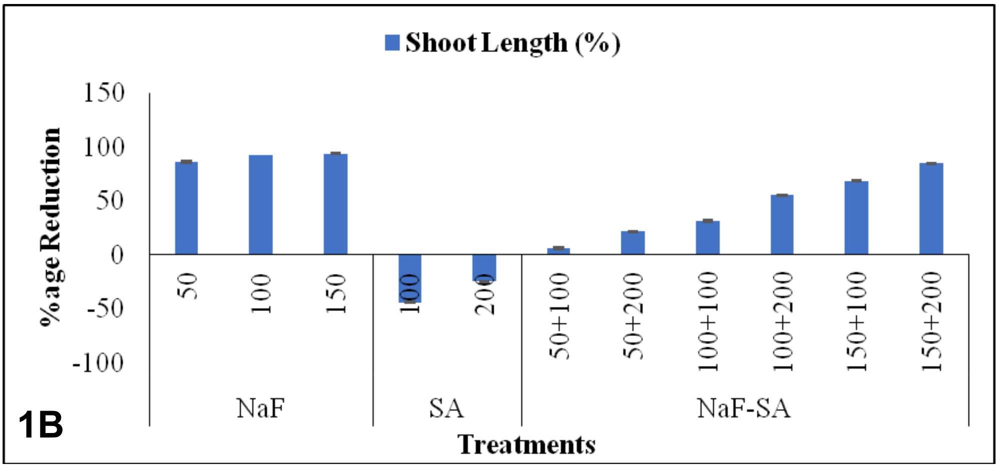


Figure 1B. Pattern of percentage reduction in Shoot Length in A. esculentus L. at 70 DAS (1B) under Sodium fluoride and Salicylic acid.

b) Trend of root length: As shown in Figures 2A and 2B, the percentage reduction was highest in the case of NaF-150 (74.74%) compared to NaF-50 (61.82%) and NaF-100 (67.11%). In comparison to 200ppm, salicylic acid at 100ppm demonstrated maximum root length (-31.01 percent and -15.74 percent, respectively). The reductions in NaF-50+SA-100 were 15.2%, NaF-100+SA-100 were 38.98 %, and NaF-150+SA-100 were 51.03 %. NaF-50+SA-200, NaF-100+SA-200, and NaF-150+SA-200, respectively, showed 23.64 percent, 44.13 percent, and 58.3 percent. Salicylic acid may have a detrimental effect on growth characteristics at greater concentrations. In comparison to 200ppm, Salicylic acid at 100ppm (-31.11%) has a beneficial effect on root length growth (-15.74 %). In comparison to NaF-50+SA-200 and NaF-50 (23.64 % and 61.82 %, respectively), root length in NaF-50+SA-100 (15.2 %) was the longest (Tables 1 and 2). At 70 DAS, with maximum root length, the same pattern of treatments was seen.

c) Number of leaves: Figures 3A and 3B depicts the maximum number of leaves that were maximum in SA-100 (-16.57%). Maximum reduction of leaves was noted at 150 ppm (71.42%) of NaF compared to 50 and 100 (57.14% and 69.14%), respectively. Salicylic acid at 100ppm advocate the maximum number of leaves over 200ppm (-2.28%). NaF-50+SA-100 showed a greater number of leaves compared to NaF-50+SA-200 and NaF-50. The percentage reduction 21.42% in NaF-50+SA-100, 31%, in NaF-100+SA-100, and 40.57% in NaF-150+SA-100 was lower and then 23.85%, in NaF-50+SA-200, 35.71% in NaF-100+SA-200, and 54.85% in NaF-150+SA-200 treatments. trend of treatments was observed at 70DAS with the maximum number of leaves (Tables 1 and 2).

d) Trend for leaf area: The reduction in leaf area for NaF-50, NaF-100, and NaF-150 was found to be 96.31, 99.04, and 99.95 (at 45 DAS) and 82.44%, 92.86%, and 95.03% (at 70 DAS) (Figures 4A and 4B). Salicylic acid improved the leaf area under stress. SA-100 (-96.84%) was more influential than SA-200 (-25.95%). Maximum leaf area was recorded at 70 DAS with the same effects of treatments (Table 2).

Table 1. Morphological growth attributes of A. esculentus L. at 45DAS under different concentrations of Sodium fluoride and Salicylic acid

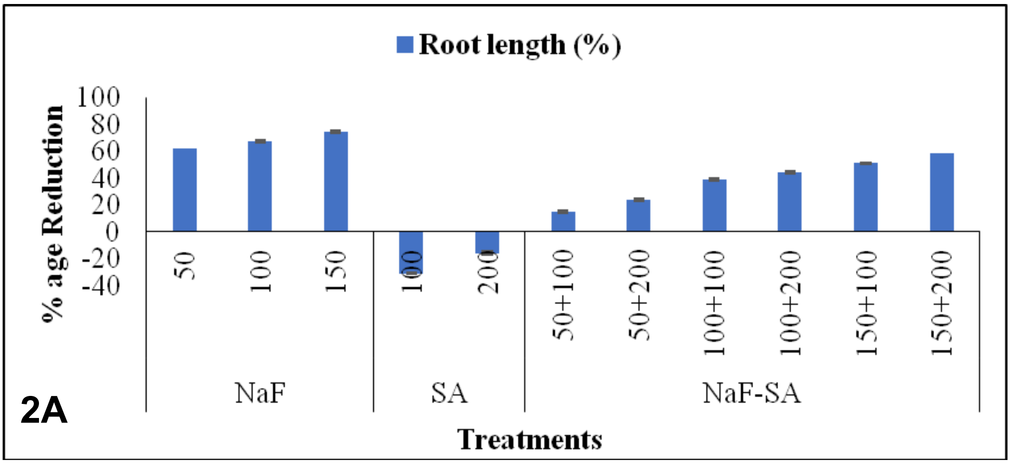
Treatment	Morphological growth attributes			
	Shoot Length (cm)	Root length (cm)	Leaf Area (cm^2)	Number of leaves
Control	14.08 ±0.85	14.93 ±0.72	45.89 ±1.03	7.0 ±0.28
NaF-50	1.92 ±0.8	5.70 ±0.206	1.69 ±0.86	3 ±0.76
NaF-100	1.08 ±0.22	4.91 ±0.98	0.44 ±0.23	2.16 ±1.04
NaF-150	0.87 ±0.6	3.77 ±0.97	0.02 ±0.01	2 ±0.28
SA-100	20.24 ±0.87	19.56 ±0.47	90.33 ±0.76	8.16 ±0.72
SA-200	17.81 ±0.72	17.28 ±0.93	57.80 ±0.55	7.16 ±0.60
NaF-50+SA-100	13.22 ±0.91	12.66 ±0.99	37.34 ±0.86	5.5 ±0.28
NaF-50+SA-200	11.03 ±0.79	11.4 ±0.71	24.17 ±0.54	5.33 ±0.60
NaF-100+SA-100	9.65 ±0.85	9.11 ±0.62	15.44 ±1.02	4.83 ±0.33
NaF-100+SA-200	6.3 ±0.56	8.34 ±0.59	9.79 ±0.11	4.5 ±0.57
NaF-150+SA-100	4.41 ±0.68	7.31 ±0.54	4.84 ±0.99	4.16 ±0.60
NaF-150+SA-200	2.14 ±0.38	6.25 ±0.14	3.28 ±0.87	3.16 ±0.44

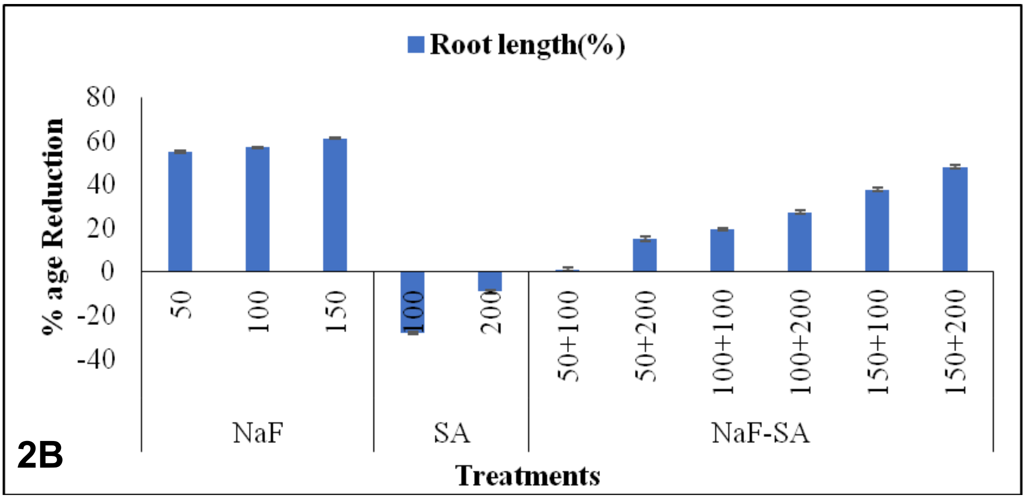
Mean ± S.E. (Mean is sum of three replicates); NaF: Sodium fluoride; SA: Salicylic acid; NaF-SA; Sodium Fluoride-Salicylic acid.

Table 2. Morphological growth attributes of A. esculentus L. harvested at 70DAS under different concentrations of Sodium fluoride and Salicylic acid

Treatments	Morphological growth attributes			
	Shoot Length (cm)	Root length (cm)	Leaf Area (cm^2)	Number of leaves
Control	26.36 ±0.34	19.17 ±0.92	83.36 ±0.97	9.33 ±0.72
NaF-50	11.64 ±0.67	8.61 ±0.52	14.63 ±0.87	2.66 ±0.60
NaF-100	9.5 ±0.69	8.26 ±0.36	5.95 ±0.80	2 ±0.28
NaF-150	7.77 ±0.64	7.47 ±0.28	4.14 ±0.97	1.67 ±0.44
SA-100	32.11 ±0.77	24.59 ±0.39	130.98 ±0.11	12.33 ±0.60
SA-200	28.96 ±0.66	20.95 ±0.67	101.52 ±0.51	10.66 ±0.88
NaF-50+SA-100	24.83 ±0.75	18.99 ±0.93	71.99 ±0.63	8.83 ±0.92
NaF-50+SA-200	23.80 ±0.66	16.29 ±0.95	45.38 ±0.67	7.35 ±0.61
NaF-100+SA-100	17.76 ±0.75	15.44 ±0.70	37.80 ±0.88	6.83 ±0.88
NaF-100+SA-200	15.36 ±0.54	13.94 ±0.81	35.92 ±0.87	5.0 ±0.5
NaF-150+SA-100	14.22 ±0.90	11.95 ±0.70	26.11 ±0.87	4.83 ±0.33
NaF-150+SA-200	12.52 ±0.87	9.95 ±0.65	16.81 ±0.57	3.51 ±0.56

Mean± S.E. (Mean is sum of three replicates); NaF: Sodium fluoride; SA: Salicylic acid; NaF-SA; Sodium Fluoride-Salicylic acid.





Figures 2A and 2B. Percentage reduction in Root Length in A. esculentus L. at 45 DAS (2A) and 70 DAS (2B) under concentrations of Sodium fluoride and Salicylic acid.

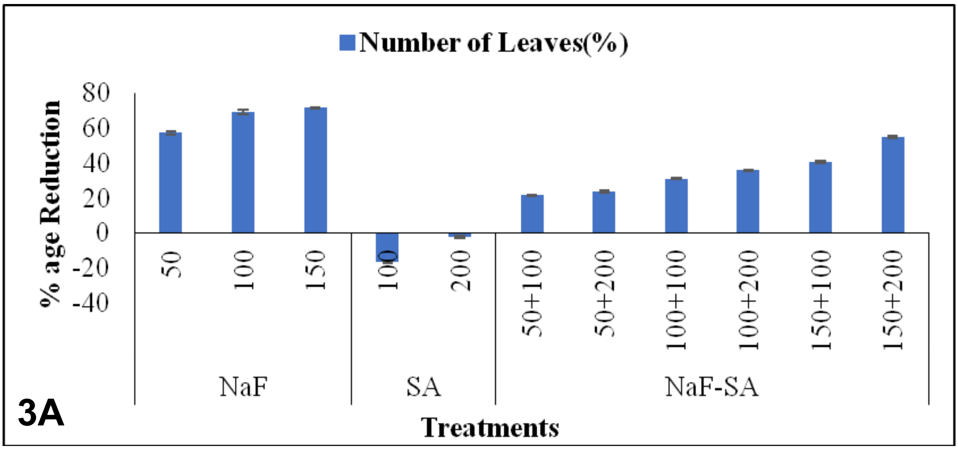


Figure 3A. Percentage reduction in Number of Leaves in A. esculentus L. at 45 DAS (3A) under Sodium fluoride and Salicylic acid.

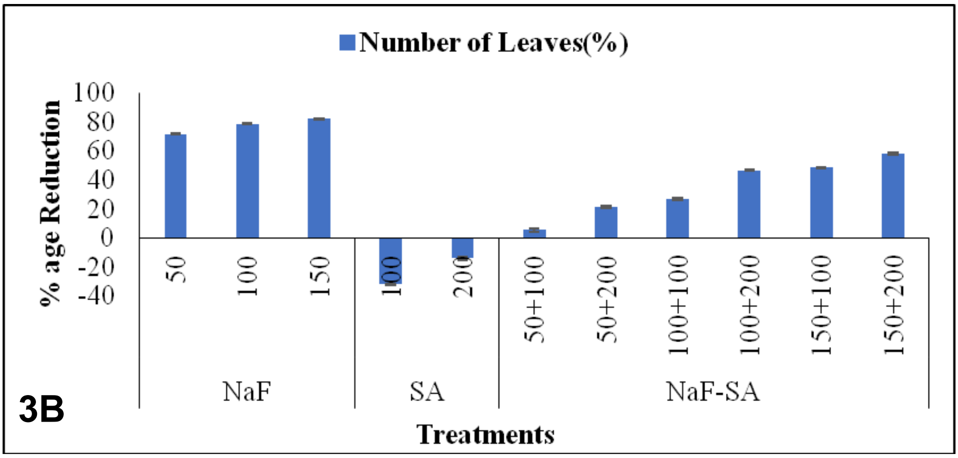
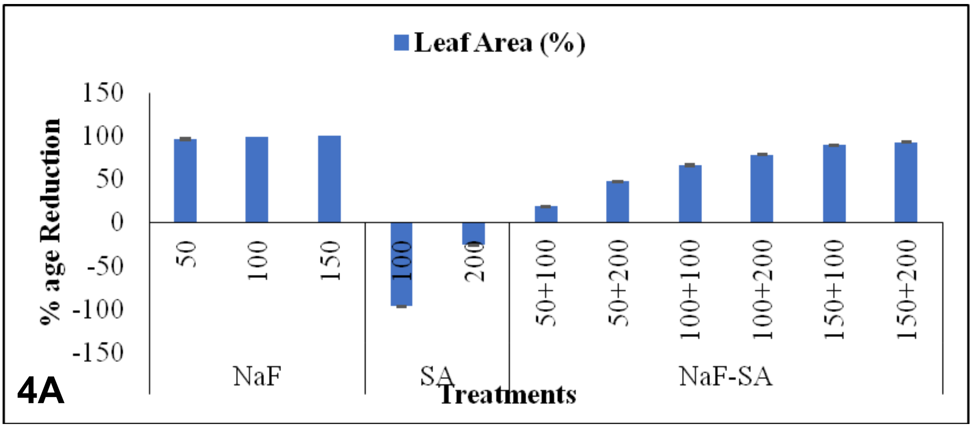
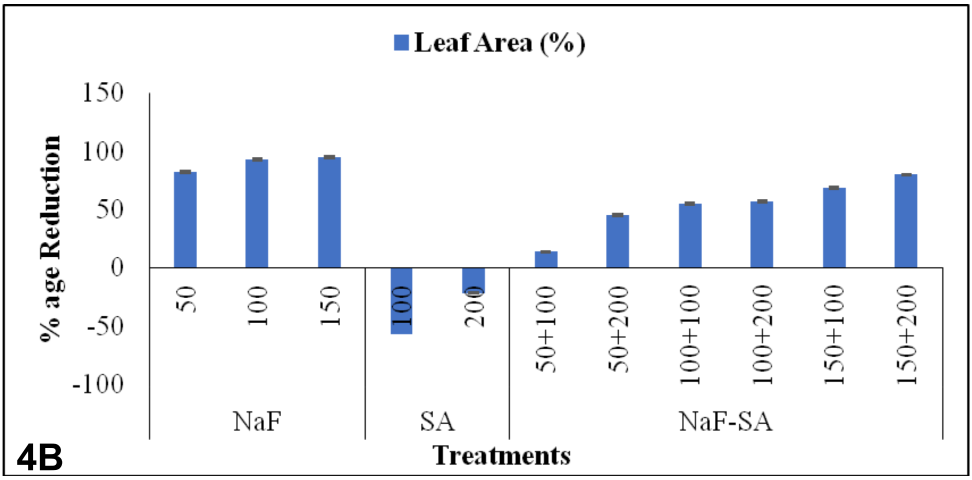


Figure 3B. Percentage reduction in Number of Leaves in A. esculentus L. at 70 DAS (1B) under Sodium fluoride and Salicylic acid.





Figures 4A and 4B. Percentage reduction in Leaf Area in A. esculentus L. (Okra) harvested at 45 DAS (4A) and 70 DAS (4B) under different concentrations of Sodium fluoride and Salicylic acid.

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e) Trend of biochemical attributes: Table 3 is depicting the values of photosynthetic pigments at 45DAS. SA-100 showed maximum values for Chlorophyll *a* (-6.12%), chlorophyll *b* (-9.52%), total chlorophyll (-8.18%) and carotenoids (-46.12%). Highest reduction was observed at 150ppm concentration of NaF. Those plants which were not treated with Salicylic acid showed a pale yellowish colored patterns on the leaves. %Age Reduction in "chlorophyll *a*" at 50ppm, 100ppm and 150ppm of NaF was 28.97%, 34.28% and 44.89% recorded (Figure 5). While "chlorophyll *b*" showed 45.23%, 47.61% and 59.52% reduction at 50,100 and 150ppm. Carotenoids and total chlorophyll followed the same trend for different concentrations of Sodium fluoride. % Age reduction was found to be maximum at 150ppm (73.52% and 56.81%) for carotenoids and total chlorophyll, respectively. 100pm of Salicylic acid was found to be more effective.

Table 4 is depicting the values of protein content at 45 DAS. SA-100 showed maximum protein content such as -10.52%. Maximum reduction was observed at 150ppm of NaF i.e., 50%. Percentage reduction at 50ppm and 100ppm was recorded 42.1% and 47.73% respectively (Figure 6). Minimum reduction was noted in all the treatments applied in combination with 100ppm. NaF-50+SA-100, NaF-50+SA-200 and NaF-50 showed a % age reduction of 7.89%, 10.52% and 42.1%. NaF-50+SA-100 was noted as 7.89%, 13.15% as NaF-100+SA-100, and 26.31% in NaF-150+SA-100 treatments compared to 10.52% in NaF-50+SA-200, 18.42% in NaF-100+SA-200 and 34.21% in NaF-150+SA-200. Hence SA-200 showed more reduction than 100ppm comparatively.

The levels of ascorbic acid in *Abelmoschus esculentus* was found to be increased with increasing concentration of Sodium fluoride salt stress as depicted in Figure 6. It was because of defensive mechanism which gets activated under any of stress conditions. Highest age reduction was found in SA-100 (28.88%) compared to that of SA-200 (15.55%). As there was not any stress at 100ppm of Salicylic acid that's why content of Ascorbic acid was reduced. Lowest percentage reduction was noted at 150ppm of NaF (-113.33%). Because at 150ppm of Sodium fluoride stress was maximum which activated the ascorbic acid. While % age reduction at 50ppm and 100ppm was recorded as -84.445 and -95.5%.

NaF-50+SA-100 showed a % age reduction of -17.77% whereas NaF-100+SA-100 as -40%, and NaF-150+SA-100 showed -66.66% respectively compared to NaF-50+SA-200 showed -33.33%, NaF-100+SA-200 showed -48.88%, NaF-150+SA-200 showed -75.55%. Compared to other parameters the reduction was more in case of 100ppm than 200ppm of Salicylic acid.

Table 3. Plant pigments of *A. esculentus* L. harvested at 45DAS under Sodium fluoride and Salicylic acid

Treatments	Plant Pigments			
	Chlorophyll "a" (mg g^{-1} FW)	Chlorophyll "b" (mg g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)
Control	0.0245 ±0.08	0.042 ±0.04	0.044 ±0.03	1.934 ±0.98
NaF-50	0.0175 ±0.01	0.023 ±0.8	0.025 ±0.07	0.668 ±0.44
NaF-100	0.0161 ±0.01	0.022 ±0.8	0.024 ±0.7	0.595 ±0.38
NaF-150	0.0135 ±0.01	0.017 ±0.07	0.019 ±0.6	0.512 ±0.33
SA-100	0.0260 ±0.1	0.046 ±0.05	0.0476 ±0.04	2.826 ±0.976
SA-200	0.0252 ±0.1	0.043 ±0.04	0.045 ±0.03	2.397 ±0.86
NaF-50 +SA-100	0.0238 ±0.6	0.039 ±0.4	0.041 ±0.3	1.493 ±0.703
NaF-50 +SA-200	0.0229 ±0.5	0.038 ±0.4	0.040 ±0.034	1.394 ±0.707
NaF-100 +SA-100	0.0219 ±0.07	0.035 ±0.2	0.037 ±0.2	1.242 ±0.63
NaF-100 +SA-200	0.0208 ±0.07	0.032 ±0.02	0.034 ±0.02	1.113 ±0.60
NaF-150 +SA-100	0.0194 ±0.16	0.025 ±0.8	0.027 ±0.7	1.014 ±0.55
NaF-150 +SA-200	0.0186 ±0.1	0.024 ±0.8	0.026 ±0.07	0.807 ±0.46

Mean± S.E. (Mean is sum of three replicates); NaF: Sodium fluoride; SA: Salicylic acid; NaF-SA; Sodium Fluoride-Salicylic acid.

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Table 4. Protein and Ascorbic acid content of A. esculentus L. harvested at 45DAS under Sodium fluoride and Salicylic acid

Treatments	Protein and Ascorbic acid content	
	Protein (mg/g FW)	Ascorbic Acid (mg/g FW)
Control	0.38 ±0.05	0.45 ±0.08
NaF-50	0.22 ±0.5	0.83 ±0.1
NaF-100	0.21 ±0.08	0.88 ±0.1
NaF-150	0.19 ±0.05	0.96 ±0.1
SA-100	0.42 ±0.05	0.32 ±0.08
SA-200	0.40 ±0.14	0.38 ±0.08
NaF-50 +SA-100	0.35 ±0.08	0.53 ±0.08
NaF-50 +SA-200	0.34 ±0.08	0.60 ±0.8
NaF-100 +SA-100	0.33 ±0.05	0.63 ±0.05
NaF-100 +SA-200	0.31 ±0.5	0.67 ±0.5
NaF-150 +SA-100	0.28 ±0.5	0.75 ±0.05
NaF-150 +SA-200	0.25 ±0.5	0.79 ±0.05

Mean ± S.E. (Mean is sum of three replicates); NaF: Sodium fluoride; SA: Salicylic acid; NaF-SA; Sodium Fluoride-Salicylic acid.

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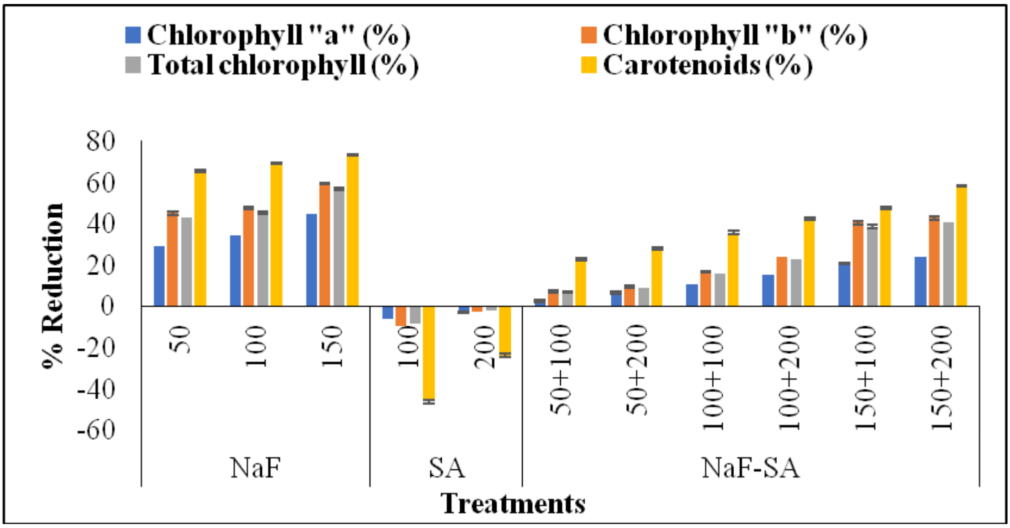

Figure 5. Showing pattern of percentage reduction in Chlorophyll a, b, Total Chlorophyll, Carotenoids in A. esculentus L. at 45 DAS under different concentrations of Sodium fluoride and Salicylic acid.

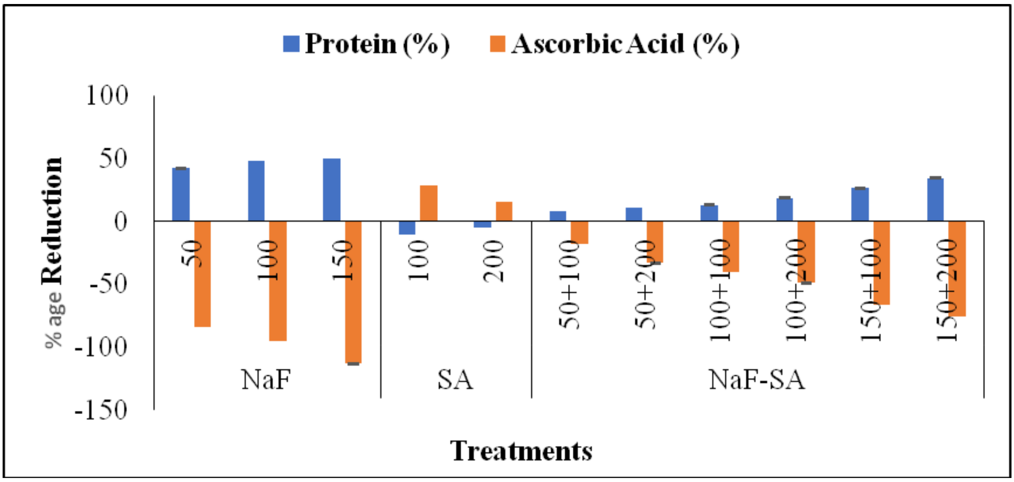

Figure 6. Showing pattern of percentage reduction in Protein content and Ascorbic acid in A. esculentus L. (Okra) harvested at 45 DAS under different concentrations of Sodium fluoride and Salicylic acid.

DISCUSSION

Fluoride is the Earth's crust’s 13th most abundant negatively charged element. Fluoride levels in the soil have risen due to the widespread use of phosphate-based fertilizers. Exogenous treatments of Salicylic acid can help plants grow faster. In the presence of Salicylic acid, plants’ stress defense mechanisms are activated. With increased fluoride concentrations, plant growth in terms of growth and biochemical properties was lowered, according to the findings of this study. At 150ppm NaF, the smallest amount of plant development was seen. Salicylic acid was used to promote the growth of a stressed plant (100ppm). However, at 200ppm, it may have a toxic effect on plant growth. Sodani et al. reported that an enhanced fluoride concentration in the germination medium caused a reduction in germination and germination related parameters [35]

Ahmed et al. found that when the concentration of sodium fluoride increased, the number of leaves, length of the plant, and area of the leaves in *Pisum sativum* decreased [36]. They also found that a lower concentration of Salicylic acid (100ppm) was useful for general stress relief compared to 300ppm, which was found to be damaging to plant growth. The results of the current study's shoot and root lengths were similar to those of a previous study by Ahmed et al. [37]. Previous research work, by Rhaman et al., also recorded SA enhanced germination percentage increased coleoptile length and weight, shoot and root length, and seed vigor index in Okra plant [38]. According to them, fluoride exposure shortened the length of turnip roots and shoots. Ahmed et al. found that *Solanum melongena* has had its root, shoot length, number of leaves, and surface area reduced by sodium fluoride [39]. At 500ppm and 600ppm of sodium fluoride, the reduction was greatest. These growth characteristics decreased as the concentration of sodium fluoride increased. According to Chakrabarti et al.[40], fluoride accumulation at 30mg NaF/L reduced the root (51.12 percent) and shoot length (47.5 percent) in *Cicer arietinum* (Bengal gramme). It was well documented that fluoride stress also reduced the dry and fresh weight of roots and shoots in Okra plants considerably [41].

Salinity stress is a major threat to agricultural productivity and sustainability as it can cause irreversible damage to photosynthetic apparatus at any developmental stage. However, the capacity of plants to become photosynthetically active under adverse saline conditions remains largely untapped [42]. Negative effect of salt stress on chlorophyll content might be attributed to the formation of proteolytic enzymes such as chlorophyllase, which is responsible for chlorophyll degradation [43]. Santos [44] also reported that the decrease in chlorophyll content in highly NaCl-stressed leaves was mainly attributable to a reduction in ALA (5-aminolinolic acid) synthesis. This acid is a precursor of protochlorophyllide, which when exposed to light, transforms to chlorophyll.

In accordance with the results of the present experiment, where chlorophyll *a*, *b*, and total chlorophyll protein content were decreased with an increasing concentration of sodium fluoride, reaching a maximum %age reduction at 150ppm. And this stress was ameliorated by using foliar applications of SA-100ppm. Ahmed et al.[45] noticed that with an increase in the concentration of sodium fluoride 50 ppm, 100 ppm, 150 ppm, 200 ppm, 250 ppm, and 300 ppm in Okra, the chlorophyll *a*, chlorophyll *b*, total chlorophyll along with carotenoid decreased. Ghaffar et al. [46] also advocated that fluoride had a negative effect chlorophyll content that decreased the crop productivity of *Zea mays* under stress. Protein was found to decline with an increasing concentration of sodium fluoride. They also noticed that the levels of ascorbic acid increased with an increase in fluoride. Ascorbic acid initially decreases at 10mg NaF/L and at 20mg NaF/L, then it increases at 30mg NaF/L (22% more than that of 20mg NaF/L). Because ascorbic acid oxidase binds to F at greater fluoride levels, it prevents ascorbic acid from being broken down, causing its levels to rise under stress. This was in line with our findings, which showed that plants under 150ppm stress had the highest levels of ascorbic acid. Iram and Khan found that when Okra seedlings were exposed to fluoride, their protein content was lower [47]. Esan et al. found that salicylic acid had a positive impact on the photosynthetic activity of Okra exposed to NaCl stress [48].

Adverse effect of fluoride contaminated irrigation water on ascorbate peroxidase on pea plants reported by Verma et al.[49]. They concluded that salicylic acid treatments increased the antioxidant enzymes and pigment levels of the plant.]

El-Nasharty et al.[50 found that foliar treatment of 400ppm Salicylic acid increased chlorophyll content. Same was the case with 200ppm of Salicylic acid in the present experiment under stress of Sodium fluoride.

CONCLUSIONS

The growth and metabolic parameters decreased as the concentration of NaF increased. The biggest percentage reduction was seen when NaF was used at a concentration of 150ppm. Fluoride stress decreased all growth characteristics (shoot length, root length, leaf area, and number of leaves), as well as biochemical properties (chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, and proteins), while increasing ascorbic acid levels. Salicylic acid at a concentration of 100ppm was found to be beneficial in reducing fluoride stress. Moreover bioremediation can be used in future to overcome the salinity to prevent the negative influences of higher concentrations of sodium fluoride on growth and biochemical parameters of crops.

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