

ASSESSMENT OF THE EFFECTS OF SALICYLIC ACID ON THE YIELD AND THE YIELD CHARACTERISTICS OF *PISUM SATIVUM* L. UNDER FLUORIDE STRESS

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ABSTRACT: The aim of the present investigation was to ascertain the impact of salicylic acid on the productivity of the pea plant, *Pisum sativum* L., grown under sodium fluoride-induced stress. The yield and the yield characteristics were studied in two varieties of *Pisum sativum* L. (RKS-510 and Classic) during the growth season 2015–2016, subjected to sodium fluoride (NaF) stress by the application of NaF, in concentrations of 50, 100, 150, and 200 ppm, as a soil drench on a biweekly basis and the biweekly exogenous application of salicylic acid as a foliar application, in concentrations of 100, 200, and 300 ppm, both alone and in combination with the NaF soil drench. Control plants were treated biweekly with tap water. The NaF caused toxic effects on the biochemical processes of the plants, with a dose-related gradual reduction in the yield, which was alleviated by the salicylic acid in concentrations of 100 and 200 ppm but not 300 ppm. We concluded that the foliar application of salicylic acid, in concentrations of 100 and 200 ppm, increased the salt tolerance of the RKS-510 and Classic cultivars of *Pisum sativum* L.

Keywords: Fluoride stress; *Pisum sativum* L., Salicylic acid; Yield.

INTRODUCTION

Salinity is one of the major hazards in irrigated and non-irrigated areas of arid and semi-arid regions of Pakistan and causes a reduction in productivity.¹ More than 6.7 Mha, out of the total irrigated area of Pakistan of 16.2 Mha, is affected due to the presence of a high concentration of salt.² Fluoride, the thirteenth most prevalent naturally occurring element in the earth's crust, is an important environmental pollutant.³ The range of the level of fluoride present in soil is approximately 20 to 1000 µg/g of soil.⁴ It usually depends on the clay fraction and the soil colloid level. The soluble fluoride content in soil is very important biologically and its toxicity is dependent on its concentration, the exposure time, and the sensitivity of the plant species. The lethal effects of fluoride have been explored for more than hundred years.⁵

Fluoride may contaminate not only water and soil but also vegetation.⁶ The potassium and sodium salts of fluorine are extremely poisonous. Acidic soils contain more fluorides than alkaline soils. The major environmental sources of fluoride are natural sources such as volcanoes, seawater, and weathering of rocks and the fumes from anthropogenic sources such as oil refineries and the aluminum, cement, phosphate fertilizer, glass, brick, pottery, and ceramic industries. These various sources of fluoride can cause an increase in concentration of fluoride in soils.⁴ The high use of phosphate fertilizers and the aerial deposition of fluoride from ceramic industries and brick kilns may contaminate agricultural land and irrigation water.⁷

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Pea (*Pisum sativum* L.) is an edible leguminous crop whose seeds contain 18–20% dry matter, including 5–8% protein and 10–12% carbohydrate. The low glycemic index and high fiber content in pea make it suitable for diabetics. It is an annual plant produced in the cool season and is commonly used as fresh vegetable although it is also canned and frozen. A high salinity level may effect the percentage yield of the pea plant.⁸

The aim of the present investigation was to ascertain the impact of salicylic acid on the yield and yield characteristics of two varieties, RKS-510 and Classic, of the pea plant, *Pisum sativum* L., grown under sodium fluoride-induced stress.

MATERIALS AND METHODS

Two varieties of *Pisum sativum* L., RKS-510 and Classic, were selected for the experiment which was conducted for 4 months, from November 2015 to March 2016, in the Botanical Garden, University of the Punjab, located at southern part of Lahore, Pakistan, (74°21-00-E, 31°35-00-N), on a 21×15 m plot. Certified seeds of the varieties RKS-510 and Classic were obtained from Punjab Seeds Corporation and Arain Seed Corporation, Lahore, respectively. Unhealthy, wrinkled, and infected seeds were discarded and the healthy seeds selected for planting were sorted to ensure an equality of size and stored in paper bags for the further experimental work. Analytical grade sodium fluoride (NaF), salicylic acid (SA) were purchased from the market.

The experimental site was surrounded with wires and netting to protect the plants from attack by birds and animals. The soil was prepared by mixing sandy soil with loam in a 1:3 ratio. Farm yard manure and leaves were also added to the soil. The normal agricultural practices were followed. Thoroughly cleaned earthen pots, 360 in number (30 cm in length and 25 cm), were used for the field experiment. Each pot was filled with 5 kg soil. Pebbles were added to the pots to partially close the holes located at the bottom to prevent the loss of soil water and extreme drainage. A randomized complete block design (RCBD) was followed to allow the plants to be treated under similar environmental conditions. NaF, prepared in concentrations of 50, 100, 150, and 200 ppm, was used to create the stress condition. Salicylic acid (SA), prepared in concentrations of 100, 200, and 300 ppm, was used to examine its ability to alleviate the NaF-induced stress. The different concentrations required for the treatments were prepared by mixing the required amounts of sodium fluoride and salicylic acid in distilled water. Initially four seeds were sown per pot with the seeds being soaked for 24 hours in clean tap water prior to being sown. After seedling emergence, two equal sized and healthy seedlings were selected in each pot by manual thinning. The pots were examined on a regular basis throughout the season to protect them from any pathogen attack. Weeds were removed from the plant surroundings manually during the experimentation.

The NaF and SA were applied biweekly (i.e., every two weeks) during the 2015–2016 growing season, starting at 25 days after sowing (DAS), with the NaF being given as a soil drench (150 mL/pot) and the SA being applied exogenously as a foliar spray (6 mL/pot). The control plants were given tap water biweekly.

The yield parameters measured at 105 DAS were: number of flowers, pod length, number of pods per plant, number of seeds per pod and per plant, and the weight of pods and seeds per plant.

The data collected data from the experiment were statistically analyzed for mean and standard error as well as Duncan's Multiple Range Test by using software COSTAT (version 3.03).⁹

RESULTS

Number of flowers: The RKS-510 variety showed the maximum reduction in the flower number (61.5%) at the highest level of sodium fluoride, i.e., 200 ppm (Figure 1). The foliar spray of salicylic acid (SA), at 100 and 200 ppm, significantly improved the number of flowers under the sodium fluoride stress condition. 100 and 200 ppm SA enhanced the number of flowers (17.3 and 12.1%, respectively) while SA 300 showed a 10.5% reduction in the number of flowers. NaF-50 + SA-100, NaF-100 + SA-100, NaF-150 + SA-100, and NaF-200 + SA-100 showed a lesser effect of NaF due to the ameliorative effect of SA while NaF-50 + SA-200, NaF-100 + SA-200, NaF-150 + SA-200, and NaF-200 + SA-200 showed a minor increase in the flower number. The treatments with NaF-50 + SA-300, NaF-100 + SA-300, NaF-150 + SA-300, and NaF-200 + SA-300 showed greater reductions in the number of flowers due the stress of both NaF and SA.

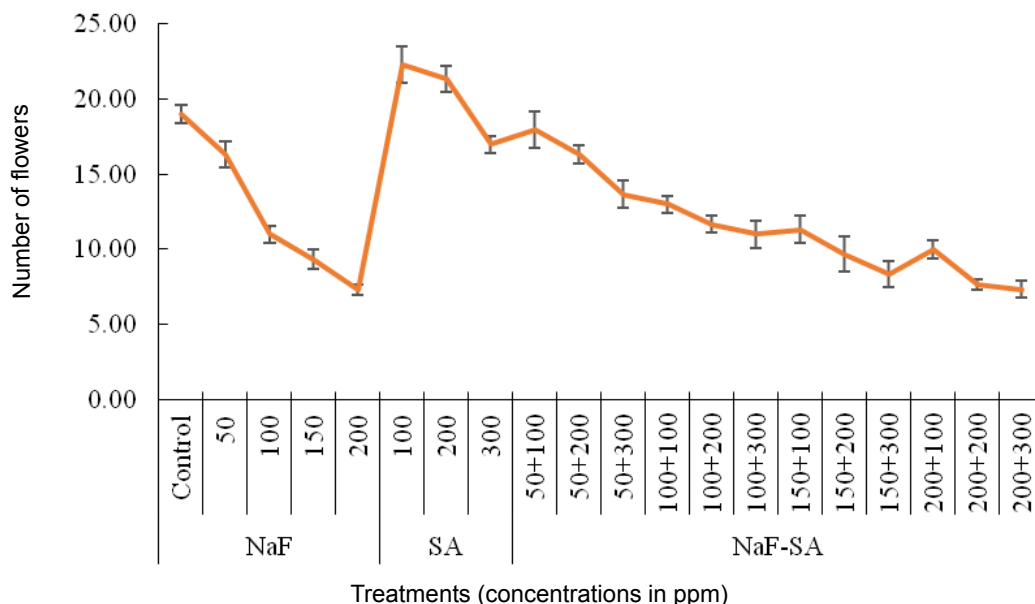


Figure 1. Showing the pattern of the number of flowers in pea variety RKS-510 under different treatments with sodium fluoride and salicylic acid. NaF=sodium fluoride, SA=salicylic acid, NaF-SA=sodium fluoride + salicylic acid.

For the variety Classic, the number of flowers was highest with the SA-100 treatment (a 15.3% increase compared to the control). Treatment with NaF, in concentrations of 50, 100, 150, and 200 ppm, resulted in a progressive reductions in the number of flowers (11.3, 22.2, 35.1, and 53%, respectively) (Figure 2).

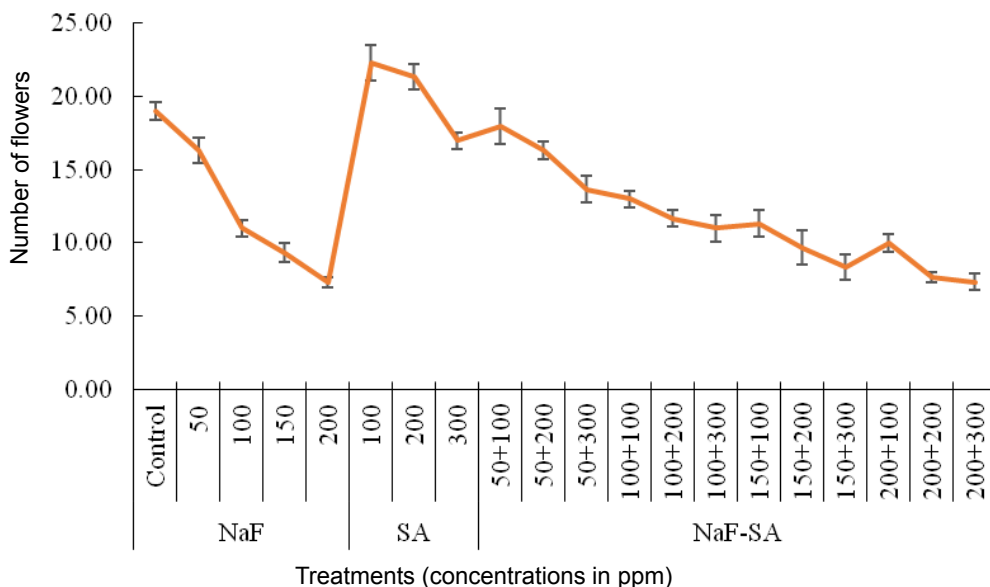


Figure 2. Showing the pattern of the number of flowers in pea variety Classic under different treatments with sodium fluoride and salicylic acid. NaF=sodium fluoride, SA=salicylic acid, NaF-SA=sodium fluoride + salicylic acid.

Pod length: The RKS-510 variety showed maximum increase, compared to the control, of 17.3% in pod length with SA-100 (Figure 3 and Table 1). 50, 100, 150 and 200 ppm concentrations of NaF resulted in a gradual decrease in pod length, compared to the control (26, 40.5, 49.2, and 57.9%, respectively). The maximum reduction (64.2%) was observed with the treatment NaF-200+SA-300, due to the stress of both NaF and SA.

The Classic variety showed a 25% increase in pod length, compared to the control, with SA-100 treatment. 50, 100, 150 and 200 ppm concentrations of NaF resulted in a gradual decrease in pod length, compared to the control (14.3, 26.2, 43.5, and 61%, respectively) (Figure 4 and Table 2).

Number of pods per plant: For both varieties of pea plant, the total number of pods was maximum in SA-100 (18 and 20.4%, for RKS-510 and Classic varieties, respectively) and reduced gradually with treatment with 50, 100, 150, and 200 ppm NaF (4, 14.5, 30.9, and 49% for the RKS-510 variety and 13.1, 25.9, 37, and 55.1% for the Classic variety, respectively) (Figures 3 and 4 and Tables 1 and 2).

Number of seeds per pod and per plant: For both varieties of pea plant, the number of seeds per pod was maximum with the SA-100 treatment (increases compared to the control of 27.9 and 52.6% for the RKS-510 and Classic varieties, respectively) while the minimum was in the NaF-200 group (decreases compared to the control of 43.2 and 57.8% for the RKS-510 and Classic varieties, respectively). The number of seeds per plant was also measured and this showed the same pattern of a reduction under NaF stress and amelioration by SA, in concentrations of 100 and 200 ppm, of the NaF-induced stress.



Figure 3. Showing the pods of the pea variety RKS-510 harvested at the destructive phase at 105 days after sowing (DAS) under the different treatments with sodium fluoride and salicylic acid. NaF=sodium fluoride, SA=salicylic acid, NaF-SA=sodium fluoride + salicylic acid. The control was treated with tap water. The concentrations are in ppm. The metal ruler in the photograph is marked for 12 inches and has a total length of 320 mm.

Table 1. Yield parameters of *Pisum sativum* L., variety RKS-510, harvested at the destructive phase at 105 days after sowing (DAS) using different sodium fluoride and salicylic acid concentrations during the growth season 2015–2016. Values are mean±SE. (NaF=sodium fluoride; SA=salicylic acid; NaF-SA=sodium fluoride+salicylic acid.)

Treatment	Yield parameter					
	Pod length (cm)	No. of pods per plant	No. of seeds per pod	No. of seeds per plant	Weight of pods per plant (g)	Weight of seeds per plant (g)
Control	6.90b ± 0.15	11.00b-d ± 0.58	6.67c ± 0.88	28.67ab ± 2.33	14.00bc ± 0.21	9.73a-c ± 0.15
NaF-50	5.10cde ± 0.12	10.60c-f ± 0.31	5.50d-g ± 0.32	26.00b-d ± 1.15	12.30c-f ± 0.85	8.60c-f ± 0.31
NaF-100	4.10fg ± 0.12	9.43d-g ± 0.30	5.20d-h ± 0.25	25.00c-f ± 0.58	10.67e-h ± 0.88	7.37f-i ± 0.32
NaF-150	3.57g-j ± 0.32	7.60hi ± 0.70	4.47h-j ± 0.24	23.00e-g ± 0.58	9.23hi ± 0.79	6.70h-k ± 0.06
NaF-200	2.90j ± 0.06	5.67jk ± 0.33	3.80i-k ± 0.12	19.30h ± 0.35	7.83ij ± 0.60	5.40kl ± 0.32
SA-100	8.10a ± 0.12	13.00a ± 0.58	8.57a ± 0.30	30.33a ± 1.45	16.00a ± 1.53	10.50a ± 0.76
SA-200	7.00b ± 0.00	12.33ab ± 0.33	7.57b ± 0.30	29.63a ± 0.32	14.93ab ± 0.64	10.00ab ± 0.12
SA-300	6.53b ± 0.27	9.57d-g ± 0.30	6.00c-e ± 0.06	26.67b-d ± 0.88	12.30c-e ± 0.65	9.00b-e ± 0.55
NaF-50+ SA-100	5.60cd ± 0.31	11.63a-c ± 0.68	6.80bc ± 0.15	27.00bc ± 0.58	13.60b-d ± 0.87	9.40a-c ± 0.32
NaF-50+ SA-200	5.67c ± 0.09	11.00b-d ± 0.58	6.07cd ± 0.09	25.63c-f ± 0.68	13.37b-d ± 0.19	9.10b-e ± 0.31
NaF-50+ SA-300	4.97de ± 0.58	8.60gh ± 0.31	5.00f-h ± 0.00	25.00c-f ± 1.15	11.90d-g ± 0.12	8.73b-e ± 0.59
NaF-100+ SA-100	4.57ef ± 0.30	10.20c-f ± 0.20	5.73d-f ± 0.15	26.27b-d ± 0.25	12.00d-g ± 0.58	8.30d-g ± 0.30
NaF-100+ SA-200	4.20fg ± 0.12	9.33fg ± 0.67	5.13e-h ± 0.24	25.83b-e ± 0.09	11.70d-g ± 0.12	7.87e-h ± 0.09
NaF-100+ SA-300	3.83gh ± 0.12	8.56gh ± 0.29	4.87f-h ± 0.09	24.43c-f ± 0.30	10.30e-h ± 0.35	7.10g-j ± 0.06
NaF-150+ SA-100	4.03f-h ± 0.09	8.33gh ± 0.88	5.10e-h ± 0.10	24.50c-f ± 0.26	10.63e-h ± 0.20	7.00g-j ± 1.15
NaF-150+ SA-200	3.67g-i ± 0.09	7.67hi ± 0.67	4.80gh ± 0.12	23.80d-f ± 0.15	9.47hi ± 0.23	6.60h-k ± 0.31
NaF-150+ SA-300	2.97j ± 0.09	7.30hi ± 0.25	4.60g-i ± 0.23	22.83fg ± 0.44	8.80hi ± 0.15	6.50h-k ± 0.29
NaF-200+ SA-100	3.40h-j ± 0.21	6.67ij ± 0.33	4.33h-j ± 0.33	21.00gh ± 1.15	9.30hi ± 0.35	6.13i-l ± 0.09
NaF-200+ SA-200	3.00ij ± 0.12	5.33jk ± 0.33	3.70jk ± 0.21	20.00h ± 0.58	8.10ij ± 0.17	5.83j-l ± 0.12
NaF-200+ SA-300	2.47j ± 0.24	4.67k ± 0.33	3.13k ± 0.19	16.17i ± 0.73	6.20j ± 0.44	4.70l ± 0.35

Within each parameter, values not followed by the same lower case letter are significantly different with Duncan's multiple range test and p=0.05.

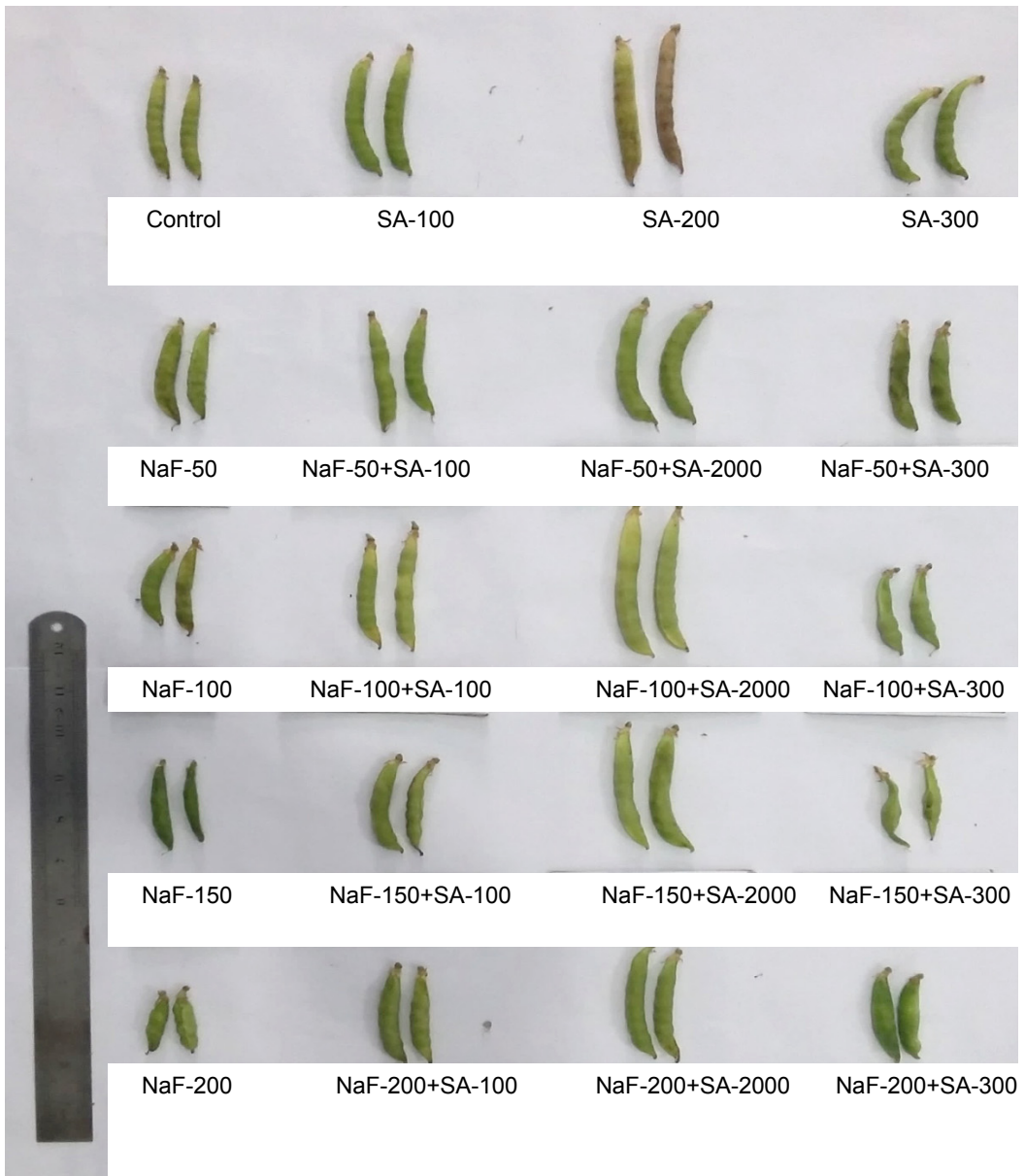


Figure 4. Showing the pods of the pea variety Classic harvested at the destructive phase at 105 days after sowing (DAS) under the different treatments with sodium fluoride and salicylic acid. NaF=sodium fluoride, SA=salicylic acid, NaF-SA=sodium fluoride + salicylic acid. The control was treated with tap water. The concentrations are in ppm. The metal ruler in the photograph is marked for 12 inches and has a total length of 320 mm.

Table 2. Yield parameters of *Pisum sativum* L., variety Classic, harvested at the destructive phase at 105 days after sowing (DAS) using different sodium fluoride and salicylic acid concentrations during the growth season 2015–2016. Values are mean±SE. (NaF=sodium fluoride; SA=salicylic acid; NaF-SA=sodium fluoride+salicylic acid.)

Treatment	Yield parameter					
	Pod length (cm)	No. of pods per plant	No. of seeds per pod	No. of seeds per plant	Weight of pods per plant (g)	Weight of seeds per plant (g)
Control	6.50b ± 0.12	12.67bc ± 0.88	5.67c ± 0.33	30.33bc ± 1.20	15.30b ± 0.35	11.40bc ± 0.83
NaF-50	5.57cd ± 0.18	11.00c-e ± 1.15	4.33d ± 0.33	27.00d-f ± 0.58	13.17cd ± 0.38	9.53d-f ± 0.69
NaF-100	4.80e ± 0.06	9.33e-g ± 0.88	3.33f-h ± 0.33	24.83fg ± 0.44	11.73ef ± 0.15	8.83e-g ± 0.24
NaF-150	3.67g ± 0.34	8.00g-i ± 0.58	3.00g-l ± 0.00	21.00ij ± 1.73	9.53gh ± 0.27	6.60hi ± 0.31
NaF-200	2.53j ± 0.32	5.67kl ± 0.33	2.33j ± 0.33	17.00lm ± 1.15	7.57j ± 0.41	4.90j ± 0.56
SA-100	8.13a ± 0.28	15.30a ± 0.65	8.67a ± 0.33	32.73a ± 0.37	18.00a ± 0.58	13.07a ± 1.10
SA-200	7.70a ± 0.12	13.00b ± 0.58	6.67b ± 0.33	31.83ab ± 0.44	16.90a ± 0.06	12.57ab ± 0.30
SA-300	5.80c ± 0.06	11.00c-e ± 1.00	5.33c ± 0.33	28.67c-e ± 0.88	14.43bc ± 0.18	10.63cd ± 0.68
NaF-50+ SA-100	6.73b ± 0.09	12.87b ± 0.09	5.73c ± 0.09	29.87bc ± 0.66	15.17b ± 0.18	10.23c-e ± 0.39
NaF-50+ SA-200	6.33b ± 0.18	11.90b-d ± 0.10	5.20c ± 0.26	29.13cd ± 0.19	13.77cd ± 0.03	9.90d-f ± 0.06
NaF-50+ SA-300	5.20de ± 0.06	9.97ef ± 0.58	3.93d-f ± 0.52	26.47ef ± 0.57	12.67de ± 0.15	9.10e-g ± 0.26
NaF-100+ SA-100	5.87c ± 0.12	10.70de ± 0.12	4.23de ± 0.20	27.43de ± 0.23	13.30cd ± 0.35	9.80d-f ± 0.12
NaF-100+ SA-200	5.27de ± 0.09	10.10ef ± 0.10	3.83d-f ± 0.09	25.07fg ± 0.18	12.73de ± 0.67	9.23d-f ± 0.24
NaF-100+ SA-300	4.20f ± 0.06	8.87f-h ± 0.09	2.97g-l ± 0.03	22.00hi ± 0.23	11.57ef ± 1.11	8.50fg ± 0.15
NaF-150+ SA-100	5.20de ± 0.06	9.50e-g ± 0.26	3.50e-g ± 0.12	24.17gh ± 0.27	10.90f ± 0.67	7.77gh ± 0.19
NaF-150+ SA-200	4.93e ± 0.09	8.70f-i ± 0.12	3.33f-h ± 0.09	22.23hi ± 0.79	10.60fg ± 0.31	7.00hi ± 0.58
NaF-150+ SA-300	3.17h ± 0.09	7.53h-j ± 0.27	2.83g-l ± 0.09	19.30jk ± 0.35	9.57gh ± 0.30	6.00ij ± 0.06
NaF-200+ SA-100	3.80fg ± 0.06	7.00i-k ± 0.58	3.13f-l ± 0.09	20.00i-k ± 0.12	8.83hi ± 0.12	5.80ij ± 0.15
NaF-200+ SA-200	2.23j ± 0.15	4.33l ± 0.33	1.97j ± 0.03	15.00m ± 1.15	6.53j ± 0.15	4.50j ± 0.31
NaF-200+ SA-300	2.47j ± 0.24	4.67k ± 0.33	3.13k ± 0.19	16.17i ± 0.73	6.20j ± 0.44	4.70l ± 0.35

Within each parameter, values not followed by the same lower case letter are significantly different with Duncan's multiple range test and p=0.05.

Weight of pods and seeds per plant: For the RKS-510 variety, the use of 100 ppm of SA resulted in an increase, compared to the control, in the weight of the pods and of the seeds, 14.3 and 8.2%, respectively. The use of 50, 100, 150, and 200 ppm of NaF resulted in a gradual decrease, compared to the control, in the weight of the pods (12.1, 23.5, 34, and 44%, respectively) and of the seeds (11.3, 24, 31.2, and 44.4, respectively) (Table 1).

For the Classic variety, The maximum weight of the pods and the seeds, compared to the control, was observed with the SA-100 treatment (17.6 and 14.6%, respectively). The use of 50, 100, 150. and 200 ppm concentrations of NaF resulted in a gradual reduction, compared to the control, in the weight of the pods (13.9, 23.4, 37.7, and 50.5%, respectively) and of the seeds (16.4, 22.5, 42.1, and 57.0, respectively) (Table 2).

DISCUSSION

Fluoride causes environmental pollution even in low amounts by contaminating soil, water, and vegetation. *Pisum sativum* L. (pea) is a leguminous crop used for human nutrition as a fresh vegetable, as dry pulses, and after canning and freezing.¹⁰ Investigations have found high concentrations of fluoride adversely affect the yield and yield components of plants.^{4,11} Acidic soils may have moderate to high levels of various kinds of fluorides which are poisonous and cause a stress condition for plants. In the present study, the impact of fluoride-induced stress on the productivity of the pea plant was determined by applying various concentrations of NaF.

Significant yield reductions in yield were observed with increases in the NaF concentration as compared to the control plants. The literature indicates that fluoride in mesophyll cells may affect various morphological and physiological parameters, such as the number of leaves, the height of a plant, fruiting, biomass, and yield, by disturbing the metabolism of various minerals and a reduction in the chlorophyll content. It has been also been found that high concentrations of fluoride cause necrosis and chlorosis of leaves, reduced growth, and ultimately a lower yield of *Hordeum vulgare* var-RD 2683. It has been reported that a high level of salt reduces the number of perfect flowers and the fruit setting as well as resulting in the production of imperfect fruit. Fluoride pollution not only affect the setting of the pods but also decreases the dry weight of the pods resulting in low productivity.^{5,12-15}

CONCLUSION

In the present study, we found that NaF produced detrimental effects on plant yield when applied to pea plants on a biweekly basis as a root drench. Pakistan, an agrarian country, is being contaminated with NaF from various sources. This contamination is a matter of serious concern from agricultural point of view due to the tremendous yield losses the pollution produces. Although plants cannot express their response verbally, they show their response in the form of a reduced yield. We found that salicylic acid, given as a foliar spray in concentrations of 100 and 200 ppm, was effective in reducing NaF-induced stress in *Pisum sativum* L. and led to an enhanced yield. However, if given at the higher concentration of 300 ppm, salicylic acid can cause additional stress in NaF-stressed plants.

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