

ASSESSING THE EFFECT OF FOLIAR AND SOIL FLUORIDE STRESS ON TURNIP (*BRASSICA RAPA* L.)Shakil Ahmed,^{a,*} Irma Saleemi,^a Fareeha Jabeen,^a Afia Zia,^b M Nauman Ahmad,^b Azeem Haider^c, Aftab Ahmad Khan,^d Syed S Alam Syed^e

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ABSTRACT: The production of crops in Pakistan is severely affected by the widely prevalent problem of salinity in the soil with salts including fluoride. The aim of the present study was to examine, in a pot experiment, the effects of sodium fluoride (NaF), applied as a foliar spray and as a soil drench, on the growth and other attributes of turnip (*Brassica rapa* L.). The percentage reduction in root length after treatment with a soil drench and a foliar spray was found to be at a maximum in plants treated with 300 ppm NaF, 62.6% and 51.02%, respectively, at 50 days after sowing (DAS) and 66.4% and 54.59%, respectively, at 70 DAS. The percentage reductions in the number of leaves, at 50 and 70 DAS, was 50.03% and 65.23%, respectively, for plants having an application of 300 ppm sodium fluoride by soil drench, while the corresponding reductions for the application of the 300 ppm NaF by foliar spray were 50.03% and 58.7%, respectively. At 50 DAS, the least percentage reduction in leaf area, 2.18%, occurred when the plants were given 50 ppm NaF by foliar spray and the highest value, 59.87%, was for the plants given 300 ppm NaF by soil drench. After 50 DAS, the percentage decrease in shoot fresh weight was found to be highest, 78.84%, when 300 ppm NaF was used as a soil drench, with the corresponding figure for the foliar spray application being 76.65%. Similarly, at 70 DAS, the percentage reduction in fresh shoot weight increased gradually with higher concentrations of NaF and was a maximum at 300 ppm NaF with both methods of application. We concluded that the application of NaF as a soil drench caused a greater reduction in the various parameters of turnip plant growth than did application as a foliar spray.

Key words: Fluoride; Foliar spray; Growth; Soil drench; Turnip (*Brassica rapa* L.); Pakistan.**INTRODUCTION**

Salts in the soil and water, like fluoride, can markedly reduce the growth and yield of plants and yield, especially in arid and semi-arid regions.^{1,2} The effects of the halides chloride and iodide, on organisms has been well documented.^{3,4,5} Another halide, the fluoride anion (F) has the highest electronegativity, is the smallest. and has distinctive chemical and biochemical characteristics. However, there is only an incomplete knowledge about the effects of fluoride on plant cells. As well as occurring in air, soil, and water,⁶ fluoride is a commonly found mineral in the layers of earth with a mean concentration of approximately 0.32 g F/kg of the crust.⁷ Moreover in the earth's crust or in the underground water, the presence of fluorides and their abundance varies according to the part of the soil or water where they are found. The concentration of fluoride in the ground is about 10–1,000 ppm⁷ and in water bodies it is about 125 μ M to 100 mM.^{8,9} Fluoride is one of abundant anions present in groundwater worldwide and creates a major problem in safe drinking water supply.⁶ Fluoride is highly toxic in the environment and its toxicity to terrestrial plants has been studied scientifically in laboratory, greenhouse, and controlled field plot experiments.¹⁰

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Fluoride enters the human body mainly through water and food and may lead to fluoride toxicity including dental, skeletal, and non-skeletal fluorosis.¹¹ The basic injury effects on plants due to fluoride attack are chlorotic leaves and black necrotic spots appearing on the periphery.¹² The plasmalemma contains phosphoproteins and once fluoride enters the plant tissue it directly alters the phosphorylation of these proteins,¹³ the functioning of enzymes,^{12,13,14} and the biochemical characteristics of the plant metabolism.^{15,16}

Turnip (*Brassica rapa* L.) shows more resistance to salt stress at germination as compared to the mature plant.¹⁷ The history of the turnip goes back to the countries linked to the Mediterranean sea and the Central Asian countries,¹⁸ and it was introduced later to East Asian countries.¹⁹ Turnip is a genetically variant crop having a lot of varieties differing in form of the root they produce, the size, and the color. The vegetable is also employed as a food crop for humans as well as animals in European countries.¹⁸ Turnip has a lot much benefit to humans as it is a reservoir of various nutrients including the vitamins A, C, E, B6, and folic acid, the elements calcium and copper, and nutritional fiber. Both the leaves and the roots may be used.²⁰

The aim of the present study was to examine, in a pot experiment, the effects of effects of NaF, applied as a foliar spray and a soil drench, on growth and other attributes of turnip (*Brassica rapa* L.)

MATERIALS AND METHODS

Certified hybrid seeds of turnip (*Brassica rapa* L.) variety White Globe Purple Top were procured from Punjab Seeds Corporation, Lahore. The Botanical Garden of the University of the Punjab, Quaid-e-Azam campus, Lahore, was chosen as the experimental site. The garden is situated in the southern area of Lahore (74° 21-00-E, 31° 35-00-N), measures approximately 21×15 m, and is in the outlying part of the city near open wide plots. The period during which experiment was performed was from December 2016 to March 2017. The plants grown in the experiment were carefully treated and protected from other environmental hazards and pests by keeping them in a netting frame of steel wire. The soil used as the growing medium was a combination of agricultural soil and farm yard manure in the 3:1 ratio used for normal agricultural practices. Eighty-four plastic pots, 7 inches in diameter and properly washed, were used in the study. The pots were placed in the wire enclosure according to a randomized complete block design (RCBD) so that the impacts of the surrounding atmosphere did not influence the results. Ten mL of a sodium fluoride solution, containing 50, 100, 150, 200, 250, and 300 ppm NaF, was applied as a foliar spray on the turnip plants and as a soil drench twice a week during experimental season. The plants were maintained in the experimental area throughout the growing season of turnip by using the normal agricultural practices of the local people and farmers.

Each treatment was conducted in triplicate and harvested at 50 and 70 days after sowing (DAS). The plants were harvested in such a way that the roots remained intact and were then rinsed thoroughly with water. The detached plants were then placed in polythene bags which were properly labeled. These were then brought into the laboratory and further parameters such as root length, shoot length, and the number of leaves were recorded.

The biomass assessment was done at 50 and 70 days DAS. The plants were oven dried by placing them in an electric oven (Wiseven, Model WOF-105, Korea) on blotting paper or in labeled bags of paper and maintaining the temperature of 70°C for 3 days. Their dry weights were then recorded using an electric balance (Sartorius GMBH, Type 1216MP 6E, Gottingen, Germany).

The 1965 Carleton and Foote formula was used for leaf area calculation:

$$\text{Leaf area} = \text{Length} \times \text{Maximum width} \times 0.75 \text{ (correction factor)}$$

The results were recorded and checked statistically by measuring the treatment mean, the standard error and the Duncan's Multiple Range Test (DMRT), according to the description.²¹ The analysis was done in the laboratory by employing a software package, Costat (version 3.03), on laboratory computers.

RESULTS

The shoot length at 50 DAS decreased regularly as the concentrations of NaF were increased from 50 to 300 ppm with both methods of application. The maximum percentage reduction in the shoot length of the plants at 50 DAS, as compared to untreated plants, was 43% which occurred in the plants given 300 ppm NaF by soil drench (Figure 1).

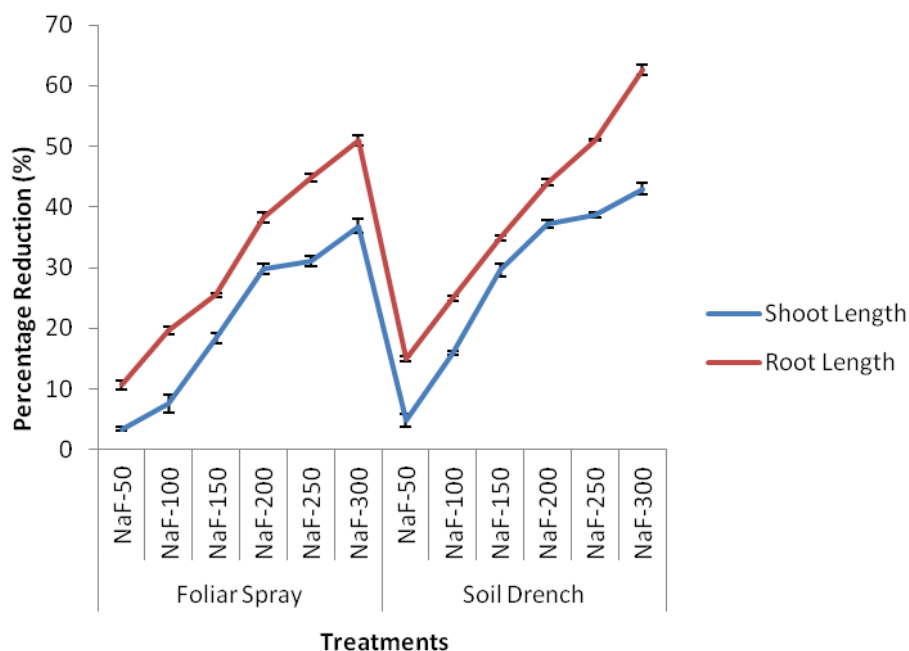


Figure 1. Root and shoot length percentage reductions in *Brassica rapa* L. harvested at 50 days after sowing (DAS) under different sodium fluoride treatments by using soil drench and foliar spray. (NaF-50=50 ppm NaF, NaF-100=100 ppm NaF, NaF-150=150 ppm NaF, NaF-200=200 ppm NaF, NaF-250=250 ppm NaF, NaF-300=300 ppm NaF).

The plants which were given a sodium fluoride concentration of 50 ppm by soil drench showed a percentage reduction shoot length of 4.8%. clearly verifying the less harmful effects of this concentration on plants (Figure 1). In the same way, the shoot length of the plants which were given a concentration a NaF of 300 ppm as foliar

spray was measured as 36.83% (Figure 1). The plants which were given a concentration of 50 ppm of NaF by the same method showed a percentage reduction in shoot length at 50 DAS of only 3.43% which demonstrates the very minor impact of this concentration on plants, compared to the effects of higher concentrations (Figure 1). The values of percentage reduction recorded for shoot length in plants at 50 DAS which were exposed to other concentrations of NaF of 100, 150, 200 and 250 as soil drench were 15.85%, 29.63%, 37.25%, 38.61%, respectively, and the corresponding values for the foliar application of these other concentrations of NaF of 100, 150, 200 and 250 on the plants at 50 DAS were observed to be 7.57%, 18.33%, 29.48%, 31.16%, respectively.

Similar observations were made at 70 DAS after the plants been treated twice weekly by given the application of the various concentrations of sodium fluoride grades. The maximum percentage reduction in the shoot length of the plants at 70 DAS given sodium fluoride by both methods of application, soil drench and exogenously by foliar spray, was observed to occur in the plants given 300 ppm NaF with the values being 40.56% and 36.02%. respectively (Figure 2).

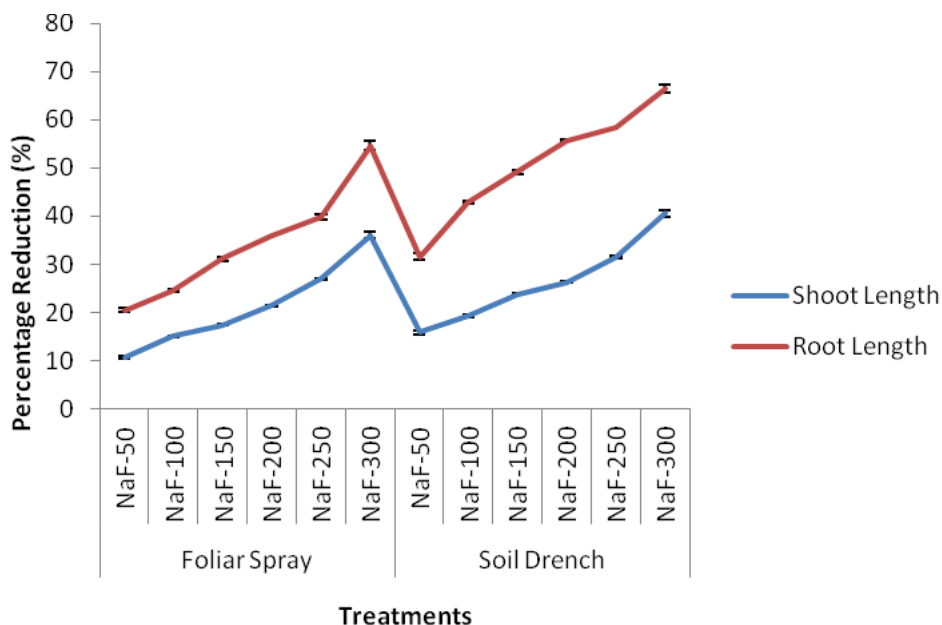


Figure 2. Root and shoot length percentage reductions in *Brassica rapa* L. harvested at 70 days after sowing (DAS) under different sodium fluoride treatments by using soil drench and foliar spray. (NaF-50=50 ppm NaF, NaF-100=100 ppm NaF, NaF-150=150 ppm NaF, NaF-200=200 ppm NaF, NaF-250=250 ppm NaF, NaF-300=300 ppm NaF).

For the plants which were given a concentration of 50 ppm NaF by soil drench and exogenously by foliar spray, the percentage reductions in shoot length at 70 DAS were 15.9% and 10.62%, respectively (Figure 2). The percentage reduction was less for foliar spray compared to that for the soil drench. Plants exposed to the other concentrations of NaF of 100, 150, 200, and 250 at 70 DAS by soil drench had percentage reductions in shoot length at 70 DAS of 19.31%, 23.86%, 26.36% and 31.47%, respectively (Figure 2). The corresponding percentage reductions in shoot length for the plants at 70 DAS given foliar spray of with the other concentrations of

NaF of 100, 150, 200, and 250 were 15%, 17.44%, 21.42%, and 26.93%, respectively (Figure 2).

The maximum percentage reductions in the root length of the plants at 50 DAS, in the plants treated with soil drench and foliar spray, compared to the untreated plants, were 62.6% and 51.02%, respectively, and occurred in the plants given 300 ppm NaF (Figure 1). The corresponding maximum percentage reductions in the root length of the plants at 70 DAS, in the plants treated with soil drench and foliar spray, compared to the untreated plants, were 66.4% and 54.59%, respectively, and occurred in the plants given 300 ppm NaF (Figure 2). There was a gradual increase in the percentage reductions in the root lengths of the plants at both 50 and 70 DAS as the concentration of the NaF used in the treatment increased from 50 to 300 ppm (Figures 1 and 2).

The maximum percentage reductions in the number of leaves of the plants at 50 and 70 DAS, in the plants treated with soil drench, compared to the untreated plants, were 50.03% and 65.23%, respectively, and occurred in the plants given 300 ppm NaF (Figures 3 and 4). The maximum percentage reductions in the number of leaves of the plants at 50 and 70 DAS, in the plants treated with foliar spray, compared to the untreated plants, were 50.03% and 58.7%, respectively, and occurred in the plants given 300 ppm NaF (Figures 3 and 4). The minimum percentage reduction in the number of leaves of the plants, 10.89%, occurred in the plants at 50 DAS treated with 50 ppm NaF given as a foliar spray, while the maximum percentage reduction in the number of leaves of the plants was recorded in plants at 70 DAS treated with 300 ppm NaF given as a soil drench (Figures 3 and 4).

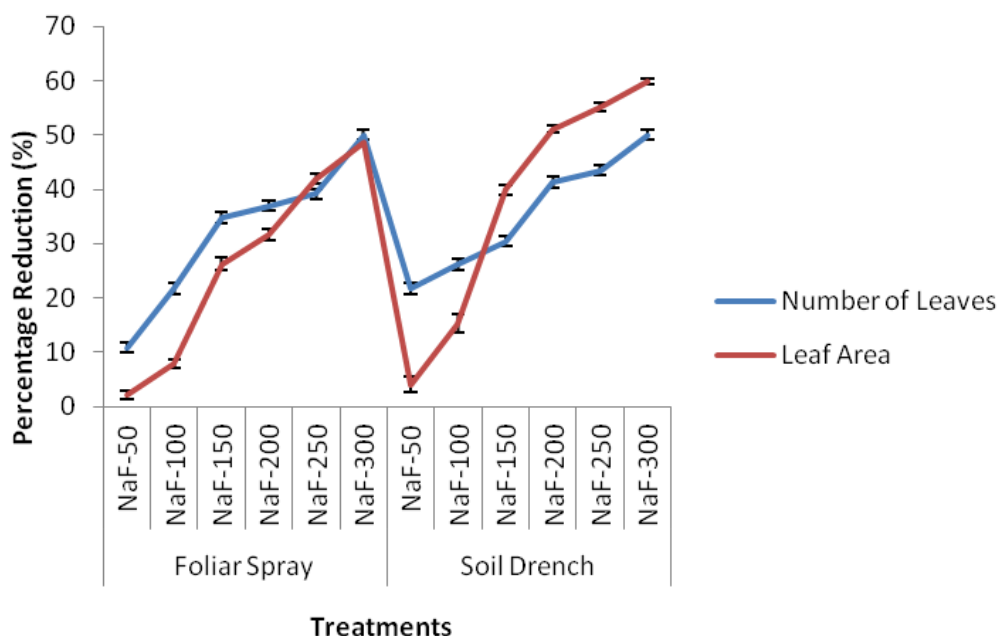


Figure 3. Number of leaves and leaf area percentage reductions in *Brassica rapa* L. harvested at 50 days after sowing (DAS) under different sodium fluoride treatments by using soil drench and foliar spray. (NaF-50=50 ppm NaF, NaF-100=100 ppm NaF, NaF-150=150 ppm NaF, NaF-200=200 ppm NaF, NaF-250=250 ppm NaF, NaF-300=300 ppm NaF).

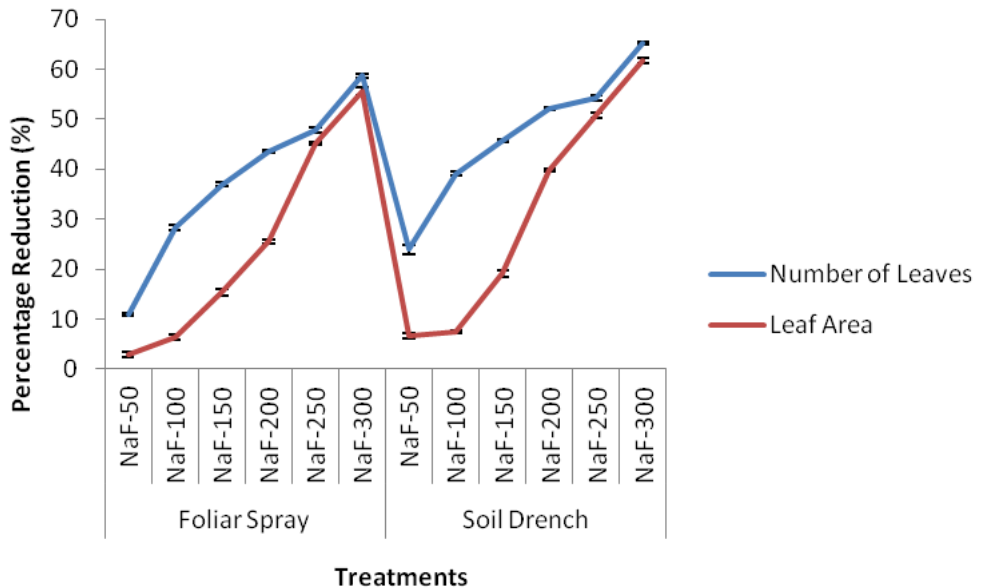


Figure 4. Number of leaves and leaf area percentage reductions in *Brassica rapa* L. harvested at 70 days after sowing (DAS) under different sodium fluoride treatments by using soil drench and foliar spray. (NaF-50=50 ppm NaF, NaF-100=100 ppm NaF, NaF-150=150 ppm NaF, NaF-200=200 ppm NaF, NaF-250=250 ppm NaF, NaF-300=300 ppm NaF).

At 50 DAS, the least percentage reduction in the leaf area of 2.18% was found in the plants given 50 ppm sodium fluoride by foliar spray and the highest value of 59.87% was present in the plants given 300 ppm NaF by soil drench (Figure 3). Similar outcomes were observed in the plants at 70 DAS (Figure 4). A greater leaf area was observed in the plants treated with the lower concentrations of NaF compared to those treated with higher concentrations of NaF (Figures 3 and 4). A foliar spray of 300 ppm NaF affected the leaf area seriously and created a percentage reduction of 55.6% at 70 DAS while it was 61.72% for the soil drench of the same concentration. More toxic effects of NaF on the leaf area were seen when its method of application was soil drench and less serious effects were observed when the method of application used was foliar spray (Figures 3 and 4). At 70 DAS, reduction in the leaf area of the plants which were given 50 ppm NaF by soil drench was 6.63% while it was 3% for plants given the foliar spray of same concentration (Figure 4). At 70 DAS the leaves of plants under stress of a 300 ppm NaF soil drench had a smaller leaf area of 61.72% than the plants treated with the foliar spray of same concentration who had a reduction in the leaf area of 55.6% (Figure 4).

The highest percentage decrease in the shoot fresh weight of 78.84% was found when 300 ppm NaF was used for a soil drench and the corresponding value for treatment with a foliar spray of 300 ppm was 76.65% (Figures 5 and 6). Similarly, the highest percentage reduction in the root fresh weight of 84.05% was found when the plants were exposed to a soil drench of 300 ppm NaF and the corresponding value for treatment with a foliar spray of 300 ppm was 77.67% (Figures 5 and 6).

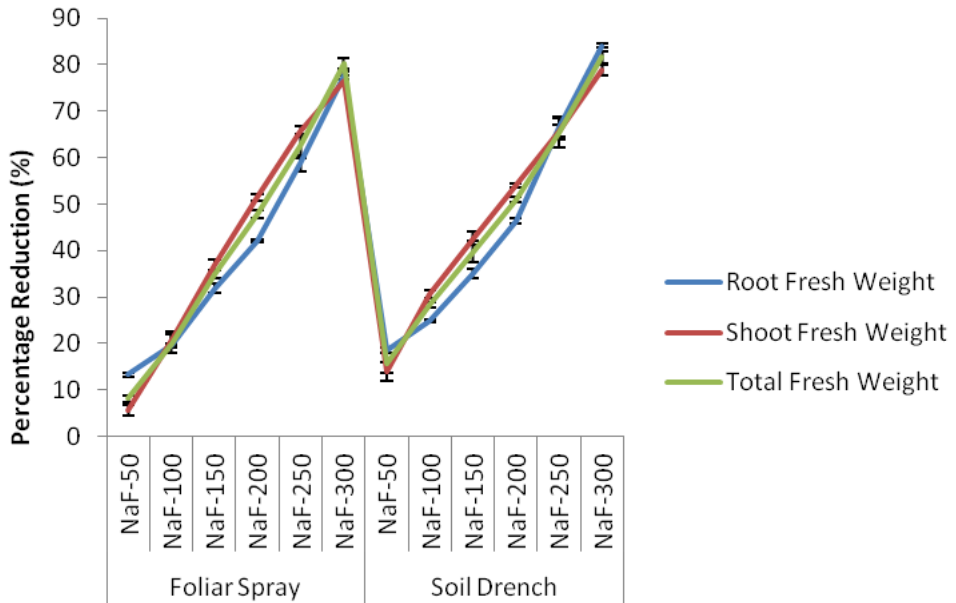


Figure 5. Shoot and root fresh weight percentage reductions in *Brassica rapa* L. harvested at 50 days after sowing (DAS) under different sodium fluoride treatments by using soil drench and foliar spray. (NaF-50=50 ppm NaF, NaF-100=100 ppm NaF, NaF-150=150 ppm NaF, NaF-200=200 ppm NaF, NaF-250=250 ppm NaF, NaF-300=300 ppm NaF).

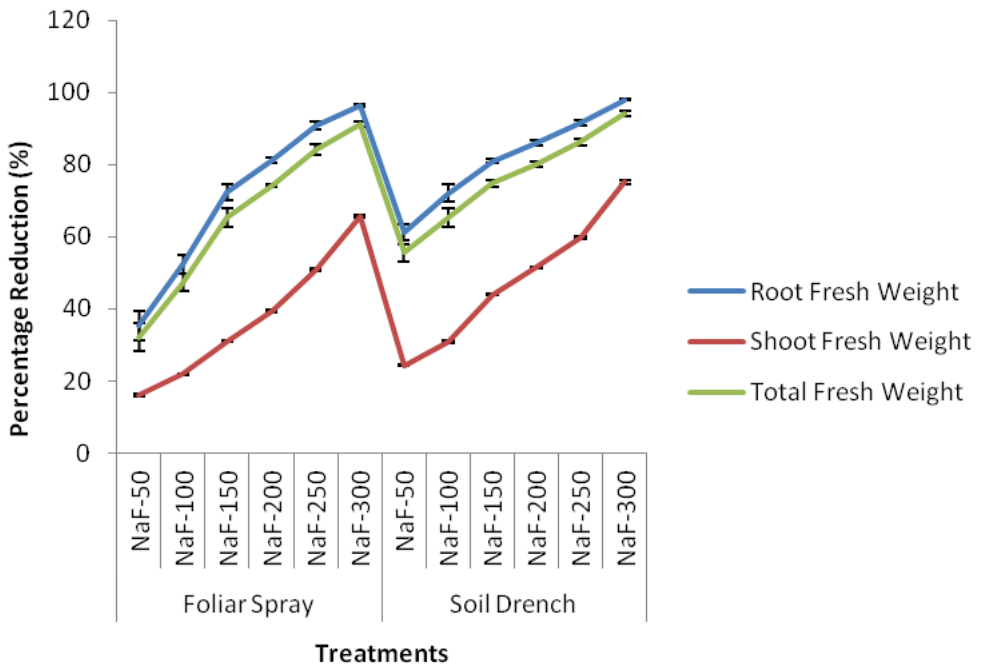


Figure 6. Shoot and root fresh weight percentage reductions in *Brassica rapa* L. harvested at 70 days after sowing (DAS) under different sodium fluoride treatments by using soil drench and foliar spray. (NaF-50=50 ppm NaF, NaF-100=100 ppm NaF, NaF-150=150 ppm NaF, NaF-200=200 ppm NaF, NaF-250=250 ppm NaF, NaF-300=300 ppm NaF).

The percentage decrease of the root dry weight at 50 DAS was recorded as 49.74% after treatment with a 50 ppm NaF as a soil drench while the corresponding figure when it was applied as a foliar spray was 46.31%. (Figure 7). The percentage reductions of shoot dry weight at 50 DAS was observed to be 10.23% for a soil drench of 50 ppm NaF and 0.51% for a foliar spray of 50 ppm NaF (Figure 7). The percentage reduction increased gradually with higher concentrations of NaF and became the maximum at 300 ppm NaF for both soil drench and foliar spray (Figure 7).

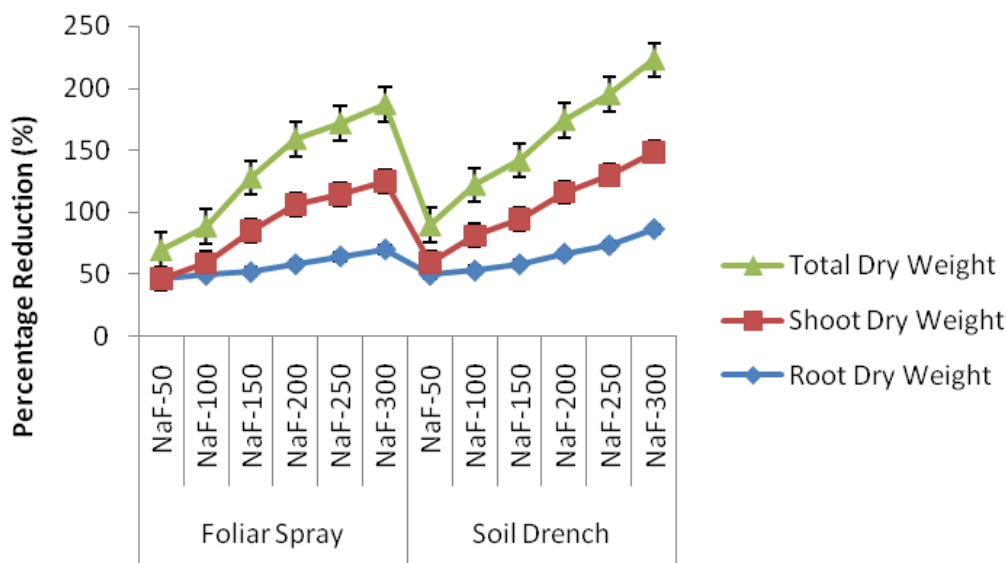


Figure 7. Shoot and root dry weight percentage reductions in *Brassica rapa* L. harvested at 50 days after sowing (DAS) under different sodium fluoride treatments by using soil drench and foliar spray. (NaF-50=50 ppm NaF, NaF-100=100 ppm NaF, NaF-150=150 ppm NaF, NaF-200=200 ppm NaF, NaF-250=250 ppm NaF, NaF-300=300 ppm NaF).

At 70 DAS, the root dry weight exhibited same declining trend with treatment with 50–300 ppm NaF as compared to the control group (Figure 8). The mean root dry weight for the control at 70 DAS was 6.60 g and after treatment with NaF as a soil drench, in concentrations of 50, 100, 150, 200, 250, and 300 ppm, the mean root dry weight showed decreasing values of 4.30, 3.36, 2.30, 1.66, 1.16, and 0.56 g, respectively (Figure 8). Similarly the recorded data for the root dry weight at 70 DAS treatment with a foliar spray, in concentrations of 50, 100, 150, 200, 250, and 300 ppm, also followed a decreasing pattern with value of 5.06, 4.10, 3.20, 2.20, 1.63, and 1.20 g, as compared to the control where the root dry weight was 6.60 g.

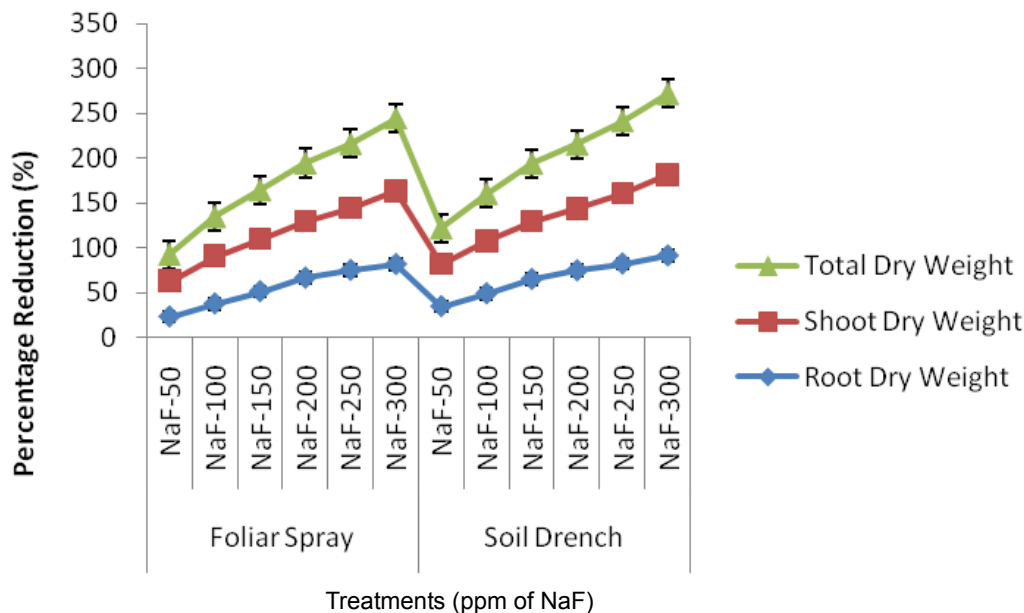


Figure 8. Shoot and root dry weight percentage reductions in *Brassica rapa* L. harvested at 70 days after sowing (DAS) under different sodium fluoride treatments by using soil drench and foliar spray. (NaF-50=50 ppm NaF, NaF-100=100 ppm NaF, NaF-150=150 ppm NaF, NaF-200=200 ppm NaF, NaF-250=250 ppm NaF, NaF-300=300 ppm NaF).

DISCUSSION

The conclusion drawn from the study are that the turnip showed a marked decrease in its growth when the concentrations of salts were elevated in its surroundings or in the rhizosphere. These results are consistent with the findings of researchers on various crops including maize²² and wheat.²³ The changes in the growth pattern of turnip due to the high levels of salt toxicity demonstrated in the present study were due to changes in a number of biochemical and physiological characteristics involving the salt tolerance mechanism, nutrient homeostasis, photosynthesis, accumulation of compatible solutes, and antioxidant enzymes activities, etc.²³ Studies by Jain²⁴ and Jangid²⁵ found that fluoride accumulates more in plant roots than in other parts of the plant. The results of the present study clearly show that a high concentration of sodium fluoride in the root zone of the turnip plant significantly reduced plant growth. Fluoride exposure caused a decrease in the root and shoot lengths of turnip due to an unbalanced nutrient uptake by seedlings.²⁶ A decrease in root and shoot length with fluoride exposure has also been reported with *Pisum sativum*,²⁶ *Brassica juncea*,²⁷ *Oryza sativa*,²⁸ and *Triticum aestivum*.²⁹ Similar results have also been obtained with other species including moth bean (*Vigna aconitifolia* L.),³⁰ radish plant (*Raphanus sativus* L.),³¹ cowpea (*Vigna unguiculata* L.),³² and *Vigna mungo* L.³³ The height of the plants was sharply decreased with elevated levels of NaF.

Our findings were also consistent with those of Jamil et al.³⁴ found that treatment of cabbage (*Brassica oleracea capitata* L.) and *Brassica oleracea botrytis* L., with different concentrations of NaF, had a negative effect on leaf number. Similarly,

Mathur et al.³⁰ reported that the stress given to the moth bean plant (*Vigna aconitifolia* L.) with increasing concentrations of sodium fluoride led to a decrease in leaf area.

Toxic concentrations of fluorides can cause a loss of yield and biomass, a decline in photosynthetic capacity and respiration rate, and a deterioration of the plant metabolism.³⁵ With increasing fluoride concentrations, the fresh and dry weights decreased due to fluoride acting as a metabolic inhibitor and resulting in reduced metabolic activity including germination.³⁶

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