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GROWTH AND YIELD RESPONSES OF LACTUCA SATIVA L. TO DIFFERENT CONCENTRATION OF SODIUM FLUORIDE

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ABSTRACT: Fluoride is abundant in the environment and toxic to all living organisms above a certain threshold. Fluoride exposure causes physiological, molecular, and biochemical changes in plants. The current study was initiated to determine the response of plants grown under fluoride stress administered as a soil drench. During the growth season of October 2019 to December 2019, the *Lactuca sativa* L. was experimented. Sodium fluoride (NaF) concentrations of 50, 100, 150, 200, 250, and 300 ppm were applied to plants twice a week as soil drench to create stressed conditions. With increasing NaF concentration, there was a gradual reduction in morphological, biomass, and yield attributes and at the highest concentration, i.e, 300ppm, the growth was least. By observing the negative effects of NaF on plants, the current pot experiment concludes that Lettuce is sensitive to NaF stress. Future researchers may direct their studies to alleviate NaF stress by understanding the biochemical changes occurring in the plant during stress conditions.

Keywords: Growth; Lactuca sativa L.; Sodium Fluoride stress; Yield.

INTRODUCTION

Soil salinization poses a serious threat to arable lands as its elevation decreases crop productivity and leaves it marginal ^[1]. Salinized soil is not merely an ultimatum to the forestry and agriculture but also decreases the soil quality and utility rate ^[2]. According to FAO, salinity has affected more than 6% total land. At present, approximately one third of the world agricultural area is affected by salinization, and due to poor irrigation practices and excessive use of agrochemicals, ^[3]. Morphological and biochemical responses of plants are adversely affected by salinity. Reduced seed germination and decreased plant growth and the resulting low yield are induced by stress ^[4]. Salinity also compromises the photosynthetic machinery and ultimately reduces the chlorophyll content and alters the structure of chlorophyll ^[5,6].

Fluoride is 13th most occurring element on earth and is considered among toxic pollutants ^[7]. In the Earth's crust, several rocks and minerals have fluorides and they leach out by weathering of such rocks and by precipitation ^[8]. Wastewaters from steel, semiconductors, aluminum, fertilizer and insecticide producing industrial activities are other major sources of entering of fluoride into the environment ^[9]. Fluoride (F) is widely distributed in soils (150–400 mgkg⁻¹) ^[10]. In normal soils, fluoride uptake is minimal as it is adsorbed by soil but with soils adversely polluted fluoride its uptake is heightened and internal damages to plant may occur ^[11]. Fluoride acts as an accumulative poison in plant foliage though its accumulation is

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gradual. Fluoride triggers the inhibition of photosynthesis and other processes. It will move in the transpiration stream from stomata or by roots and accumulate in leaf margins ^[12].

Lettuce is a very popular leafy vegetable and ranks high in both production and economic value among all the vegetables ^[13]. Lettuce is originated from Western Asia and South Europe and its early forms were used in Egypt around 4500 BC. As early as in the beginning of the Christian era, the Romans grew types of lettuce resembling the present Romaine cultivars. China also used the crop in 7th century A.D ^[14]. Lettuce is an excellent source of vitamins A, C, B, E, K, folate, and iron ^[15]. It also contains protein, carbohydrate, and vitamins like vitamin C and in 100 g of edible portion of lettuce contains 93.4% moisture, 2.1g protein, 0.3g fat, 1.2g minerals, 0.5g fibre, 2.5g carbohydrates, 310 mg calcium, 80mg phosphorus, 2.6mg iron, 1650 I.U. vitamin A, 0.09mg thiamine, 0.13mg riboflavin and about 10.0mg vitamin C ^[16]. *Lactuca sativa* L. (Lettuce) is in category of moderately salt tolerant ^[17]. More than 2.6 dS m⁻¹ and 2.0 of salinity levels can reduce lettuce plant growth and fresh yield, respectively ^[18]. The lettuce is sensitive to sodium chloride which significantly decreases those indices of physiology and growth ^[19].

The aim of this study was to understand the effect of NaF on Lettuce as it is among the burgeoning salt in soil to deteriorate the normal functioning of plants. The effects of morphological growth patterns and yield has been studied. Furthermore, the techniques to mitigate stress conditions can be helpful for appropriate growth of this plant.

MATERIALS AND METHODS

Plant material and experimental site: Healthy and verified seedlings of Lettuce (*Lactuca sativa* L.), variety Grand Rapids were obtained from Roshan Seeds Corporation, Lahore. Five pots containing about 30-40 seedlings were purchased. The fresh and uninfected seedlings were chosen for transplantation. The location chosen for the experimental setup was Botanical Garden, Southern Region of Lahore city (74°21'-00"-E, 31°35'-00"-N), Punjab University, Quaid-e-Azam Campus. The experimental work was initiated in October 2019, and it lasted till December 2019. The experimental plants were grown in a Wire House to protect the plants from heavy rainfalls and intense sunlight. The plants were grown in natural conditions without any anti-fungal or pesticidal sprays.

Soil and pot preparation: Loam and Sand were mixed in ratio of 1:3 respectively. The soil was also supplemented with natural organic matter like farm manure and humus to make soil rich in organic content and to increase its fertility. The experiment was performed in 42 clay pots. Arrangement of pots was according to Randomized complete block design (RCBD). 42 pots were arranged in a way that there were 3 replicates per harvest. Two harvests were taken for this experiment. The pots were arranged according to their respective treatments. Each treatment had six replicates. The pots were labelled in accordance with their treatments.

Solution preparation: In the experiment, Sodium Fluoride (NaF) salt is used in six different ppm concentrations. The NaF solutions were made by combining the solute

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(NaF) with a weighed amount of distilled water. Control, NaF 50, NaF 100, NaF 150, NaF 200, NaF 250, and NaF 300 are the solutions (treatments) prepared.

Transplantation and application of sodium fluoride (NaF) solution: The seedlings were transplanted to the experimental pots one at a time, with five seedlings placed in each of the 45 pots. After 15 days of transplantation, the plants that were able to withstand the stress were chosen and the others were removed from the pot. In this experiment, the NaF solution was used as a soil drench. After 23 days of transplantation, the first treatment was administered. The plants were treated with NaF solution twice a week. As a soil drench, each pot received 100mL of the appropriate concentration solution.

Determination of morphological parameters: The plants were carefully extracted from the pots with their roots. To measure the root and shoot length, the roots were separated from the shoot after thorough washing to clean out dirt, so to evaluate the effect of sodium fluoride (NaF) on them. The morphological parameters that were recorded were: Length of Shoot (cm), Length of root (cm), Leaf Number, Leaf area (cmð). The area of leaf was calculated by Carleton and Foote ^[19]. formula.

Leaf Area= Leaf Length P Leaf width P0.75 (correction factor)

Biomass assessment: Using Electrical Balance Sartorius GMBH, type 1216 MP 6E Gottingen, Germany, the dry and fresh weight were measured. The samples were dried using the drying oven (Wiseven, Model WOF-105, Korea). The following characters were noted for biomass assessment, Fresh weight of Shoot (g), Shoot Dry weight (g), Fresh weight of root (g), Root dry weight (g), Total Fresh weight (g).

Yield parameters evaluation: The parameters of yield were as follows, Fresh weight of leaves (g), Leaves Dry weight (g).

Statistical analysis: The data were statistically analyzed by means of one-way ANOVA, using SPSS software. Duncan's multiple range test was used for splitting of means for significant treatment at $p \le 0.05$. The values are means of 3 replications \pm SE.

RESULTS

Determination of morphological parameters: Increase in the concentration of Sodium Fluoride caused a linear decline in the morphological growth pattern of Lettuce (*Lactuca sativa* L.). The shoot length gradually decreased in both harvests, at Control the shoot length was longest 13.2cm in first harvest and 15.63cm in second harvest as depicted in Table 1. And the shoot length was least at the highest level of stress, i.e., 300pm. Marked reduction in root size was noticed in plants under higher levels of stress. Maximum decrease in root length was seen at 300 ppm for both harvests. The root length decreased from 11.63cm to 7.1cm after 59 DAT (Table 1). As shown in Table 1 number of leaves in lettuce also depicted adverse effects of NaF salinity stress under various concentrations. At first harvest, highest count of leaf number was noted in Control that were 15.33 by average. The plants that were treated with NaF-300 ppm concentration showed least number of leaves, this shows that Lettuce is sensitive to greater concentration of NaF. Same trend was observed for final harvest.

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The length and width of leaves were also affected by the varying concentration of Sodium fluoride. At 39 DAT, the maximum leaf area was calculated in Control plants that was 89.3cm² and then the leaf area started to decrease as various concentrations of NaF were given to plants and the least was observed at highest level of stress for both the harvests (Table 1).

 Table 1. Morphological growth parameters of Lactuca sativa L. harvested at 39 DAT and 59 DAT under 7 treatments of Sodium fluoride during growth season of October 2019- December 2019

Treatments	Parameters							
	39 DAT			59 DAT				
	Shoot Length (cm)	Root Length (cm)	No. of Leaves	Leaf Area (cm²)	Shoot Length (cm)	Root Length (cm)	No. of Leaves	Leaf Area (cm²)
Control	13.2	11.6	15.33	93.37	15.63	11.63	20.66	155.77
	±0.30a	±0.30a	±0.39a	±0.30a	±0.18 a	±0.34a	±0.33a	±1.81a
50 ppm	13.0	10.63	13.66	88.42	13.63	10.7	17.33	109.29
	± 0.05a	±0.18b	±0.33b	±0.23b	±0.40b	±0.20b	±0.28b	±4.25b
100 ppm	12.8	9.90	13.0	81.65	12.16	9.56	13.66	81.25
	±0.05a	± 0.05c	± 0.15b	±0.44c	±0.52c	±0.12c	±0.33c	±2.58c
150 ppm	11.83	8.46	11 ±	78.65	11.06	9.13	9.33	73.97
	±0.16b	±0.08d	0.23c	±0.39d	±0.23d	±0.27cd	±0.19d	±1.92d
200 ppm	11.36	8.2	9.33	68.68	10.8	8.73	8.33	61.37
	±0.23b	±0.11de	±0.31d	±0.41e	±0.30d	±0.14de	±0.33de	±1.37e
250 ppm	10.7	7.56	6.66	66.82	10.26	8.23	7.33	57.87
	±0.11c	±0.29e	±0.12e	±0.27f	±0.14d	±0.14e	±0.39e	±2.42 f
300 ppm	10.3	5.3	4.66	60.87	9.16	7.1	5.66	43.27
	±0.05c	±0.30f	±0.07f	±0.32g	±0.58e	±0.15f	±0.25f	±1.21g

Data exhibit means \pm SE of 3 replicates. Non-identical letters point to significant difference between the treatments (P \leq 0.05) of One-Way ANOVA.DAT= Days after transplantation.

Biomass assessment: NaF salt concentration negatively affected biomass of Lettuce (*Lactuca sativa* L.) When the amount of sodium fluoride increased, biomass of shoot decreased linearly. This trend was observed in both harvests (Table 2).

As in Table 2 maximum shoot weight was observed in plants with Control treatment with weight of 42.44g and 126.46g for both harvests, respectively. Lettuce has fibrous root system and dense roots develop approximately two weeks after sowing. Sodium Fluoride negatively affected the growth of roots with increasing concentration. The fresh weight of root decreased to 0.93g and 4.36g at 300ppm for both harvests respectively. Total fresh weight was ultimately decreased as it includes the combined weight of shoot and root.

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The dry weight of shoot also followed the similar pattern and with increasing salt concentration, the shoot dry weight decreased. At 39 DAT, the Control plants had the maximum. At 59 DAT, the plants grew to their mature stage, but the trend of their growth was quite visible and the plants that were kept under Control had the highest shoot dry weight as compared to those treated with various concentration of NaF. At 39 DAT, the dry weight of root was maximum at Control that was 0.46g but with the amount of salt increased, the root dry weight reduced as 0.45g, 0.41g, 0.36g, 0.17g, 0.13g, and 0.11g at 50ppm, 100ppm, 150ppm, 200ppm, 250ppm, and 300ppm, respectively (Table 2). At 59 DAT, the roots observed in Control plants flourished well but as the concentration of NaF increased, the roots became feeble and fragile, also their bulk decreased (Plates 1 and 2).



Plate 1. Growth of Lettuce under different concentrations of sodium fluoride using soil drench at 39 days after transplantation (DAT) during growth season October 2019-December 2019.

Plate 2. Growth of Lettuce under different concentrations of sodium fluoride using soil drench at 59 days after transplantation (DAT) during growth season October 2019-December 2019.

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Table 2. Biomass Assessment of Lactuca sativa L. harvested at 39 DAT and 59 DAT under 7
treatments of Sodium fluoride during growth season of October 2019- December 2019.

Treatments		Parameters						
		Shoot Fresh Weight (g)	Root Fresh Weight (g)	Total Fresh Weight (g)	Shoot Dry Weight (g)	Root Dry Weight (g)	Total Dry Weight (g)	
	Control	42.44 ±0.38a	3.1 ±0.05a	45.54 ±0.40a	3.73 ±0.12a	0.46 ±0.03a	4.2 ±0.15a	
39 DAT	50 ppm	40.5 ±0.05b	2.82 ±0.02b	43.33 ±0.07b	3.45 ±0.02b	0.45 ±0.02a	3.9 ±0.05b	
	100 ppm	36.37 ±0.12c	2.69 ±0.00c	39.07 ±0.12c	2.86 ±0.03c	0.41 ±0.02ab	3.27 ±0.04c	
	150 ppm	22.56 ±0.23d	1.5 ±0.02d	24.06 ±0.24d	1.41 ±0.01d	0.36 ±0.02b	1.77 ±0.03d	
	200 ppm	15.86 ±0.18e	1.36 ±0.02e	17.23 ±0.20 e	1.26 ±0.12d	0.17 ±0.01c	1.43 ±0.11e	
	250 ppm	12.39 ±0.02f	1.28 ±0.04e	13.67 ±0.03f	0.88 ±0.01e	0.13 ±0.00c	1.01 ±0.02f	
	300 ppm	9.24 ±0.02g	0.93 ±0.01f	10.18 ±0.04g	0.63 ±0.03f	0.11 ±0.00c	0.74 ±0.02 g	
	Control	126.46 ±0.31a	8.6 ±0.1a	135.06 ±0.41a	5.16 ±0.08a	0.63 ±0.12a	5.8 ±0.11a	
59 DAT	50 ppm	89 ±0.25b	7.5 ±0.08b	96.5 ±0.33b	4.33 ±0.14b	0.62 ±0.04a	4.98 ±0.17b	
	100 ppm	49.5 ±0.37c	5.1 ±0.17c	54.66 ±0.08c	3.86 ±0.13c	0.5 ±0.02a	4.36 ±0.11c	
	150 ppm	43.53 ±0.34d	4.96 ±0.28c	48.5 ±0.23d	3.6 ±0.05c	0.34 ±0.03b	3.94 ±0.04d	
	200 ppm	40.33 ±0.33e	4.53 ±0.29c	44.7 ±0.12e	3.16 ±0.12d	0.28 ±0.01bc	3.44 ±0.12e	
	250 ppm	33.96 ±0.31f	4.43 ±0.29c	38.4 ±0.25f	3.06 ±0.06d	0.2 ±0.01c	3.27 ±0.05e	
	300 ppm	32.1 ±0.15g	4.3 ±0.27c	36.46 ±0.42g	2.67 ±0.16e	0.14 ±0.03c	2.81 ±0.15f	

Data exhibit means \pm SE of 3 replicates. Non-identical letters point to significant difference between the treatments (P \leq 0.05) of One-Way ANOVA.DAT= Days after transplantation.

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Yield parameters evaluation: Lettuce is a leafy vegetable, and the leaves of Lettuce are used for consumption. The flowering in Lettuce occurs in the summers but once the crop flowers it is not used for consumption as the flowering in Lettuce is only beneficial for getting seeds and for the continuity of the next generation. So, the leaves of this crop are known to be its yield. At 39 DAT, the lettuce leaves were young and green in Control and the weight was 39.5g, at 50, 100, 150, 200, 250, and 300ppm the leaves fresh weights were 33.98g, 31.66g, 19.9g, 13.6g, 10.83g, and 8.86g respectively. The fresh weight of leaves decreased in gradual manner with the escalating salt concentration. At 59 DAT, the similar gradual decrease in fresh weight of leaves was noted (Figure 1 and Plate 3).

Figure 1. Showing mean values Leaves Fresh Weight in *Lactuca sativa* L. harvested at 39 DAT and 59 DAT under 7 treatments of Sodium fluoride during growth season of October 2019-December 2019.

Plate 3. Leaves Fresh Weight of Lettuce under different concentrations of sodium fluoride using soil drench at 39 DAT during growth season October 2019-December 2019.

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Plate 4. Leaves Fresh Weight of Lettuce under different concentrations of sodium fluoride using soil drench at 59 DAT during growth season October 2019-December 2019.

The dry weight of leaves showed the same trend of decline with increasing salt concentrations. The highest leaves dry weight was noted in Control plants in both harvests. The highest leaves dry weight was manifested in Control plants that valued 3.33g and 4.96g at 39DAT and 59 DAT, respectively (Figure 2).

Figure 2. Showing mean values Leaves Dry Weight in *Lactuca sativa* L. harvested at 39 DAT and 59 DAT under 7 treatments of Sodium fluoride during growth season of October 2019-December 2019.

DISCUSSION

Among abiotic stress factors that limit plant growth, salinity is remarkable, especially in semi-arid and arid fields ^[20, 21]. Almost all crops are sensitive to salt stress, thus excessive salinity causes a decrease in yield and biomass ^[22]. During this study, the Lettuce plants were exposed to sodium fluoride stress and similar results were obtained. The effects of NaF on various morphological parameters and biomass attributes are shown in Tables 1 and 2. They clearly show that all the growth characters of the plant are adversely affected by the elevating levels of NaF. These

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outcomes are in conformity with the results of Kimambo et al.^[9] and Singh et al.^[23] who studied *Abelmoschus esculentus* L and *Raphanus sativus* L, respectively, and reported inhibition of root and shoot elongation and decrease in biomass due to varying levels of NaF stress.

Shoot and root length decreased as the concentrations of NaF were increased because fluoride acts as a phytotoxin at higher concentration and inhibits all the essential reactions in plant and thus inhibits growth. Similar reductions in growth were observed in other crops like cluster beans ^[2] and wheat ^[22]. Kumar and Singh ^[24] demonstrated a decrease in Gossypium hirsutum L. root biomass to about 73% when exposed to 1000ppm of fluoride contaminated irrigation water. Shoot length, root length, total plant weight, and leaf number were significantly changed when plants were exposed to the salt stress conditions, similar results were observed in the present study. For shoots, in the first week, there was a reduction in length by 12%, 25%, 28%, and 33% respectively under 50 mM, 100 mM, 150 mM, and 200 mM salt treatment compared to the control plants as observed by Ahmed et al.^[25]. These results are same as Lettuce crop which showed an overall decrease in biomass of root and shoot. Twenty % reduction in Prosopis juliflora biomass has been observed by Saini et al. ^[26] when given a fluoride dose of 100mg/kg of soil. Fluoride stress may initiate irreversible biochemical changes in plant which might be a cause of reduction of overall growth of plant. Studying and reporting these changes can be a point of concern for future researchers to mitigate the fluoride toxicity in plants.

CONCLUSIONS

This study concludes that NaF has a negative impact on lettuce biomass, yield characteristics, and morphological growth parameters. At 39 DAT and 59 DAT, responses to sodium fluoride resulted in extreme reductions in growth parameters. Lettuce productivity was reduced in the treated plants. Because NaF loading harms soils in Pakistan, removing high sodium fluoride levels from soils is an important consideration in irrigated lands. Future researchers may focus their efforts on alleviating NaF stress by better understanding the biochemical changes that occur in the plant during stressful conditions.

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