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Effect of Sodium Fluoride on Seed Germination, Seedling Growth and Biochemistry of *Abelmoschus esculentus*

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Abstract

Fluoride contamination in water, soil and plants is a serious health problem throughout the world. We studied the effect of aqueous solutions of 0, 10, 20, 30, 40 and 50 ppm sodium fluoride on seed germination, seedling growth and biochemical parameters of *Abelmoschus esculentus*. The experiment was conducted as factorial with completely randomized design with five replicates. The percentage of seed germination decreased up to 62 percent with increasing sodium fluoride treatment. The seedling growth parameters (root length, shoot length and vigor index) and biochemical parameters i.e., chlorophyll, nitrogen and protein content as well as the Fluoride uptake by the germinated seedlings were estimated after 20 days of treatment.

Keywords: Vigor index; Chlorophyll; Nitrogen and protein; Fluoride; Translocation factor

Introduction

If fluoride contaminated ground water is used for irrigation it's adversely affects crop growth especially in beginning of seedling growth [1,2]. Fluoride affects the enzymatic activity and growth by slow the rate of cellular division and expansion [3]. This paper reports results of laboratory investigation to study the effect of F on the germination of the lady finger seeds. The seedling growth parameters (root length, shoot length, root weight, shoot weight, germination percentage and vigor index) and biochemical parameters i.e., chlorophyll, nitrogen and protein content as well as the Fluoride uptake. Response of fluoride depends upon some factors such as dose, duration of exposure, age and genotypes of plants [4].

Materials and Methods

Abelmoschus esculentus (Lady Finger) var. SOH-198 seeds soaked in distilled water for 24 hr. The seedlings were then transferred to Petri dishes containing filter paper, moistened from below with sterilized cotton pads and treated with 0, 10, 20, 30, 40 and 50 mg/l NaF prepared from a stock solution. After 20 days the experiments were terminated, the seedlings were studied for percent seed germination, and the shoot and root length and their dry weight were determined. Biochemical parameters i.e., chlorophyll, nitrogen and protein content as well as the Fluoride uptake were also analyzed. Fluoride was analyzed by Selective Ion Meter. Five seedlings from each petridishes were taken and weighed to get the fresh weight.

Data analysis

Germination percentage:

$$G.P = \frac{\text{Number of normally germinated seeds}}{\text{Total number of seeds}} \times 100$$

Vigor index: Vigor index was calculated at each NaF concentration as per the equation by following equations 2,3.

Vigor Index=(Root length+Shoot length) × Germination Percentage (1)

Biochemical parameters: Following formula will be used to calculate the amount of chlorophyll a and b:

Chlorophyll a mg/l=12.7 × A663-2.69 × A645

Chlorophyll b mg/l=22.9 × A6454.68 × A663 Chlorophyll (a+b) mg/l=8.02 × A663+20.20 × A645 (2)

The amount of nitrogen will be calculated as follows:

% Nitrogen=(T-B) × N × 1.4/S (3)

Where T=Sample titration in ml.

B=Blank titration in ml.

N=Normality of titrant (0.01 NHCl).

S=Weight of plant material in g.

The protein content will be calculated as follows

%Protein=%nitrogen × 6.25 (4)

Fluoride uptake and translocation factor: The roots and shoots of seedlings were separated and extracts were prepared in 0.1 M per chloric acid. Water extractable F was measured by ion selective electrode method from all test seedlings. The translocation factor (TF) of F in plants were calculated by this equation

$TF=(C_{SHOOT}/C_{ROOT})$

Where C_{SHOOT}=concentration of fluoride in plant's shoot (mg/kg) and C_{ROOT}=concentration of fluoride in plant's root (mg/kg).

Results and Discussion

The observed effects of different doses of F on seed germination, shoot length, root length, shoot weight, root weight and vigor index on *Abelmoschus esculentus* (var. SOH -198) are given in Tables 1-6.

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Level	Root length	%reduction	Shoot length	%reduction
Control	3.182 ± 0.190	-	3.136 ± 0.171	-
10 PPM	2.951 ± 0.101	7.3	2.674 ± 0.050	14.74
20 PPM	2.734 ± 0.017	14.08	2.342 ± 0.102	25.32
30 PPM	2.444 ± 0.182	23.2	2.274 ± 0.133	27.49
40 PPM	2.328 ± 0.114	26.84	2.118 ± 0.082	32.47
50 PPM	1.976 ± 0.106	37.91	1.741 ± 0.091	44.42

Table 1: Root and shoot lengths (cm) and percent reduction of *Abelmoschus esculentus* seedlings (mean ± SE).

Level	Root weight	%reduction	Shoot weight	%reduction
Control (D.W.)	12.9 ± 0.13	-	31.26 ± 0.09	-
10 PPM	9.178 ± 0.10	29	24.35 ± 0.08	22.11
20 PPM	8.022 ± 0.09	37.82	20.168 ± 0.09	35.51
30 PPM	7.092 ± 0.09	45.03	16.32 ± 0.08	47.80
40 PPM	5.212 ± 0.08	59.60	11.256 ± 0.10	64
50 PPM	4.258 ± 0.09	67	8.222 ± 0.07	73.37

Table 2: Root and dry weights (mg) and percent reduction of *Abelmoschus esculentus* seedlings (mean ± SE).

Level	Germination %	Vigor index
Control (D.W.)	100	631.8
10 PPM	90	562.4
20 PPM	78	507.6
30 PPM	63	471.8
40 PPM	42	444.6
50 PPM	38	371.6

Table 3: Germination (%) and vigor index of *Abelmoschus esculentus* seeds.

Level	Chlorophyll a	Chlorophyll b	Chlorophyll (a+b)
Control (D.W.)	6.14 ± 0.131	9.259 ± 0.013	15.337 ± 0.013
10 PPM	5.142 ± 0.015	8.059 ± 0.033	13.257 ± 0.016
20 PPM	4.144 ± 0.016	7.271 ± 0.012	11.328 ± 0.014
30 PPM	3.98 ± 0.012	6.868 ± 0.017	10.867 ± 0.017
40 PPM	3.842 ± 0.016	6.471 ± 0.015	10.49 ± 0.016
50 PPM	3.231 ± 0.014	5.917 ± 0.017	9.895 ± 0.015

Table 4: Chlorophyll content of *Abelmoschus esculentus* seedlings (mean ± SE).

Level	% Nitrogen	% Protein
Control (D.W.)	1.695 ± 0.018	10.597 ± 0.116
10 PPM	1.366 ± 0.017	8.542 ± 0.11
20 PPM	1.265 ± 0.018	7.907 ± 0.11
30 PPM	1.050 ± 0.019	6.564 ± 0.119
40 PPM	0.983 ± 0.013	6.369 ± 0.485
50 PPM	0.868 ± 0.02	5.427 ± 0.129

Table 5: Percent nitrogen and protein content of *Abelmoschus esculentus* seedlings (mean ± SE).

Level	Root (Fluoride)	Shoot (Fluoride)	Translocation Factor
Control (D.W.)	ND	ND	-
10 PPM	6.874 ± 0.16	3.78 ± 0.13	0.549
20 PPM	8.022 ± 0.09	4.754 ± 0.16	0.592
30 PPM	8.96 ± 0.16	6.424 ± 0.16	0.716
40 PPM	10.276 ± 0.16	7.368 ± 0.09	0.717
50 PPM	12.068 ± 0.15	9.258 ± 0.12	0.767

Table 6: Concentration of fluoride in roots and shoot (mg/kg) of *Abelmoschus esculentus* seedlings (mean ± SE).

Fluoride uptake

Fluorine is not considered as an essential element for plants. Previous studies describe that most cases of acute fluoride intoxication have resulted from the ingestion of large doses of fluoride compounds. The severity of the symptoms depends upon the irritating properties and to the amount of the compound that has been ingested [5]. The uptake in the *Abelmoschus esculentus* increased many fold with increasing NaF concentrations. In root it ranges from 6.874-12.068 mg/kg and shoot part it ranges from 3.78 to 9.258. Translocation Factor (TF) i.e., the ratio of F concentration in plant's shoot parts to that of plant's root parts is quite high (0.549-0.767) indicating that the shoot tissues have less capabilities to hold F. In seedlings of *Phaseolus* *vulgaris* (minus cotyledons) have determined the fluoride taken up at three stages of growth in different strengths of fluoride [6]. There was little difference in uptake by the plants between light and dark growing conditions. Similar study has been studied in *Acacia georqinae* to determine the concentration of synthesizes fluoroacetate [7]. In 1 to 4 days it reached 8 ppm in the aerial parts and 16 ppm in the roots. Total fluoride was greater than inorganic fluoride. More "organic" fluoride was present generally in the roots than in the aerial parts. With higher concentration of fluoride, 10.5 to 75 mM (200 to 300 ppm), much larger amounts of fluoride were taken up, especially by the roots, and much more apparent organic fluoride was formed.

Solutions of sodium fluoride were found to cause inhibition of seed germination at lower concentrations. Uptake of fluoride, as well as growth and yield of plants vary with both plant species and plant varieties within a species. Exposure of seeds to solutions of sodium fluoride and hydrogen fluoride during germination was used as a guide to establish the nature of the inhibiting effects of the inorganic fluoride oxidizing agents. In contrast, even very low concentrations of ClF_3 or BrF_5 in air are very damaging. The damage appears to result from a rapid reaction between the interhalogens and the plant or seed surfaces. Fresh weight of seedlings decreased monotonically with increasing fluoride concentration due to reduction of metabolic activity in presence of fluoride, because germination is a one kind of metabolism and fluoride acts as a metabolic inhibitor [2,8,9]. Table 3 shows that vigor index was decreased (58.81%) with higher concentration of fluoride treatment over control. Similarly reduction in vigor index has also been reported in *Triticum aestivum* [1].

As shown in the Table 4 chlorophyll -a, chlorophyll-b, total chlorophyll content of leaves decreased monotonically at 50 ppm, concentration of NaF/L both chlorophyll a, and chlorophyll b decreased as 52.62%and 63.90% whereas reduction in total chlorophyll content was 64.51% this may be due to the breakdown of chlorophyll during stress or due to inhibition of chlorophyll biosynthesis which is a primary symptom of fluoride induced chlorosis. While, in some studies inhibitory effect of fluoride on chlorophyll accumulation was noticed in cereals [9] sunflower [10], *Triticum aestivum* [1], *Pisum sativum* [2] and *Oryza sativa* [8], while in case of wheat there is increase in chlorophyll content due to initial treatments of sodium fluoride and then decreases the chlorophyll content as fluoride treatments goes on increasing [11-15]. Thus, the effect of fluoride on chlorophyll content is obscure.

Conclusion

In the present study NaF concentrations disturbs the seed germination and early growth of seedlings further it was also reported that germination percentage and vigor index also decreased by NaF from 100% to 38%, in addition to morphological features such as root, shoot length and weight and some biochemical features such as photosynthetic pigments such as chlorophyll a and b, %nitrogen and protein content were also adversely affected by increased NaF concentration. The high root-to-shoot translocation factor of Fluoride in the seedlings indicates possible accumulation of Fluoride in the edible plant parts such as fruit. Fluoride present as a natural earthy material, its accumulation affects plants growth and therefore fluoride may also be considered as an additional stress.

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