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Evaluating the Influence of Fluoride Intoxication on *Amaranthus dubius*

B. SRI SUYAMBULINGA PERUMAL*[1], N. REXIN ALPHONSE[2], P. SRI RENGANATHAN[3] and D. VENKATESH[4]

[1, 3] P. G. and Research Department of Chemistry, Rani Anna Government College for Women (Affiliated to Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli - 627 012, Tamil Nadu), Tirunelveli - 627 008, Tamil Nadu, India
[2] Department of Chemistry, Sarah Tucker College (Autonomous), Tirunelveli - 627 007, Tamil Nadu, India
[4] Department of Chemistry, Vel Tech High Tech Dr. Rangarajan and Dr. Sakunthala Engineering College, Avadi, Chennai - 600 062, Tamil Nadu, India

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Abstract

The toxic effect of fluoride in plants has been investigated to the various morphological parameters on *Amaranthus dubius.* The various concentrations of sodium fluoride taken such as 1, 2, 5, 10, 25 and 50 ppm were treated along with control sample. The morphological parameters such as root and stem lengths, height, seed germination, fresh weight, number of leaves and fluoride uptake were examined for 55 days of sodium fluoride treatment. The percentage of seed yield and number of leaves has been reduced to 50% with increasing concentrations of sodium fluoride. 33% of height and 15% of biomass yield have decreased with increase the concentration of NaF. The adverse effects can be caused by the transportation of fluoride from soil to plant roots and further shifted stem to organs of plant. At 50 ppm of NaF, the uptake of fluoride increased and height, number of leaves of Amaranthus Dubius noticeably decreased.

***Key words*:** NaF, Toxicity, *Amaranthus dubius*, Soil contamination, Fluoride uptake, Translocation factor

In recent times, globalization and industrialization causes major threat to the soil and ground water. Soil could be ruined by various contaminates such as heavy metals, chemicals, pesticides, insecticides, organic pollutants, fluorides and so on. Among these pollutants, fluoride causes toxic effects on environment, plants, animals and human. Ground water containing dissolved fluorides in high concentrations displayed serious damages in the environment and also stress effects on some physiological and biochemical confines to the plants. Fluoride concentration in the drinking water is between 0.9 and 1.2 mgL^{-1} has a detrimental effect on tooth enamel and may cause mild dental fluorosis [1]. The unique chemical and biological properties of fluoride are due to its size and reactivity [2]. The major natural source of inorganic fluorides in the soil is parent rock [3]. Fluoride released naturally through the weathering of minerals, volcano emissions and marine aerosols [4]. Some fluoride mineral such as cryolite is readily broken-down during weathering under acidic conditions [5]. Minerals such as calcium fluoride and fluorapatite are dissolved slowly [6]. Most of the fluorides are insoluble in soil and less available to plants. Clay soil has higher fluoride content than sandy soils [7]. Fluoride retention is greater at pH 5.5 and decreased at both lower and higher pH levels [8]. At high pH an unfavourable electrostatic potential decreases retention of fluoride on the soil and increases fluoride concentration in soil solution. The increase of fluoride uptake and accumulation in the plants and vegetables cause serious health risks to human. Recent industrial expansion recognized the series toxicity to vegetation [9]. These findings are similar to observation of other researchers in *Lycopersicum esculentum* [10], *Brassica rapa* [11-12] and *Cicer arieninum* [13]. The gradual reduction of height with increasing the fluoride ion concentration [14]. Similar result was demonstrated for wheat (*Triticum aestivum*), Bengal gram (*Cicer arietinum* L), mustard (*Brassica juncea*) and tomato (*Lycopersicon esculentum*) [15]. The height growth decreased with increasing accumulation of NaF for *Prosopisjuliflora* [16]. The reduction in growth of height of *Triticum aestivum* due to this high concentration of fluoride [17].

There are numerous studies has been performed at the laboratory scale regarding the impact of fluoride on ground water, soil and biochemical changes of different plants. The impact of sodium fluoride toxicity in wheat and gram has been recorded to reduce the growth and yield. 100-200 ppm NaF produces very harm to the plants [18]. 100-250 ppm of NaF doses made to diminish the growth and yield of the Urd bean and Mung bean. The chlorophyll content has reduced by the accumulation of NaF on Urd bean var. T9 for 60 days and Mung bean var. P.S.-16 for two years [19]. The fluoride concentration has been accumulated in the shoot of *Atractylis serratuloides* with the quantity of 252 mg/L through photo extraction [20]. The root and shoot growth has been diminished by uptake of fluoride content in *Abelmoschus esculentus* and also causes the reduction of chlorophyll a and b, nitrogen uptake and protein content in plants [21-22] and yield loss of soybean reached 30% at F levels 375 mg kg^{-1} or greater. Singh *et al* [23] studied that increasing F above 50 mg L^{-1} decreased the yield of rice. Zuo *et al.* [24] observed the tendency of fluorides to get accumulate in

***Correspondence to:** B. Sri Suyambulinga Perumal, E-mail: bsslperumal@gmail.com; Tel: +91 9445886845

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the leafy portions of plants which badly affected the pattern of growth and crop productivity. Saroj *et al* [25] reported the biomass reduction in *Vigna aconitifolia* L due to F saline stress. Sabal *et al.* [26] also reported similar decline in biomass of plants. The present study was undertaken to examine the impacts of NaF on root and stem growth and other morphological parameters of *Amaranthus dubius* in a soil bed condition.

MATERIALS AND METHODS

The experiment was carried out in natural climates and soil bed condition with cultivars of Red Lettuce seeds of *Amaranthus dubius*. Certified seeds were collected from Agriculture Department, Government of Tamil Nadu, Tirunelveli. A stock solution of 1000 mg/L was prepared by dissolving NaF crystals in distilled water.

Seven plastic pots were chosen for this study with the diameter of 8 inches. Each pot had filled with 4000 gm of fertile soil and 500 gm of cow dung mixed together, kept it for three days. Certified *Amaranthus dubius* seeds were moistened for 8 hrs and 20 seeds were sowing with adequate space in the soil of each pot. 50ml of distilled water and 50ml of various concentration of sodium fluoride solution such as 1, 2, 5, 10, 25, 50 ppm were used to watering to the plants at the morning for control and treated sample for 55 days respectively. Germination was completed in 15 days. Every 5 days once two saplings were uprooted carefully from these pots and record the fluoride uptake, height, fresh weight and number of leaves of control and treated samples. After 55 days the experiment was terminated and yield of seeds were recorded from harvested plants.

Data analysis

Fluoride uptake and translocation factor

The roots and stems of plants were separated and dissolved separately in 0.1 M perchloric acid. Water extractable fluoride from roots and stems of plants were determined by ion selective electrode. The translocation factor (TF) of F in these plants were calculated by the following equation [21].

$$TF = (C_{Stem} / C_{Root})$$

Where;

C_{Stem} = concentration of fluoride in plant's stem (mg/kg) and

C_{Root} = concentration of fluoride in plant's root (mg/kg)

Fig 1 The morphological parameters studied using the various concentration of sodium fluoride such as control, 5ppm, 10 ppm, 25ppm and 50 ppm on *Amaranthus dubius* in pot experiments

Height of plants

Height of the plants has measured with a ruler 5 days once from day 15.

Number of leaves in plants

Number of leaves in plants has counted one by one by naked eyes for 5 days once from day 15.

Fresh weight of plants

The root parts of the uprooted saplings from pots have washed in distilled water dried in air and weighed using a Shimadzu AU series unibloc semi-micro / analytical electronic balance.

Seed yield of plants

The seeds were separated from spikelet and counted in a flat plate using some forceps slowly and carefully.

RESULTS AND DISCUSSION

The effects of fluoride toxicity studied for control and different concentrations of sodium fluoride such as 1, 2, 5, 10, 25 and 50 ppm treated on height, fresh weight, number of leaves, yield of seeds and fluoride uptake of *Amaranthus dubius*. The pot experiments of fluoride toxicity in *Amaranthus dubius* can be depicted in (Fig 1). Maximum results were recorded in control and the minimum results were recorded in higher concentration of NaF (50ppm) treated in all characters except fluoride uptake.

The maximum height (cm), number of leaves per plant and fresh weight (g)/plant were found maximum in control plants while minimum results were found in 50 ppm concentrated NaF treated. The maximum seed yield was also found in control and minimum seed yield was found in 50 ppm NaF treated.

Fluoride uptake and translocation factor

The fluoride compounds are not very essential to the plants. The results of previous studies on fluoride intoxication in plants observed for consumption of high concentration of fluoride compounds. The fluoride uptake in the *Amaranthus dubius* has been increased with increasing NaF concentrations of 1-50 ppm as depicted in (Fig 2). The results of fluoride uptake of root part observed the ranges from 4.141, 4.918, 5.516, 6.476, 8.825 and 11.206 mg/kg and stem part from 2.031, 2.482, 3.344, 4.139, 6.124, and 8.568 mg/kg at different concentration of 1, 2, 5, 10, 25 and 50 ppm sodium fluoride solutions. As presented in (Table 1), the translocation factor is ranged from 0.4904 to 0.7645 for *Amaranthus dubius* plant. The results revealed that the fluoride uptake in root part is higher than the stem part at 50 ppm of sodium fluoride solutions. The stem part is minimum ability to hold the fluoride ion rather than the root part at different strengths of NaF [26-27].

Table 1 Effect of different concentration of sodium fluoride in root and stem (mg/Kg) of *Amaranthus dubius* plants

Analyzing data	Control*	1 ppm	2 ppm	5 ppm	10 ppm	25 ppm	50 ppm
Root (F mg/Kg)	NA	4.141	4.918	5.516	6.476	8.826	11.206
Stem (F mg/Kg)	NA	2.031	2.482	3.344	4.139	6.124	8.568
Translocation factor	-	0.4904	0.5046	0.6062	0.6391	0.6915	0.7645
Number of seeds	231	211	202	183	159	137	116

*-NA – Not applicable

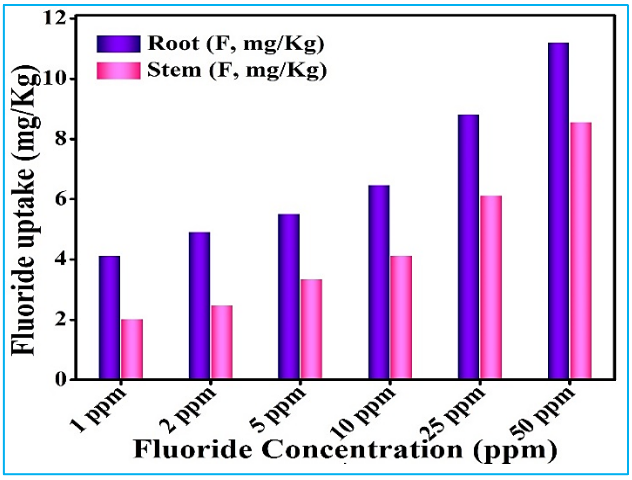

Fig 2 Fluoride uptake analysis of *Amaranthus dubius* roots and stem by using various concentration of sodium fluoride such as 1ppm, 2ppm, 5ppm, 10ppm, 25ppm and 50 ppm

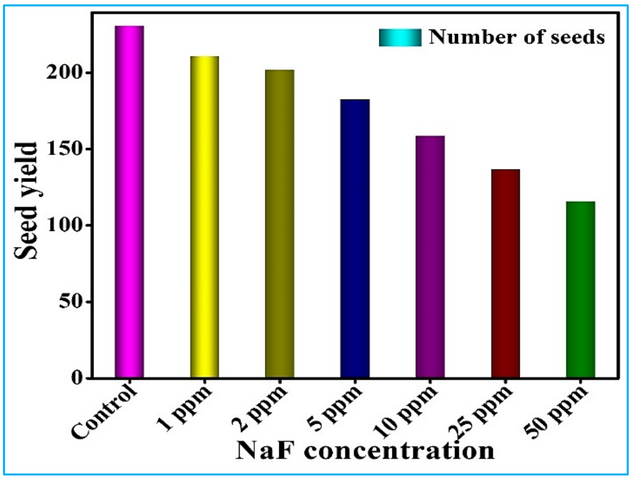

Fig 3 Variation of seed yield analysis of *Amaranthus dubius* by using various concentration of sodium fluoride such as 1ppm, 2ppm, 5ppm, 10 ppm, 25ppm and 50 ppm.

Table 2 The results of variation in height of *Amaranthus dubius* plant at various concentrations of NaF for 55 days

NaF concentration	Number of days								
	15	20	25	30	35	40	45	50	55
Control	7.5	14.0	16.5	19.0	21.5	23.5	26.25	28.25	30.2
1 ppm	7.5	13.5	16.1	19.5	21.0	23.0	26.0	28.0	30.0
2 ppm	7.25	13.1	15.5	18.9	20.5	22.4	25.5	27.25	29.5
5 ppm	7.0	12.2	14.8	18.1	19.5	21.1	23.5	25.8	28.0
10 ppm	6.5	11.1	13.4	17.0	18.0	19.2	20.8	22.5	25.5
25 ppm	6.0	9.9	12.1	15.8	16.0	17.3	18.5	20.2	22.4
50 ppm	5.5	8.7	10.8	14.3	13.5	15.4	17.0	18.5	20.2

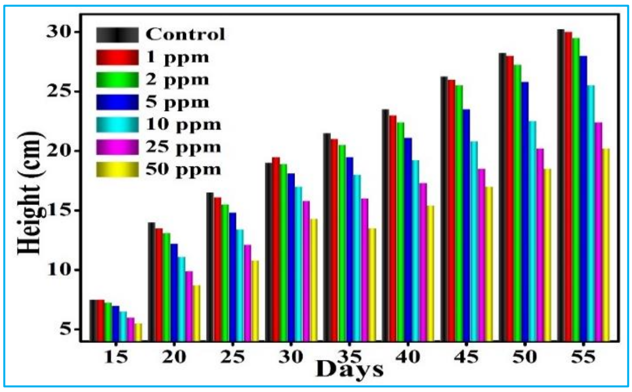

Fig 4 Variation of height analysis of *Amaranthus dubius* by using various concentration of sodium fluoride such as 1ppm, 2ppm, 5ppm, 10 ppm, 25ppm and 50 ppm

Seed yield

The seed yield of *Amaranthus dubius* plants decreased as the increased concentration of sodium fluoride as presented in (Fig 3). The control sample gave the seed yield of 231 and 116 observed for 50 ppm of NaF solution treated after 55 days as presented in the (Table 1). The reduction of seed yield percentage observed as 9%, 13%, 21%, 31%, 41% and 50% for 1, 2, 5, 10, 25 and 50 ppm sodium fluoride treatment over control respectively. This could be observed due to number of ears/plant and less number of spikelet/plant [28-31].

Height of the plants

The growth analysis of plants was measured using control and different concentrations of sodium fluoride treated for 55 days as presented in (Fig 4). The results were obtained as 30.2, 30, 29.5, 28, 25.5, 22.4, and 20.2 (cm) for plants. Fluoride toxicity causes reduction in height of the spinach due to unbalanced nutrient uptake by seedlings [26]. The reduction in height was due to decrease in size as well as number of the cells [29]. The data depicted in (Table 2) shown that the height reduced percentage 0.7%, 2%, 7%, 15%, 29% and 33% to the 1, 2, 5, 10, 25 and 50 ppm sodium fluoride treatment over control respectively.

Number of leaves

The reduction in number of leaves was observed at higher concentration of 50 ppm sodium fluoride treated. The obtained results are 20, 18, 16, 14, 12, 11 and 10 for the concentration of control, 1, 2, 5, 10, 25 and 50 ppm NaF for 55 days as depicted in (Fig 5). The number of leaves reduced arising from the effect on cell division resulting differences in cell number and on cell extension present in the plant [32]. The reduction of number of leaves percentage was observed 10%, 20%, 30%, 40%, 45% and 50% for 1, 2, 5, 10, 25 and 50 ppm sodium fluoride treatment over control as presented in (Table 3). Number of leaf of plants harshly reduced with fluoride treatment was observed [33]. Mehta *et al*. [35] reported decline in number of leaves due to high fluoride accumulation. Gama *et al*. [34] also registered that increased F concentration was responsible for declining of number of leaves. Elloumi *et al*. [36] also provided the supporting fact about reduction of leaf with increasing fluoride concentration.

Table 3 The results of variation of number of leaves of *Amaranthus dubius* plant at various concentrations of NaF for 55 days

NaF concentration	Number of days								
	15	20	25	30	35	40	45	50	55
Control	5	7	9	11	13	15	17	19	20
1 ppm	5	7	8	10	12	14	15	16	18
2 ppm	5	7	8	9	11	13	14	15	16
5 ppm	5	7	8	8	9	11	12	13	14
10 ppm	5	6	8	8	9	9	11	11	12
25 ppm	5	6	7	8	9	9	10	10	11
50 ppm	5	6	7	7	8	8	9	9	10

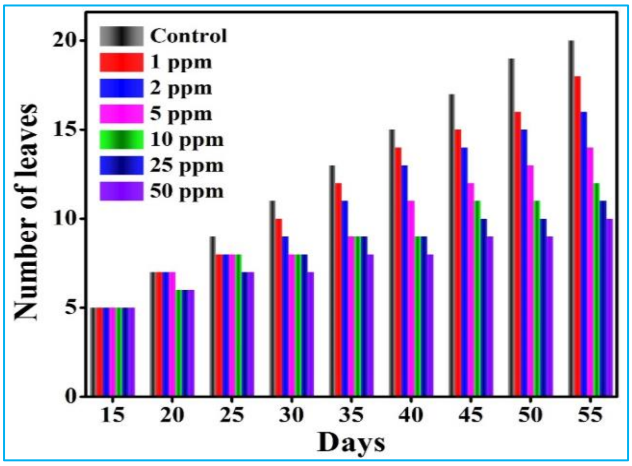

Fig 5 The variation of number of leaves analysis of *Amaranthus dubiucs* by using control and various concentration of sodium fluoride such as 1ppm, 2ppm, 5ppm, 10ppm, 25ppm and 50 ppm at different days

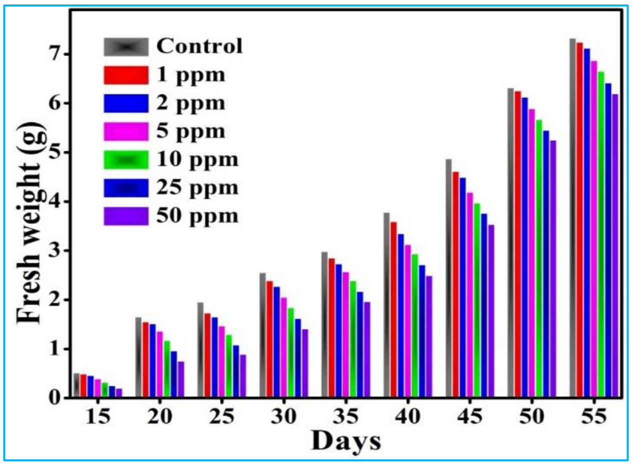

Fig 6 The variation of fresh weight analysis of *Amaranthus dubiucs* by using control and various concentration of sodium fluoride such as 1ppm, 2ppm, 5ppm, 10ppm, 25ppm and 50 ppm at different days

Table 4 The results of variation of fresh weight of *Amaranthus dubius* plant at various concentrations of NaF for 55 days

NaF concentration	Number of days								
	15	20	25	30	35	40	45	50	55
Control	0.51	1.65	1.95	2.55	2.98	3.78	4.87	6.31	7.32
1ppm	0.49	1.55	1.73	2.39	2.85	3.59	4.61	6.25	7.24
2ppm	0.46	1.51	1.65	2.27	2.73	3.34	4.49	6.12	7.12
5ppm	0.39	1.36	1.47	2.05	2.57	3.12	4.19	5.89	6.87
10ppm	0.32	1.17	1.29	1.84	2.39	2.93	3.97	5.67	6.65
25ppm	0.25	0.96	1.08	1.62	2.17	2.71	3.76	5.45	6.41
50ppm	0.20	0.75	0.89	1.41	1.96	2.49	3.53	5.24	6.19

Biomass yield

The biomass yield was critically reduced as the concentration of sodium fluoride increased. The inclination of fluoride ion collects at the leafy portions of plants, which reduces the growth of the plant and crop productivity. This study reveals the significant reduction in the biomass yield as presented in (Table 4). The reduced percentage was found as 1%, 3%, 6%, 9%, 12% and 15% for 1, 2, 5, 10, 25 and 50 ppm of sodium fluoride treatment over control respectively. The results of biomass yield indicate the plants growth and crop increments. The fresh and dry weight of the roots and shoots gradually decreased over a period of 55 days as displayed in

(Fig 6). The quantity of uptake fluoride ions increases gradually reduce the yield of plants [37]. The metabolic activity of plants can be reduced by the absorption of fluoride ion over a different concentration due to fluoride acts as metabolic inhibitor [26], [38-39].

CONCLUSION

From the afore mentioned investigation it could be concluded that the toxic effect of fluoride ion in plants revealed that an additional stress and affects the morphological parameters. The root and stem growth significantly reduced as the concentration of fluoride ion increased. The percentage of height, number of leaves, biomass, and seed yield diminished due to observed fluoride ion which accumulates on the root and stem of *Amaranthus dubius*. The fluoride ion leached into the leaves and reduced the number of cells of the plant. The seed germination of plants can significantly reduce the growth and crop development due to metabolic inhibition of fluoride ion. Therefore, further development can able to produce tolerant genotypes for ecological crop yield improvement partly supports to farmers.

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