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Studies on the Assessment of the Consequences of Water-soluble Fluoride Poisoning on Chlorophyll Pigments of *Amaranthus dubius*

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Abstract

A growing amount of fluoride is making its way into the human food and drink chain when people consume tea, wheat, spinach, cabbage, carrots and other Indian items. Therefore, while calculating total fluoride intake, food fluoride concentration should not be disregarded. The concentration of fluoride in irrigation water and soil affects the amount of fluoride in food. Therefore, in the current study, the assessment of the consequences of water-soluble fluoride poisoning on chlorophyll pigments from leaves, stems, roots and seeds were studied using various concentrations of sodium fluoride in the water used to irrigate the plant *Amaranthus dubius.* The results showed that *Amaranthus dubius* which receives only water (control) had higher amount of chlorophyll a (1.71µg/g) in its leaves and low amount of chlorophyll a (0.22 µg/g) in the stems of *Amaranthus dubius* watered with 50ppm of sodium fluoride on the 55th day of growth. The amount of chlorophyll a decrease when concentration of sodium fluoride increases. The amount of chlorophyll a (µg/g) in leaves of all experimentally challenged *Amaranthus dubius* varied from 0.22µg/g - 1.71µg/g (minimum in stems to maximum in leaves) when estimated from 15 to 55 days.

***Key words*:** Fluoride, *Amaranthus dubius*, chlorophyll a, chlorophyll b

It is well known that fluoride (F^-) is a common, extremely reactive and non-biodegradable environmental contaminant. Over the past twenty years, an ecosystem's F^- level has increased due to a variety of anthropogenic and natural processes [1]. Some of the main natural resources of F^- found in water and soil are scheelite, fluorite or fluorspar, cryolite, fluorapatite, apatite, topaz, fluormica, biotite, epidote, tremolite and hornblende. Rock weathering and volcanic eruptions are examples of atmospheric emissions that release F^- into the atmosphere naturally. Anthropogenic activities centered on agriculture and industry are on the rise these days, contributing to the addition of F^- either directly through the use of phosphate fertilizers, pesticides and fluorinated water irrigation or indirectly through air emissions from burning coal, refining oil, manufacturing bricks, producing aluminum and other industries [2-3]. F^- in low quantities helps to prevent tooth cavities and promotes the mineralization of hard tissues. However, dental, skeletal and non-skeletal fluorosis (exhibiting symptoms like gastrointestinal and urinary issues, infertility and neurological and brain damage) is brought on by excessive exposure levels, ingestion and buildup of F^- in both humans and animals [4-5]. Permissible limits for F^- in drinking water have been suggested by the World Health Organization (WHO) and Indian Standards (IS) respectively, as 1.5 and 1.0 mg L^{-1}. Extended exposure to fluoride (F^-) results in many harmful consequences for plants animals [6-7] and humans [8-9] as well as other species [10]. The primary way that F^- contaminated water, soil, gasses and dust affect plant physiology is via changing leaf physiology. The severity of the indications of F^- injury to plants, which can be either acute or chronic, depends on the concentration of F^- as well as the length and frequency of F^- exposure [6]. Plants become harmful to fluorine when fluorine slowly seeps into their subcellular components, altering the fluorine-sensitive metabolic processes. Extended exposure to F^- is linked to observable damage to the folia. Additionally, fluoride buildup hindered photosynthesis. F^- mostly impacts photosynthesis by inhibiting the Hills reaction, degrading chloroplasts and lowering chlorophyll synthesis. Additionally, the plant's photosynthetic system is compromised and the amount of chlorophyll is reduced. In the end, these led to a reduction in the production and absorption of CO_2 [11-12].

The photosynthetic electron transport pathway in plant thylakoid membranes has been examined following F^- exposure. It was discovered that the accumulation of F^- inhibits the electron transport rate of photosystem-II (PSII), which is then followed by an increase in the electron transport rate of photosystem-I (PS-I). This finding suggested that F^- toxicity is caused by state changes. Ballantyne's study stated that plants treated with F^- at 190 ppm have less pigments involved in photosynthetic processes. Additionally, it was discovered in

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Reddy and Kaur's investigation [13]. The photosynthetic capacity, concentrations of chlorophyll-a (Chl-a) and chlorophyll-b (Chl-b), total chlorophyll, carotenoids and leaf area of plants grown on contaminated soil with F^- are all reduced [14-15]. The decrease in chlorophyll contents in the plants may be caused by F^- reducing the chlorophyll biosynthesis. It is likely that the amount and activity of the enzyme chlorophyllase, which degrades chlorophyll, increases after F^- accumulation [16]. In the semi-arid region, plants grown on contaminated soil demonstrated the same effects [17]. In addition to being a staple aliment for people all over the world, spinach is used as a model monocot species in molecular biology studies. Groundwater irrigation is primarily used for all spinach production operations, including germination, seedling, growing, transplanting, and other associated tasks in the main field.

Numerous research works on F^- uptake and its impact on various plant species have been published. On the other hand, little is known about how F^- poisoning affects chlorophyll pigments found in the leaves, stems, roots and seeds of plants that are widely grown by Indian farmers in this area. Consequently, the aim of this investigation was to comprehend and evaluate the effects of fluoride poisoning in water on F^- chromolybdate pigments in *Amaranthus dubius*, a significant crop. The study's conclusions are significant and practical for researchers, farmers and agricultural specialists.

MATERIALS AND METHODS

The experiment was conducted using Red Lettuce cultivars from *A. dubius* seeds in natural weather and soil bed conditions. Certified seeds were gathered from the Tamil Nadu government's Agriculture department in Tirunelveli. To prepare a 1000 mg/L stock solution, NaF crystals were dissolved in distilled water. For this investigation, seven plastic pots with an 8-inch diameter were selected. Each pot was filled with 4,000 grams of rich soil and 500 grams of combined cow dung, and it was left for three days. Twenty certified *Amaranthus dubius* seeds were sown in each pot with enough room between them after being wet for eight hours. For the control and treated samples, 50 milliliters of distilled water and 50 milliliters of sodium fluoride solutions at different concentrations:1, 2, 5, 10, 25, and 50 ppm were used to irrigate the plants in the morning. The treatments lasted for 55 days each.

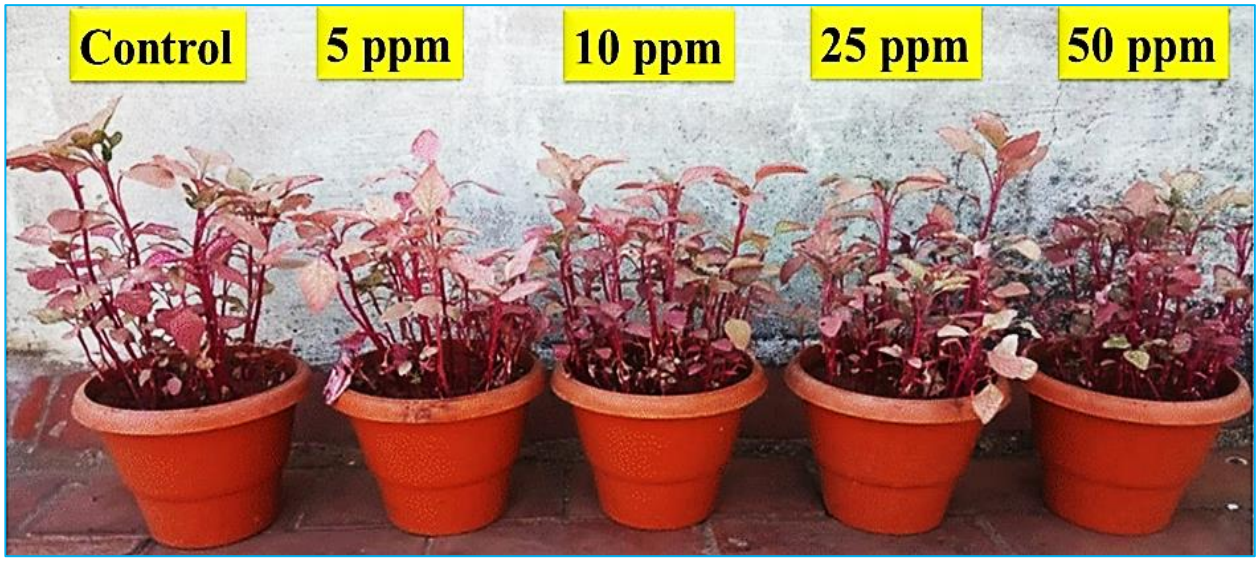

Fig 1 The chlorophyll a & b studied using the various concentration of sodium fluoride such as control, 5ppm, 10 ppm, 25ppm and 50 ppm on *Amaranthus dubius* in pot experiments

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

In fifteen days, germination was finished. Once two saplings were safely removed from these pots every five days, measurements of the fluoride intake, height, fresh weight, and number of leaves on the control and treated samples were taken. The experiment was stopped after 55 days and the amount of seeds collected from the harvested plants was noted.

Data analysis

Fluoride uptake and translocation factor

Plant stems and roots were divided and dissolved in different amounts of 0.1 M perchloric acid. Plant roots and stems were tested for water extractable fluoride using an ion-selective electrode. The following formula was used to determine the translocation factor (TF) of F in these plants [18].

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

Where;

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

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

Analysis of chlorophyll pigments (µg/g)

In the current study, the chlorophyll content of *Amaranthus dubius* cultivated in several experimental setups was extracted using acetone from the leaves, stems, roots and seeds. Using a spectrophotometer, the produced samples were

subjected to a range of wavelength-distributed light sources. The concentrations of chlorophyll a and b were calculated using the Arnon method.

Estimation of chlorophyll a (µg/g) from leaf of A. dubius

On the 55th day of plant growth, the leaves of *Amaranthus dubius*, which were examined as a control, had a maximum chlorophyll a content of 1.71µg/g. Furthermore, the amount of chlorophyll a in *Amaranthus dubius* leaves dropped as sodium fluoride concentrations in the water used to irrigate the study's experimental setups increased. On days 15 and 55 of the plant's growth, *Amaranthus dubius* leaves that were irrigated with 50 ppm sodium fluoride (0.07µg/g and 0.70µg/g) had the lowest levels of chlorophyll a. From day 15 to day 55, the concentration of chlorophyll a changes; as the plant grows, the amount of chlorophyll increases. (Table 2, Fig 2) present the chlorophyll a content of the leaves of *Amaranthus dubius* plants treated with sodium fluoride.

Table 2 Chlorophyll a (µg/g) in the leaves of *Amaranthus dubius* watered with various concentrations of sodium fluoride and harvested on various days

Days	Concentrations of sodium fluoride (ppm)						
	Control	1ppm	2ppm	5ppm	10ppm	25ppm	50ppm
15	0.33	0.30	0.27	0.24	0.21	0.17	0.07
20	0.45	0.43	0.40	0.36	0.27	0.22	0.10
25	0.56	0.54	0.51	0.48	0.33	0.26	0.15
30	0.74	0.71	0.69	0.63	0.41	0.34	0.23
35	0.85	0.82	0.80	0.71	0.48	0.52	0.31
40	1.00	0.95	0.86	0.78	0.56	0.60	0.43
45	1.40	1.32	0.98	0.82	0.74	0.69	0.55
50	1.63	1.58	1.41	1.17	0.85	0.75	0.60
55	1.71	1.63	1.48	1.24	1.06	0.88	0.70

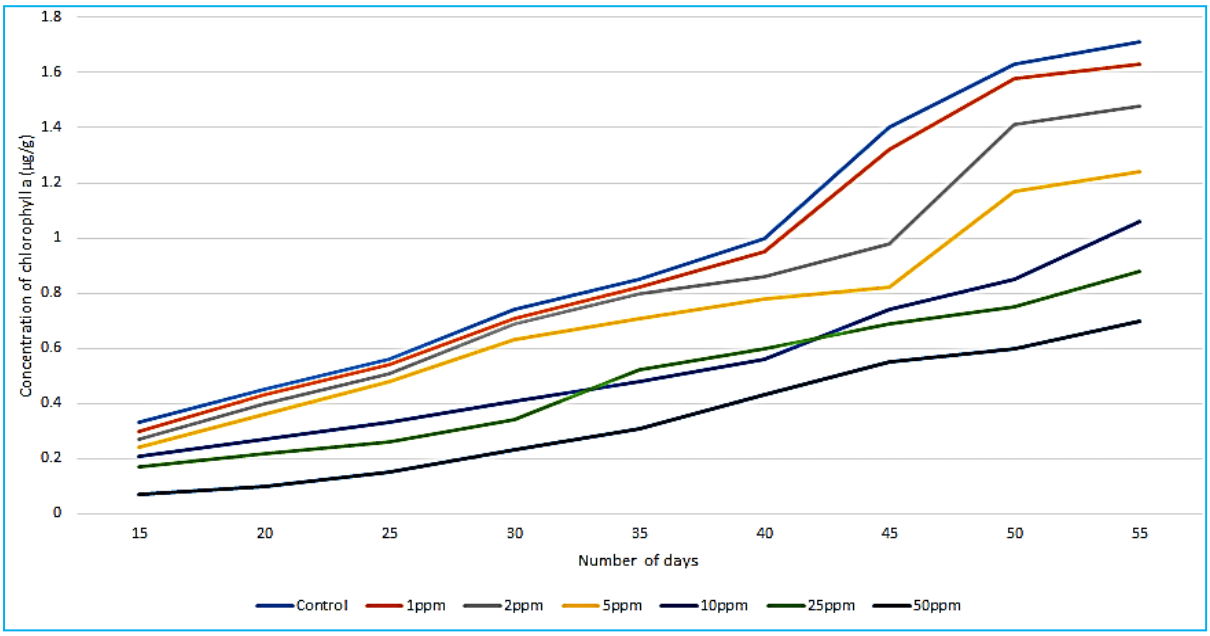

Fig 2 Chlorophyll a (µg/g) in the leaves of *Amaranthus dubius* watered with various concentrations of sodium fluoride and harvested on various days

Table 3 Chlorophyll a (µg/g) in the stems of *Amaranthus dubius* watered with various concentrations of sodium fluoride and harvested on various days

Days	Concentrations of sodium fluoride (ppm)						
	Control	1ppm	2ppm	5ppm	10ppm	25ppm	50ppm
15	0.12	0.10	0.05	-	-	-	-
20	0.18	0.13	0.11	0.03	-	-	-
25	0.26	0.20	0.16	0.09	0.04	-	-
30	0.36	0.25	0.22	0.13	0.10	0.05	-
35	0.50	0.41	0.36	0.32	0.21	0.13	-
40	0.61	0.55	0.42	0.37	0.26	0.20	0.03
45	0.73	0.61	0.58	0.50	0.35	0.27	0.10
50	0.85	0.76	0.72	0.61	0.54	0.31	0.15
55	0.95	0.86	0.79	0.70	0.65	0.38	0.22

Estimation of chlorophyll a (µg/g) from stem of Amaranthus dubius

The maximum chlorophyll concentration in the control *Amaranthus dubius* stem on day 55 of plant growth was 0.95µg/g. Moreover, the amount of chlorophyll an in the stem of *Amaranthus dubius* decreased as the sodium fluoride concentration in the water used to irrigate the experimental setups in the current study increased. The stem of *Amaranthus dubius* with 50 ppm sodium fluoride had the lowest level of chlorophyll a (0.22µg/g) on the 55th day of plant growth. Between days 15 and 55, there is variation in the concentration of chlorophyll a; as the plant develops, the amount of chlorophyll rises.

The results for chlorophyll a in the *Amaranthus dubius* stem treated with varying sodium fluoride concentrations are displayed in (Table 3, Fig 3).

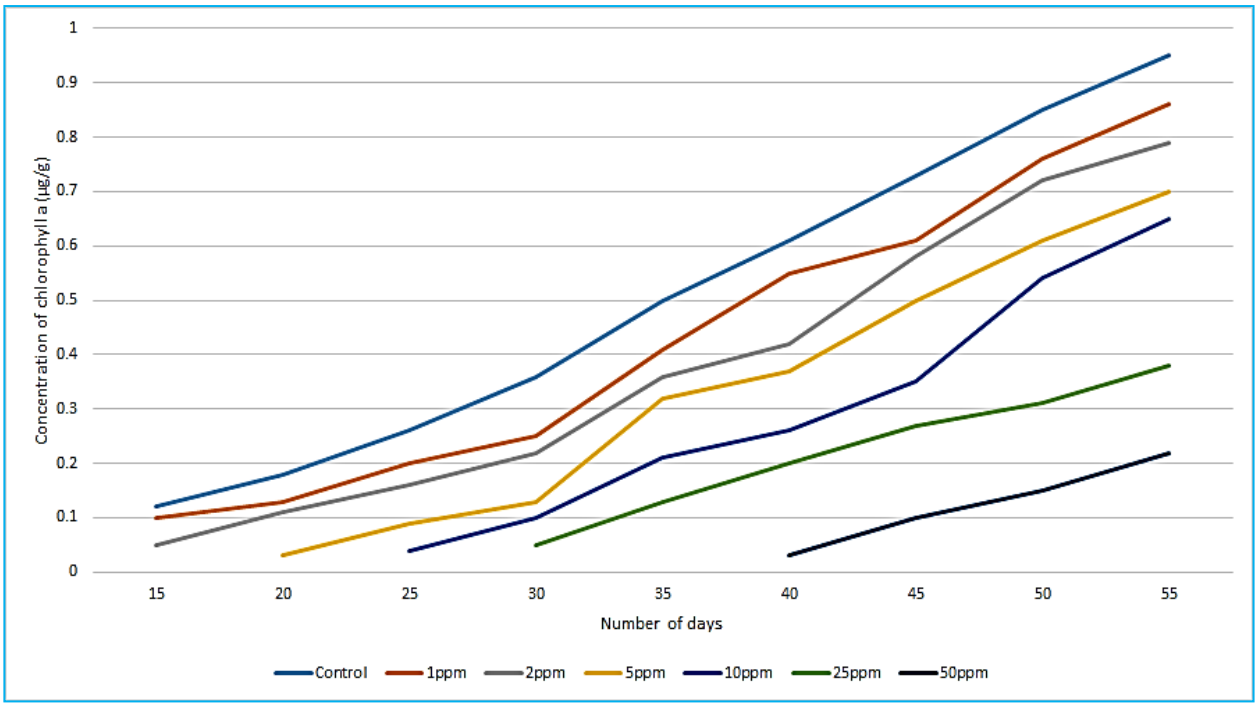

Fig 3 Chlorophyll a (µg/g) in the stems of *Amaranthus dubius* watered with various concentrations of sodium fluoride and harvested on various days

Estimation of chlorophyll a (µg/g) from root of A. dubius

Chlorophyll a levels were determined when *Amaranthus dubius* roots were treated with different amounts of sodium fluoride from day 0 to day 55. The findings showed that the roots had no detectable levels of chlorophyll a.

Estimation of chlorophyll a (µg/g) from seed of A. dubius

Chlorophyll a was examined in the seeds taken from *Amaranthus dubius* plants that were treated with different sodium fluoride concentrations from day 0 to day 55. The findings demonstrated that the seeds contained no evidence of chlorophyll a.

Estimation of chlorophyll b (µg/g) from leaf of A. dubius

As a control, the maximum chlorophyll b concentration of *Amaranthus dubius* leaves on day 55 of plant growth was 1.13µg/g. Moreover, the amount of chlorophyll b in *Amaranthus dubius* leaves decreased as the sodium fluoride concentration in the water used to irrigate the experimental settings in the current study increased. The leaves of *Amaranthus dubius*, irrigated with 50ppm sodium fluoride (0.09 µg/g and 0.62 µg/g), exhibited the lowest amounts of chlorophyll b on the 15th and 55th day of plant growth. Between days 15 and 55, there is variation in the concentration of chlorophyll b; as the plant develops, the amount of chlorophyll b increases. The levels of chlorophyll b in *Amaranthus dubius* leaves treated with sodium fluoride are displayed in (Table 4, Fig 4).

Table 4 Chlorophyll b (µg/g) in the leaves of *Amaranthus dubius* watered with various concentrations of sodium fluoride and harvested on various days

Days	Concentrations of sodium fluoride (ppm)						
	Control	1ppm	2ppm	5ppm	10ppm	25ppm	50ppm
15	0.34	0.29	0.24	0.19	0.15	0.11	0.09
20	0.42	0.36	0.29	0.25	0.22	0.17	0.15
25	0.58	0.46	0.40	0.32	0.27	0.24	0.19
30	0.65	0.61	0.58	0.48	0.36	0.36	0.21
35	0.74	0.70	0.64	0.53	0.48	0.41	0.37
40	0.85	0.82	0.73	0.65	0.53	0.49	0.40
45	0.98	0.90	0.85	0.78	0.64	0.53	0.47
50	1.05	0.99	0.93	0.83	0.75	0.62	0.55
55	1.13	1.08	1.02	0.96	0.84	0.75	0.62

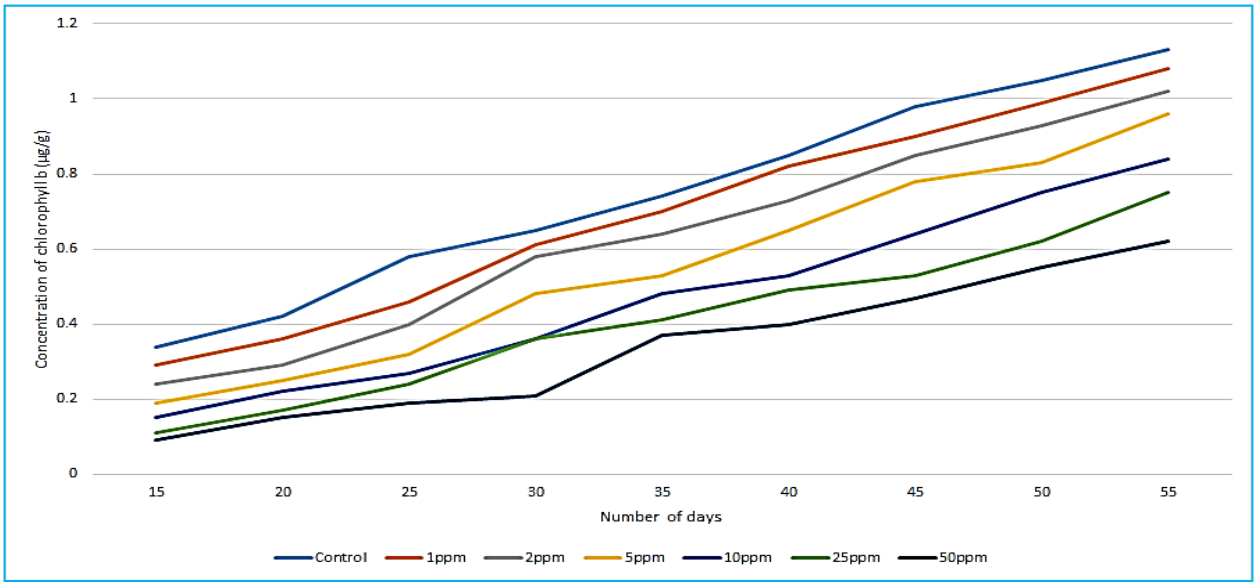

Fig 4 Chlorophyll b (µg/g) in the leaves of *Amaranthus dubius* watered with various concentrations of sodium fluoride and harvested on various days

Estimation of chlorophyll b (µg/g) from stem of A. dubius

The greatest chlorophyll b level that the *Amaranthus dubius* stem displayed in the control group on day 55 of plant growth was 0.70µg/g. Moreover, the amount of chlorophyll b in the stem of *Amaranthus dubius* dropped as the sodium fluoride concentration in the water used to irrigate the experimental settings in the current investigation increased. For a while, the plant is unable to generate chlorophyll b, particularly as the concentration of sodium fluoride rises. The stem of *Amaranthus dubius* with 50 ppm sodium fluoride had the lowest chlorophyll b level (0.28µg/g) on day 55 of plant growth and until day 40, chlorophyll b is not visible. Between days 15 and 55, there are variations in the concentration of chlorophyll b; as the plant develops, its chlorophyll content rises. (Table 5, Fig 5) show the results for chlorophyll b in the leaves of *Amaranthus dubius* treated with sodium fluoride.

Table 5 Chlorophyll b (µg/g) in the stems of *Amaranthus dubius* watered with various concentrations of sodium fluoride and harvested on various days

Days	Concentrations of sodium fluoride (ppm)						
	Control	1ppm	2ppm	5ppm	10ppm	25ppm	50ppm
15	0.14	0.07	-	-	-	-	-
20	0.17	0.12	0.05	-	-	-	-
25	0.24	0.18	0.17	0.08	-	-	-
30	0.30	0.27	0.22	0.19	0.06	-	-
35	0.42	0.35	0.26	0.23	0.18	0.07	-
40	0.52	0.43	0.33	0.29	0.25	0.14	-
45	0.64	0.50	0.39	0.35	0.31	0.25	0.09
50	0.68	0.55	0.46	0.40	0.37	0.30	0.17
55	0.70	0.62	0.58	0.49	0.44	0.36	0.28

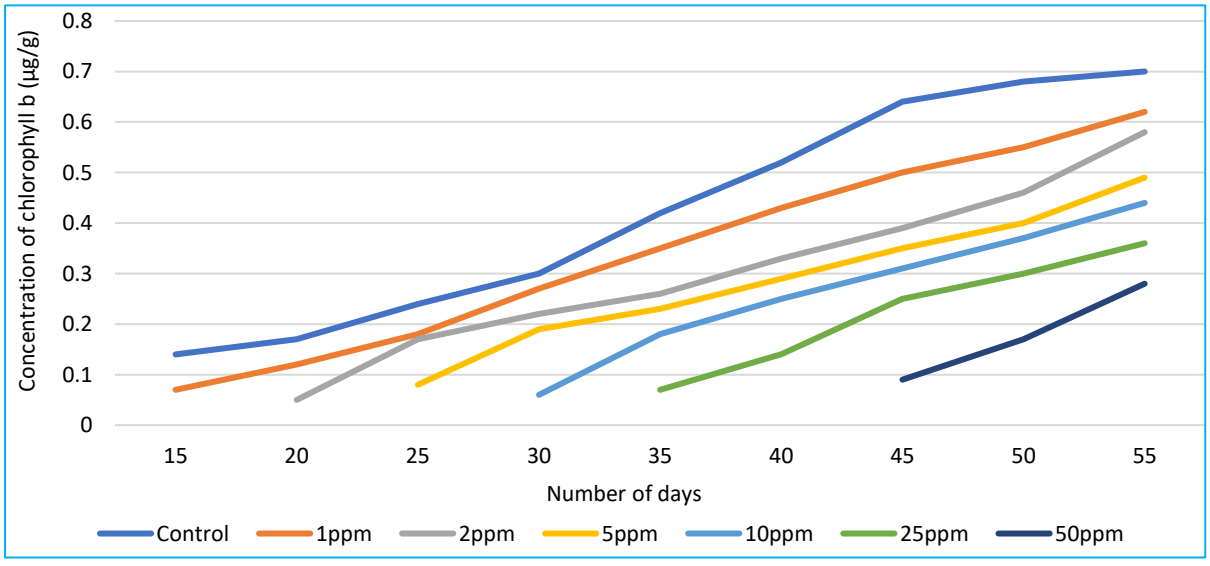

Fig 5 Chlorophyll b (µg/g) in the stems of *Amaranthus dubius* watered with various concentrations of sodium fluoride and harvested on various days

Estimation of chlorophyll b (µg/g) from root of Amaranthus dubius

Chlorophyll b level was measured when *Amaranthus dubius* roots were treated with different amounts of sodium fluoride from day 0 to day 55. The findings demonstrated that the roots lacked any signs of chlorophyll b.

Estimation of chlorophyll a and b (µg/g) from seed of Amaranthus dubius

Chlorophyll b was examined in the seeds taken from *Amaranthus dubius* plants that were treated with different sodium fluoride concentrations from day 0 to day 55. The findings demonstrated that the seeds contained no evidence of chlorophyll b.

RESULTS AND DISCUSSION

The effects of fluoride toxicity on height, fresh weight, number of leaves, seed yield and fluoride uptake in *Amaranthus dubius* were investigated for control and various concentrations of sodium fluoride, ranging from 1, 2, 5, 10, 25, and 50 ppm. Fig. 1 shows the results of the fluoride toxicity pot experiments conducted on *Amaranthus dubius*. The control group had the highest results, while the greater concentration of NaF (50 ppm) produced the lowest outcomes in all aspects except fluoride uptake.

The control plants exhibited the highest maximum height (cm), number of leaves per plant and fresh weight (g)/plant, whereas the plants treated with 50 ppm concentrated NaF showed the lowest results. The control group exhibited the highest seed yield while the group treated with 50 ppm NaF showed the lowest seed yield.

Fluoride uptake and translocation factor

Plants do not really need the fluoride chemicals. The findings of earlier research on fluoride intoxication in plants showed that eating of high fluoride compound concentrations was associated with this condition. As shown in (Fig 6), the fluoride uptake in *Amaranthus dubius* has risen with increasing NaF concentrations of 1–50 ppm.

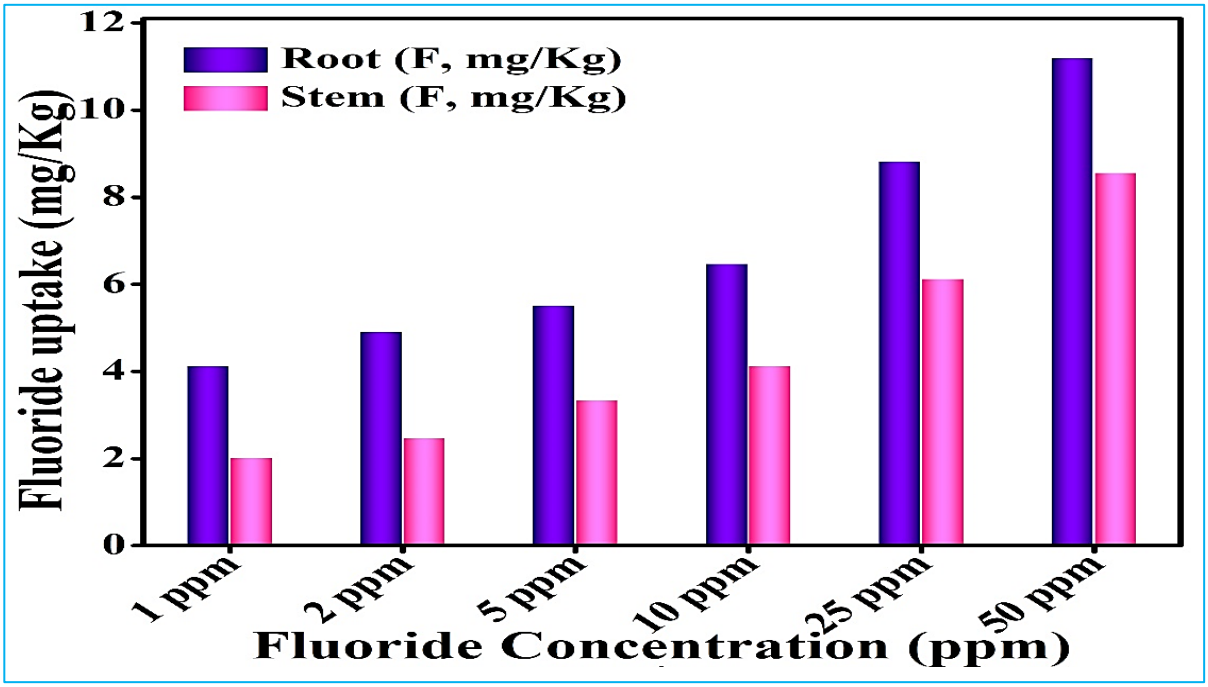

Fig 6 Fluoride uptake analysis of *Amaranthus dubius* roots and stem by using various concentration of sodium fluoride such as 1ppm, 2ppm, 5ppm, 10ppm, 25ppm and 50 ppm

At varying concentrations of 1, 2, 5, 10, 25, and 50 ppm sodium fluoride solutions, the root part's fluoride uptake ranged from 4.141, 4.918, 5.516, 6.476, 8.825, and 11.206 mg/kg, while the stem part's results ranged from 2.031, 2.482, 3.344, 4.139, 6.124, and 8.568 mg/kg. The translocation factor for the *Amaranthus dubius* plant ranges from 0.4904 to 0.7645, as shown in (Table 1). The findings showed that the root portion's uptake of fluoride

Analysis of chlorophyll pigments (µg/g) in leaf, stem, root and seed of Amaranthus dubius watered with various concentrations of sodium fluoride

As sodium fluoride concentrations in the water used to irrigate the study's experimental settings increased, the amount of chlorophyll a and b in *Amaranthus dubius* decreased. Chlorophyll a (0.07 µg/g and 0.70 µg/g) and b (0.09 µg/g and 0.62 µg/g) were lowest in *Amaranthus dubius* leaves that were irrigated with 50 ppm sodium fluoride on days 15 and 55 of the plant's growth. When the concentration of sodium fluoride increases, the *Amaranthus dubius* stem cannot produce chlorophyll a and b for a period. On day 55 of plant growth, the *Amaranthus dubius* stem with 50 ppm sodium fluoride showed the lowest levels of chlorophyll a (0.22µg/g) and chlorophyll b (0.28µg/g). Up to day 40, chlorophyll a and b are not visible. When researching the effects of fluoride in barley, Baunthiyal and Ranghar [14] also reported a decrease in chlorophyll a and b in the presence of F^-. Even at the lowest dosage of NaF (1.0 mM), there were notable reductions in the levels of all photosynthetic pigments. This decrease could be the consequence of either suppression of chlorophyll biosynthesis or rapid degradation of chlorophyll during fluoride stress, as high F^- has been demonstrated to limit the availability of Fe^{2+} ions, which are required for chlorophyll synthesis [14]. An analysis conducted recently by Ram *et al*. [16] on the growth and development of watermelon (*Citrullus lanatus*) seedlings revealed that when the concentration of NaF was increased, the amount of chlorophyll a and b in the seedlings decreased in relation to a control group.

Trapp and McFarlane [19] claim that pigment degradation results from the breakdown of chloroplasts and is made worse by F^- buildup in the organelles of the cell. Upon reaching the leaves, F^-s dense charge facilitates its easy interaction with $Mg^{2+,}$ forming the MgF^+ complex. This kind of F^- complexation is well-researched [20-22]. It causes a significant drop in pigment concentration [19], [23] by breaking down photosynthetic pigments, particularly chlorophylls. When Ca and F were applied together, the trend was reversed and larger levels of chlorophyll were evident. 1.0 and 2.5 mM NaF treatment of chickpeas resulted in stimulation of total chlorophyll (2.98 and 3.06 mg/g, respectively) over the control, in contrast to the current study [24]. The bulk of earlier research in a range of species, including another cultivar of *C. arietinum* called Anuradha [25], has not shown an increase in chlorophyll in the presence of F^-. Datta *et al*. [26], Tomar and Aery [27] observed a consistent rise in the chlorophyll levels and root and shoot lengths by 20 and 40 g/ml NaF in a study on *Triticum aestivum*. A genotype-specific reaction to fluoride stress could be the cause of this. However, it's interesting to note that in the observations by Sachan and Lal [24], a rise in NaF to 5.0 and 10.0 mM resulted in a quick drop in chlorophyll a and chlorophyll b.

CONCLUSION

The concentration of chlorophyll a and b is affected by an extra stress, as demonstrated by the lethal action of fluoride ions in *Amaranthus dubius*. The chlorophyll in stem and leaves significantly reduced as the concentration of fluoride ion increased. Because of the observed fluoride ion buildup on the stem and leaves of *Amaranthus dubius* as a result of decreased chlorophyll a and b and photosynthetic activities, the percentage of leaves, biomass, and seed yield decreased. The plant's cell count decreased as a result of the fluoride ion seeping into the leaves. Fluoride stress significantly hampers plant growth and crop development by causing metabolic inhibition. The presence of fluoride ions disrupts vital metabolic processes, leading to reduced photosynthetic activity and overall stunted growth in affected plants. Because fluoride stress breaks down photosynthetic pigments, especially chlorophylls, it results in a considerable decline in pigment content. Given these challenges, additional research is crucial to develop fluoride-tolerant genotypes that could withstand this stress. Such advancements would help farmers achieve better ecological crop yields despite fluoride stress.

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