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ORIGINAL ARTICLE

Studies on the impact of fluoride toxicity on germination and seedling growth of gram seed (*Cicer arietinum* L. cv. Anuradha)

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The influence of 0, 0.1 mM, 0.5 mM, 1.0 mM, 4.0 mM, 8.0 mM fluoride (F) concentration on seed germination, seedling growth of gram seeds (cv. Anuradha) was studied under laboratory condition. At the end of 15 days of treatment, significant reduction in root length, shoot length, dry weight, fresh weight, % of germination, protein content, catalase activity, tolerance index, vigour index, germination rate, germination relative index, mean daily germination were observed at increasing fluoride concentration. Total soluble sugar content, proline content, peroxidase activity, ascorbic acid oxidase activity, % DFC, % phytotoxicity of root and shoot increased along with gradual increment of F concentration. 4.0 mM F concentration was found to be most sensitive for gram seeds. At 8.0 mM F concentration germination occurred but plants were totally dried after completion of treatment period.

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Abbreviations: % DFC - Difference from control, SEM - Scanning electron microscope, GRI - Germination relative index, SEM (±) - Standard error mean, CV (%) - Coefficient of variance, C.D. - Critical difference, cv – cultivar, FW - Fresh weight, N.S - Not significant.

Fluoride occurs widely in the earth's crust in a very minute amount, but frequently acts as an environmental pollutant (Jacobson *et al.,* 1966).F is not only toxic for human but it is also toxic for plant body. In case of plant certain physiological processes are known to be markedly affected by F e.g., decreased plant growth (Elloumi *et al.,* 2005; Jacobson *et al.,* 1966), chlorosis (Mcnulty and Newman, 1961), leaf tip burn and necrosis (Hadujue, 1966) ,decrease in chlorophyll (Mcnulty and Newman, 1961). The importance of seed germination in plant growth is widely recognized and its study has been used as a model for investigating F toxicity by various authors (Elloumi *et al.,* 2005; Gulzar and Khan, 2001; Gupta *et al.,* 2009; Rubio-Casal *et al.,* 2003; Wang *et al.,* 1991; Wilde and Yu, 1998). F acts as a metabolic inhibitor. As germination is closely associated with

active metabolism is likely to inhibit germination and early seedling growth.

This paper reports results of laboratory investigation to study the effect of F on the germination of the gram seeds and seedlings growth with respect to their physiology, biochemistry, and phytotoxicity. The aim begin to determine the extent to which this test species can tolerate excess amount of F. The work is related to germination physiology of seeds under the influence of F in laboratory condition.

MATERIALS AND METHODS

Seed Materials and its treatment

Gram seeds (*Cicer arietinum* L.cv. Anuradha) were obtained locally and soaked in distilled water. After that seeds were sterilized by 0.1% mercuric chloride for 30 seconds to 1 minute and rinsed repeatedly with distilled water, then tap water and again distilled water. Seeds were then transferred to Petri dishes containing a sheet of blotting paper and thin layer of cotton and moisten with distilled (control) and fluoride solution (for treatment) (0.1mM, 0.5mM, 1.0mM, 4.0mM, 8.0mM). Each dishes contain 41 seeds and each treatment had replica set. Treatment was carried out for 15 days.

Parameter Studied

After 15 days root length, shoot length, fresh weight, dry weight, % of germination, peroxidase activity, catalase activity, ascorbic acid oxidase activity, total soluble sugar content, protein content, proline content, %DFC, % phytotoxicity, Tolerance index, vigour index, germination rate, mean daily germination, germination relative index were determined. Micrograph study was also done. Analysis of variance (ANOVA) was carried out to determine whether significant differences were present among their treatment under laboratory condition. SEM(±), C.D. At 5%, CV(%) were performed to study the significance of different fluoride concentration on different parameters studied.

Table 1. Effect of F on root length, shoot length, fresh weight and dry weight of seedlings

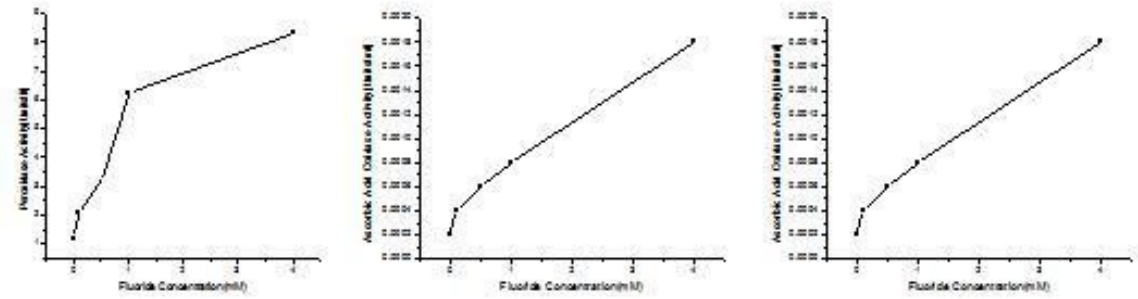
Treatments(mM)	Root length(cm)	Shoot length(cm)	Fresh weight(gm)	Dry weight(gm)
0	12.7	12.3	0.443	0.081
0.1	10.0	8.3	0.097	0.061
0.5	8.5	8.0	0.062	0.045
1.0	8.1	7.1	0.056	0.036
4.0	4.0	6.2	0.038	0.026
SEM(±)	0.254	0.110	0.018	N.S.
CV%	14.457	6.265	23.022	
C.D. at 5%	0.362	0.351	0.032	

RESULTS

Findings of seed germination and seedlings growth experiment shows a decreasing trend in root length and shoot length with increasing sodium fluoride concentration. At 0.1mM fluoride concentration root length and shoot length were 79% and 67% less than that of control respectively. At 4.0 mM fluoride concentration root length was 31% less than that of control and shoot length was 50% than that of control (Table 1). In case of fresh weight and dry weight similar trends was also shown here. At 0.1 mM fluoride concentration the fresh weight and dry weight were less than 22% and 75% respectively over control. At 4.0 mM fluoride concentration the

fresh weight and dry weight were less than 9% and 32% respectively over control. % of germination also decreased with increasing F concentration (Table 1.). At 0.1 mM fluoride concentration 80% seeds were germinated and at 4.0 mM fluoride concentration nearly 59% seeds were germinated (Table 2). Other than morphological parameters biochemical parameters like total soluble sugar and proline contents were increased with increasing sodium fluoride concentration (Table 3). But protein content in leaves of seedlings showed a gradual decrease with increasing fluoride concentration. At 4.0 mM fluoride concentration protein content was nearly 8% less than that of control (Table 3). On the other hand enzyme activity like catalase showed a decreasing trend but peroxidase and ascorbic acid oxidase were increased with increasing sodium fluoride concentration (Fig. 1).

Again % DFC and % of phytotoxicity of root and shoot of seedlings were showed similar trend as peroxidase and ascorbic acid oxidase (Table 4). Vigour index, tolerance index, germination rate and mean daily germination were decreased monotonically with increasing fluoride concentration (Table 5 and Table 6). Germination relative index on 3rd, 5th, 7th, and 10th days exhibited inverse relationship with increasing fluoride concentration (Table 7). Micrograph study showed the adverse effect of fluoride on anatomical structure of root, shoot and leaf. The results of this study indicated that gram seeds (cv. Anuradha) are highly resistant under 0.1 mM and 0.5 mM fluoride concentration but more sensitive under 4.0 mM fluoride concentration. Moreover 100% mortality of seedlings occurred under 8.0 mM fluoride concentration (Fig. 2a, b and c).



(a) (b) (c)

Fig.1. Showing variation of different enzymes activity such as (a) Catalase Activity, (b) Peroxidase Activity, (c) Ascorbic Acid Oxidase Activity

Table 2. Effect of F on % of germination of seedlings

Treatments(mM)	% of germination
0	87.805
0.1	80.487
0.5	80.487
1.0	73.170
4.0	58.53
SEM(±)	0.293
CV(%)	0.682
C.D. at 5%	0.961

MICROGRAPH STUDY

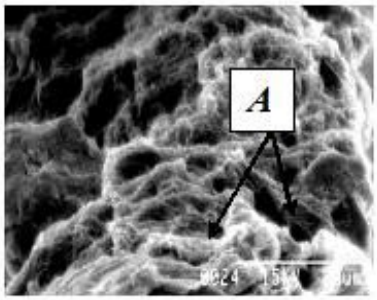


Fig.2a. SEM Study of root under 4.0mM F concentration (20µm mgnification). [A] totally distorted cell structure and it is more prominent.

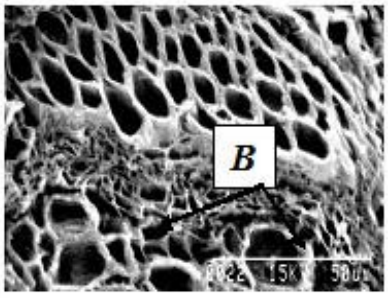


Fig.2b. SEM Study of shoot under 4.0 mM F concentration(50µm magnification).[B] cambium tissues of the vascular bundles are distorted and it is more prominent

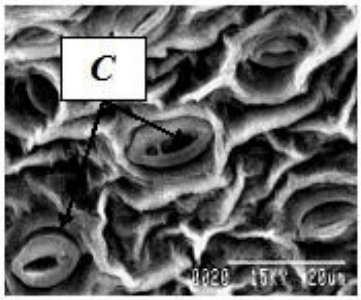


Fig.2c.SEM study of leaf under 4.0mM F concentration(20µm magnification) [C] under higher magnification stomatal structure deformed and epidermal cells are completely detached from subsidiary cell and it is very prominent.

Table 3. Effect of F on total soluble sugar content, proline content and protein content in leaves of seedlings

Treatments(mM)	Total soluble sugar content(mg/g FW)	Proline content (µg/g FW)	Protein content (mg/g FW)
0	25.74	68.966	97.4
0.1	33.44	71.234	19.18
0.5	49.70	9.326	15.58
1.0	66.86	108.462	11.98
4.0	84.14	148.226	7.58
SEM(±)	0.141	0.197	0.202
CV(%)	1.349	0.993	3.252
C.D. at 5%	0.467	0.649	0.658

Table 4. Effect of F on %DFC and % of phytotoxicity of root and shoot of seedlings

Treatments(mM)	%DFC	%phytotoxicity of root	%phytotoxicity of shoot
0.1	8.326	21.3	32.5
0.5	8.326	33.1	35.0
1.0	16.663	36.2	42.3
4.0	33.337	68.5	49.6
SEM(±)	1.565	N.S.	N.S.
CV(%)	39.837		
C.D. at 5%	5.4		

Table 5. Effect of F on Vigour index and Tolerance index (%phytotoxicity) of seedlings

Treatments(mM)	Vigour index	Tolerance index(%phytotoxicity)
0	187.014	78.740
0.1	147.297	66.929
0.5	117.515	63.780
1.0	81.147	31.496
4.0	31.021	
SEM(±)	3.698	N.S.
CV(%)	16.059	
C.D. at 5%	12.079	

Table 6. Effect of F on germination rate and mean daily germination of seedlings

Treatments(mM)	Germination rate	Mean daily germination
0	14.583	17.561
0.1	14.364	16.097
0.5	14.303	16.097
1.0	14.000	14.634
4.0	12.833	11.707
SEM(±)	2.239	1.223
CV(%)	6.221	39.352
C.D. at 5%	0.148	4.133

Table 7. Effect of F on GRI on 3rd, 5th, 7th,10th days of seedlings

Treatments(mM)	GRI on 3rd day	GRI on 5th day	GRI on 7th day	GRI on 10th day
0	408	720	1008	1440
0.1	360	660	980	1440
0.5	360	660	980	1440
1.0	336	600	868	1240
4.0	228	480	672	960
SEM(±)	2.941	4.669	4.239	4.362
CV(%)	4.258	3.666	2.303	1.659
C.D. at 5%	9.607	15.253	13.731	4.253

DISCUSSION

The increasing concentration of sodium fluoride shows phytotoxic effects on morphological, biochemical as well as phytotoxicity determining parameters. Fluoride causes reduction in root length and shoot length due to unbalanced nutrient uptake by seedlings in presence of fluoride (Sabal *et al.,* 2006). Fresh weight, dry weight and % 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 (Gulzar and Khan, 2001; Gupta *et al.,* 2009; Sabal *et al.,* 2006).Total soluble sugar and proline content in leaves initially decreased but increased with increasing fluoride concentration because there was gradual accumulation of proline during the germination period, with increasing fluoride concentration due to fresh synthesis or breakdown of proline rich proteins during stress. This might have contributed towards increase in the level of sugar and proline content for enhancing the tolerance capacity of plant under stress condition (Greenway and Munns, 1980; Yang and Miller,

1963). In case of protein content in leaves of seedlings showed gradual decrease with increasing fluoride concentration due to stress condition which was formed under fluoride treatment (Singh *et al.,* 1985). In case of enzymatic activity specifically catalase activity decreased with increasing fluoride concentration which might be attributed towards enduring the stress condition and protects the plants from oxidative damage (Wang *et al.,* 1991).

Ascorbic acid oxidase and peroxidase activity were increased gradually with increasing fluoride concentration (Wang *et al.,* 1991). Both of these enzyme activity increased for increasing the tolerance capacity of plant against the stress condition due to presence of fluoride. Same results were found in % DFC and % phytotoxicity of root and shoot of seedlings. This was probably due to the presence of fluoride germination was inhibited, so % DFC gradually increased and the higher percentage of phytotoxicity revealed the deleterious effect of fluoride on root and shoot growth due to presence of excessive fluoride in 4.0 mM fluoride concentration which exerts its toxic effect on root and shoot growth (Mishra and Choudhuri, 1999; Jamal *et al.,* 2007). But vigour index and tolerance index decreased monotonically with increasing fluoride concentration. In case of vigour index (% phytotoxicity) significantly reduction occurred due to the presence of fluoride which can inhibited the % of germination and growth of embryonic axis at 48 hours (Mishra and Choudhuri, 1999). In case of tolerance index fluoride reduced the tolerance capacity of seedlings as well as create a deleterious condition leading towards susceptibility of seedlings under highest fluoride concentration (Mishra and Choudhuri, 1999). The germination rate and mean daily germination were gradually decreased with increasing fluoride concentration due to inhibition of germination under fluoride treatment (Gulzar and Khan, 1980). Germination relative index on 3rd, 5th, 7th, 10th days exhibited inverse relationship with increasing fluoride concentration due to toxic effect of fluoride (Siddhu *et al.,* 2008).

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