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Exterminate consequence of NaF on seed germination and some morphological changes of major pulse crop *Cicer aritinum* L. Cv. Anuradha (Bengal gram)

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ABSTRACT

In sort to study the result of sodium fluoride on germination and growth of Cicer arietinum L. cv. Anuradha under fluoride stress. The experiment was conducted as factorial with completely randomized design with three replicates. The factor of the experiment are different levels of NaF concentrations (0,25,50,75, and 100ppm).the result showed that increased fluoride content would decrease the components of germination including germination percentage root length and shoot length and wet weight of the seedling. Chlorophyll contents were also decreased in seedling under fluoride stress.

Key words: NaF stress, seed germination, morphological and bio chemical changes in *Cicer aritinum* L. cv. Anuradha

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INTRODUCTION

Fluoride occur naturally in plants and widely in the earth crust in a very minute amount, the lethal dose for 70kg (154 lb) human is .estimated at 5-10g , NaF is caused by both inhalation and ingestion .NaF is an ionic compound dissolving to give Na+ and F-- Fluoride presents in soil, water, air and plants in varying concentrations but is not considered essential for the normal growth of plants (R.K.Sarkar et al 1982) It is absorbed by roots and accumulated in leaves with its adverse effect Certain fluoride salts are metabolic inhibitors and affect a wide range of plant processes. Certain plant species have been observed to be injured as a result of the accumulations of excessive fluoride from the atmosphere. The general symptoms of fluoride injury are necrotic lesions and burning, which appear first in the leaf tips and margins. The exact mechanism by which fluoride causes damage to plants is little understood. Nevertheless, certain physiological processes are known to be markedly affected by fluoride. For example, a decrease in chlorophyll (McNulty & Newman,1961), a diminished rate of photosynthesis (Thomas& Hendricks, 1956), decreased plant growth (Bonner & Thimann, 1950) and increased respira-tion in growing plants (McNulty & Newman, 1957)have been reported. Fluoride contaminated ground water is used for irrigation which adversely affects crop growth especially in early stage of seedling growth(. D. Sabal,et al 2006,) .(D. Bhargava et al 2010). 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 (Elloumiet al., 2005; Gulzar and Khan, 2001; Gupta et al.,2009; Rubio-Casal et al., 2003; Wang et al., 1991;Wilde and Yu, 1998). Fluoride reduced germination by lowering the enzymatic activity and growth by slow the rate of cellular division and expansion(B.R.Gadi et al 2012). This paper reports results of laboratory investigation to study the effect of F on the germination of the gram seeds . Various physiological processes such as water transport, photosynthesis, respiration, metabolism of hydrocarbons and lipids are inhibited by fluoride (G.W. Miller,et al , 1993), (K. Rakowski et al, 1997). Response of fluoride depends upon some factors such as dose, duration of exposure, age and genotypes of plants. India is the major growing country of the world, accounting for 61.65 % of the total world area under Bengal gram during 2002

and 68.13% of the total world production and bengal gram is a major pulse crop in India. Bengal gram belongs to family Leguminoseae. It is a small, much branched herbaceous plant. Bengal gram is widely appreciated as health food. It is a protein-rich supplement to cereal-based diets. Hence, the objective of this study was to understand and determine the phytotoxic effects of flouride on behavior of seed germination, seedlings growth, photosynthetic pigments were studied in vitro grown seedlings of *Cicer arietinum* L.

MATERIALS AND METHODS

Seeds of Bengal gram (*Cicer arietinum* L cv Anuradha) were obtained from N.G.Ranga Agricultural University, Tirupati and germination experiment was performed. Pre soaked seeds were sterilized with 0.1 N mercuric chloride for 1 min, thoroughly washed with sterilized double distilled water seeds were transferred to 9cm Petri dishes lined with two layers of whatman N o.1 filter paper. Then the seeds were placed in individual Petri dishes labeled as control and NaF concentrations were 25ppm, 50ppm, 75ppm and 100ppm.The pre-sterilized Petri-dishes were lined with two layers of whatman N o.1 filter paper, moistened with sodium fluoride solution. Three replicates were taken for each respective concentration of fluoride and control. 5-10 ml of sodium fluoride solutions were added to each petri-plate on 1^{st}, 3^{rd}, 5^{th}, and 7^{th} days of treatment. The petri-plates were incubated in B.O.D. incubator at 28°±2°C. The number of seeds germinating every day after treatment was recorded and radical and plumule length were recorded and pressured for statistical analysis.

Germination percentage was recorded on 3^{rd} day after sowing. Germination percentage (GP) was calculated according to the international seed testing association (ISTA) methods (Vahid Ghodrt et al).

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

Vigour index was calculated as per equation by Anderson et al. 1973

Vigour Index = (Root length + Shoot length) x Germination percentage.

Photosynthetic pigments (chlorophyll a, b and carotinoids) were determined by Arnon's method. For studying root and shoot elongation 15,20 seedlings were randomly selected and the length of each radical and plumule was measured, Five day old seedlings were selected for uniformity and transferred to freshly prepared earthen pots moisten control with distilled water and treatments (25,50,75,and 100 ppm) with Naf solution up to the end of the experiment. After 15 days of the treatment various morphological parameters such as percent germination, root length, shoot length, fresh weight and biological parameters such as levels of chlorophyll and carotinoids were determined by Arnon's method leaves were homogenized in 80% acetone. The extract was centrifuged at 2500 Rpm for 15 minutes and absorbance of supernatant was read at 663, 645, and 445 nm using spectrophotometer.

RESULTS AND DISCUSSION

Different environmental conditions shows its effect on physiologically complex process such as seed germination and early seedling growth the present study shows that the failure of the treated seeds to germinate at high concentrations of the applied inhibitors that may be consequence of retarded water uptake, inhibited cell divisions and enlargements in the embryo and or an overall decrease in metabolic activity relevant to these steps The blockage of any one of the phases leading to germination may, and very likely ill, completely inhibit the process of germination. The radical and plumule lengths of the treated seedlings (5 day old) of the test plants were considerably Reduced at all levels of the applied inhibitors (M.A. Shaddad et al 1989)

Table-1: Effect of NaF on germination Percentage, Radical and Plumule length of *Cicer aritinum* L. cv.Anuradha

Treatment (ppm)	Radical length (cms)	Plumule length (cms)
Control	2.26±0.11	2.66±0.29
25	1.23±0.09	0.90±0.29
50	1.10±0.04	0.40±0.04
75	0.37±0.02	0.09±0.00
100	0.00	0.00

±Standard error of mean Significant at P ≤ 0.05radical

Table .1 Shows The inhibition of the embryonic root and plumule lengths of the germinated seeds, observed in this work at high levels of the applied inhibitors, may be one aspect of the role of metabolic inhibitors in the overall phenomenon of plants growth. Evidence to support this suggestion May be obtained from the work Hesset et al.(1976), who reported a depressed root elongation in Zea mays seedlings in the presence of cadmium. A somewhat similar situation has been observed by Shahnaz (1981). fluoride causes reduction in root length and shoot length due to unbalanced nutrient uptake by seedlings in presence of fluoride (Sabal et al., 2006)

As shown in the Table. 2 root length and shoot length decreased monotonically with increasing NaF concentration at 75 ppm the average root length and shoot length were reduced to 65% and 87% respectively 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 (Gulzar and Khan, 2001; Gupta et al., 2009; Sabal et al., 2006).

Table 2: Effect of different sodium fluoride concentrations on germination percentage average root length and shoot length ,vigour index and fresh weight of *Cicer aritinum L.* cv.Anuradha **seedlings**.

±Standard error of mean significant at P ≤ 0.05

Treatment (ppm)	Germination percentage	Root length (cms)	Shoot length (cms)	Vigour index	Fresh weight (gms)
Control	93.30±2.71	18.40±0.12	32.06±0.09	4711±146	1.399±0.043
25	63.33±4.77	16.00±0.09	30.60±0.49	2936±133	1.184±0.003
50	40.00±4.71	14.96±0.11	30.26±0.11	2110±121	1.157±0.003
75	26.70±4.71	12.03±0.11	28.00±0.09	1066±104	1.083±0.004
100	0	0	0	0	0

Seed vigour comprises those seed properties which determine the potential for rapid, uniform emergence. (Anon seed vigour testing hand book 2002) Seed vigour is defined as the sum total of those properties of the seed that determines the potential level of embryo activity and performance of the seed during germination and seedling emergence. High vigour seeds allow rapid embryo growth and thus seedling establishment however, stressful field conditions reduce seed vigour of plants. Table-1 shows that vigour index was decreased (78%) with higher concentration (75 ppm) of fluoride treatment over control. Similarly reduction in vigour index has also been reported in *Triticum aestivum* (Bhargava et al 2010).

As shown in the below (Figure- 1) chlorophyll –a, chlorophyll-b ,total chlorophyll content of leaves decreased monotonically at 75 ppm ,concentration of NaF/L both chlorophyll a , and chlorophyll b decreased as 41%and 46% where as reduction in total chlorophyll content was 43% 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

Table 3: Effect of different concentrations of sodium fluoride on chlorophyll –a, chlorophyll-b, Total chlorophyll and carotinoids on *Cicer aritinum L.* cv.Anuradha seedlings.

Treatment	Chlorophyll –A	Chlorophyll -B	Total chlorophyll	Carotinoids
Control	14.86±0.05	21.44±0.33	36.36±1.07	2.43±0.02
25 ppm	10.99±0.01	14.05±0.91	25.99±0.00	2.024±0.00
50 ppm	8.18±0.05	12.19±0.06	19.90±0.03	1.651±0.00
75 ppm	6.20±0.01	09.90±0.01	15.74±0.01	1.31±0.00
100 ppm	0	0	0	0

±Standard error of mean significant at P ≤ 0.05

Caritinoids are accessory pigments in photosynthetic systems and protect chlorophyll against oxidative stress in the present experiment caritinids was decreased at 75ppm NaF concentration by 53% over control Maximum decrease in caritinoids content was observed with highest concentration of NaF under stress condition plant membranes are subjected to changes such as increase in permeability and loss of integrity.

CONCLUSION

In the present study NaF concentrations disturbs the seed germination and early growth of seedlings further it was reported that germination percentage and vigour index also decreased by NaF, in addition to morphological features such as radical and plumule length and some biochemicals such as photosynthetic pigments such as chlo-a ,chlo-b ,caritinoids were also adversely affected by increased NaF concentration

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