

DUAL EFFECTS OF FLUORIDE AND CALCIUM ON THE UPTAKE OF FLUORIDE, GROWTH PHYSIOLOGY, PIGMENTATION, AND BIOCHEMISTRY OF BENGAL GRAM SEEDLINGS (*CICER ARIETINUM* L.)

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SUMMARY: The effects of 4.0, 8.0, 10.0, and 20.0 mM aqueous sodium fluoride (NaF) were studied on Bengal gram seedlings, *Cicer arietinum* L. A significant decrease ($p < 0.01$) in the physiological growth parameters (root and shoot length, fresh root and shoot weight, dry root and shoot weight, number of primary leaves, and number of root branches) was observed with increasing F concentration after seven days of treatment. However, separate addition of aqueous calcium chloride (CaCl_2) to the NaF growth media gave little or only minor improvement in the growth physiology of the seedlings. On the other hand, the pigment, protein, and proline content showed good agreement with F stress, and addition of CaCl_2 caused improvement in all biochemical parameters. The concentration of F in the plant body increased with increasing NaF concentration, but maximum reduction of plant body F occurred with the addition of 10.0 mM CaCl_2 to the 20mM NaF growth medium.

Keywords: Bengal gram seedlings; Biochemical parameters; Calcium chloride; *Cicer arietinum* L.; Fluoride and gram seedlings; Growth physiology; Pigment content.

INTRODUCTION

Fluoride (F) is a potential air, water, and soil pollutant, and also a common phytotoxic element.¹ The main sources of accumulating F in soil are weathering of volcanic ashes,² application of phosphate fertilizer in agriculture,³ and several industrial processes, especially the aluminum and phosphate fertilizer industries.^{4,5} Some soils contain a very high level of natural F.⁶

Although the toxic effects of F on plants and animals have been known for more than a hundred years, only with recent industrial expansion have they been increasingly recognized for causing serious toxicity to vegetation. F adversely affects various physiological features of plants including causing decreased plant growth, chlorosis, leaf tip burn, and necrosis of leaf tip and leaf margin.⁷⁻⁹ Certain biochemical processes are also known to be affected by F toxicity, such as changes in enzyme level and protein and pigment content.¹⁰ The growth and productivity of many crops are also adversely affected by F.¹¹ Lower concentrations of F can initiate various physiological and biochemical changes without the appearance of visible symptoms of injury, which may have important consequences like reduction in growth and crop yield.¹²

On the other hand, the presence of certain elements such as aluminum, magnesium, and calcium in the soil or in solution have been found to inhibit the uptake of F by plants during growth.¹³ F binds or forms complexes with these elements and inhibits the transport of F into plants.¹⁴ Although most attention has been paid to F complexes of aluminum to impede the uptake of F by plants,

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calcium has also been reported to inhibit the uptake of F, and this effect is proposed as a mechanism that might ameliorate F toxicity in plants.¹⁵ Since the effects of calcium on the uptake of F by plants have not been fully investigated, the objective of this study was to examine how co-administration of calcium with F might affect not only the F uptake but also the inhibitory effects of F on the growth and biochemistry of Bengal gram seedlings.

MATERIALS AND METHODS

Dry and healthy seeds of Bengal gram (*Cicer arietinum* L.) were soaked overnight in distilled water. After sterilization with 0.1% mercuric chloride, the seeds were transferred to nine petri dishes containing moist filter paper with 25 seeds per dish. The seeds were allowed to germinate and grow for ten days with exposure to sunlight for one hr/day. After ten days, the nine dishes were divided into three sets. The first set, consisting of four petri dishes, was treated with four concentrations of aqueous NaF: 4 mM (T2), 8 mM (T3), 10 mM (T4), and 20 mM (T5). The second set of four petri dishes were also given the same treatments with NaF, but they were also treated separately with 2 mM (T6), 4 mM (T7), 5 mM (T8), and 10 mM (T9) of CaCl₂, respectively. Dish T1 was treated only with distilled water and was used as the control.

After seven days of treatment, shoot and root length, fresh shoot and root weight, dry shoot and root weight, number of primary leaves, and number of root branches were measured, and the color of root and leaf tip was also recorded. Chlorophyll 'a', 'b', total chlorophyll, and carotenoid were measured following Arnon's method.¹⁶ Protein and proline were determined by standard procedures.^{17,18} Finally, F concentrations in various plant parts were measured by the method developed by Paul et al.¹⁹

The changes of the percentage in the growth of roots and shoots and various biochemical parameters were calculated with reference to the control. A comprehensive statistical software package (SPSS 16.0) was used to make the t-calculation of the experimental data.

RESULTS AND DISCUSSION

Growth parameters: The growth of root and shoot was suppressed with F treatment compared to dual application of Ca and F (Table 1). The little improvement in root length and shoot length after addition of CaCl₂ may be due to the precipitation of CaF₂, thereby reducing the availability of F in the medium. As also seen in Table 1, a significant difference ($p < 0.05$) in the number of root branches occurred between T3 and T7 and between T5 and T9, but between T2 and T6 and T4 and T8 there was no significant difference. On the other hand, biomass is a good indicator of growth and development in plants. Here the fresh and dry weight of the roots and shoots gradually decreased over time. The same results have been reported by Pant et al.²⁰ Such a decrease is probably due to interference of F in the water uptake capacity of plants.²⁰⁻²² However, the decreasing pattern in biomass was not evident under dual application of Ca and F.

Moreover, we also observed leaf tip burn and margin necrosis in the F treated plants. Klumpp et al.²² and Kamaluddin and Zwiazek⁸ observed similar

symptoms, which may occur when F reaches the site of greatest evaporation, namely, the leaf tip and margin as water evaporates from the leaves, causing the amount of F to accumulate and induce necrosis of the leaves.²³ However, leaf tip burn and margin necrosis were less pronounced in the NaF treatment supplemented with CaCl₂. There was also a gradual decrease in the NaF treated plants in the number of primary leaves and the number root branches that were attenuated with the addition of CaCl₂.

Correlation analysis confirmed a significant ($p < 0.01$) negative impact of F on root length ($r = -0.871$), shoot length ($r = -0.809$), dry shoot weight ($r = -0.814$), number of primary leaves ($r = -0.898$), and number of root branches ($r = -0.916$).

Table 1. Variation in growth parameters in different plant parts from the various treatments

Treatment	Root length (cm)	Shoot length (cm)	Fresh root wt (g)	Fresh shoot wt (g)	Dry root wt (g)	Dry shoot wt (g)	No. of primary leaves	No. of root branches
T1	2.57 ^a	2.66 ^a	0.06 ^a	0.053 ^a	0.02 ^a	0.015 ^a	22 ^a	6 ^a
T2	2.32 ^b	2.53 ^{ab}	0.049 ^{ab}	0.052 ^a	0.019 ^a	0.008 ^b	13 ^b	4 ^b
T6	2.42 ^a	2.47 ^b	0.056 ^b	0.053 ^a	0.017 ^a	0.013 ^a	17 ^c	4 ^b
T3	2.2 ^b	2.32 ^b	0.042 ^b	0.051 ^a	0.013 ^b	0.008 ^b	8 ^b	3 ^b
T7	2.28 ^b	2.33 ^b	0.063 ^a	0.052 ^{bc}	0.012 ^b	0.011 ^b	12 ^c	4 ^c
T4	2.2 ^b	2.14 ^b	0.033 ^b	0.049 ^a	0.009 ^b	0.006 ^b	7 ^b	3 ^b
T8	2.25 ^a	2.26 ^b	0.037 ^b	0.051 ^a	0.01 ^b	0.01 ^b	10 ^c	3 ^b
T5	1.99 ^b	1.98 ^b	0.008 ^b	0.032 ^b	0.008 ^b	0.004 ^b	3 ^b	2 ^b
T9	2.07 ^a	2.06 ^b	0.043 ^c	0.042 ^c	0.01 ^b	0.008 ^b	7 ^c	3 ^c

^{a,b,c}Different letters indicate significant differences at $p < 0.01$ according to the Tukey-HSD.

Pigmentation: As seen in Table 2, a monotonic decrease in pigmentation occurred with increasing F in the form of decreasing levels of chlorophyll ‘a’, chlorophyll ‘b’, and total chlorophyll. Degradation of pigment is due to breakdown of chloroplasts, aggravated by F accumulation in the cell organelles.²⁴ As a densely charged anion, F can bind readily with Mg²⁺, forming a MgF⁺ complex, when it reaches the leaves. This kind of complexation of F destroys the photosynthetic pigments, particularly the chlorophylls, thereby significantly decreasing the concentration of pigments²⁴ and is well documented.^{12,25,26} Owing to its relation to the chlorophyll level by coordinate synthesis and accumulation mechanisms, similar detrimental reduction of carotenoid also occurs.²³ However, with dual application of Ca with F, reversal of this trend occurred, and increased levels of chlorophyll and carotenoid were evident.

Biochemical parameters: As also seen in Table 2, the protein content was highest in T1, while the F treatments showed the same reduction pattern as with pigment and carotenoid. When Ca was added in the medium, protein content was 1.7% higher in T6 than in T2. An even greater improvement occurred with Ca in

going from T7 to T9. According to Eyini et al.,²⁷ a decrease in protein with higher concentration of F may be the result of a reduced rate of protein synthesis. With increased exposure to NaF and CaCl₂, the present study also showed a non-monotonic enhancement in the content of proline, which is an important stress indicator for decreased protein synthesis.

Table 2. Variation in chlorophyll, carotenoid, protein and proline content in the plants from different treatments

Treatment	Chlorophyll 'a' (mg/g)	Chlorophyll 'b' (mg/g)	Total chlorophyll (mg/g)	Carotenoid (mg/g)	Protein (mg/g)	Proline (mg/g)
T1	1.031 ^a	0.863 ^a	2.734 ^a	0.327 ^{ac}	22.245 ^a	0.075 ^a
T2	1.690 ^b	0.613 ^b	2.608 ^a	0.306 ^b	21.257 ^b	0.117 ^b
T6	1.730 ^b	0.840 ^a	2.611 ^a	0.334 ^{bc}	21.625 ^c	0.226 ^c
T3	1.480 ^b	0.457 ^b	2.072 ^b	0.215 ^b	20.302 ^b	0.374 ^b
T7	1.560 ^b	0.625 ^c	2.400 ^c	0.311 ^a	20.615 ^b	0.001 ^c
T4	1.070 ^b	0.288 ^b	2.028 ^b	0.120 ^b	17.402 ^b	0.141 ^b
T8	1.160 ^c	0.335 ^c	2.054 ^c	0.258 ^c	17.735 ^b	0.172 ^c
T5	0.720 ^b	0.224 ^b	1.658 ^b	0.106 ^b	16.092 ^b	0.035 ^b
T9	1.010 ^b	0.305 ^c	1.758 ^b	0.222 ^a	16.407 ^b	0.153 ^c

^{a,b,c}Different letters indicate significant differences at p < 0.01 according to the Tukey-HSD

Fluoride in plant body: As expected, bioaccumulation of F in the plant parts decreased in the Ca treated samples (see Figure), since Ca⁺⁺ forms CaF₂, making less F available for plant uptake.^{15,28}

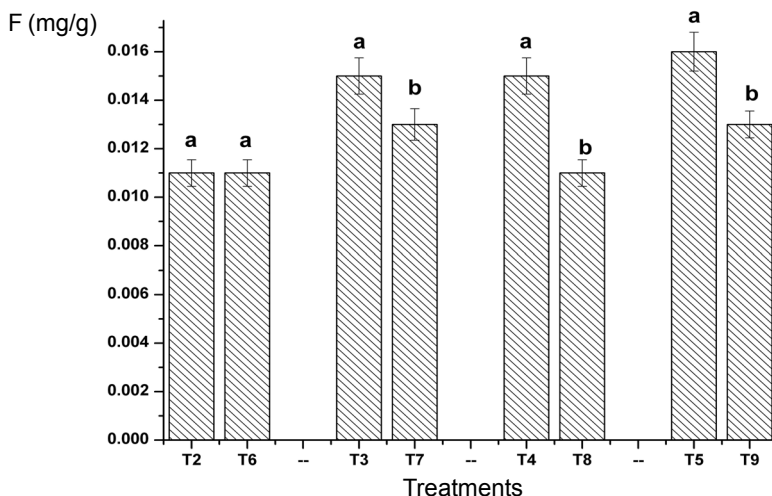


Figure. Fluoride concentrations in the seedling plants. Different letters indicate significant differences at p < 0.01 according to the Tukey-HSD.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to one of the authors, Professor Jayanta Kumar Datta, Department of Environmental Science, The University of Burdwan, for his continuous encouragement and help. We also thank the Department of Environmental Science for providing necessary laboratory facilities.

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