## EFFECT OF FLUORIDE TOXICITY ON LEAF AREA AND CHLOROPHYLLS IN RADISH (*RAPHANUS SATIVUS L*) VAR. ARKA NISHANT AS MODIFIED BY NUTRIENT AMENDMENTS

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The common foliar symptoms, of fluoride injury, are decrease in chlorophyll contents and necrosis. Effect of sodium fluoride (1.5mM) and mineral nutrient amendments on chlorophyll contents were studied in radish plants grown in clay pots. In the absence of calcium, magnesium and phosphorus, fluoride caused more injury in leaves and decreased the content of cholorophyll. Fluoride injury symptoms were not seen with the addition of calcium ( $10x10^{-3}$  M), magnesium  $4x10^{-3}$  M) and phosphorus  $2x10^{-4}$  M). Concentration of chlorophyll pigments were also more. Leaf area was reduced with fluoride. Leaf area increased when calcium, magnesium and phosphorus contents in the B<sup>a</sup> S<sup>a</sup>1 medium were  $5x10^{-4}$ ,  $2x10^{-3}$ , and  $1x10^{-14}$  M respectively.

Keywords: Chlorophyll pigments; Leaf area; Mineral nutrients; Sodium fluoride.

Fluoride occurs in nature in different forms and causes soil, water and air pollution. Fluorides cause a variety of physiological and biochemical changes in plants<sup>1</sup>. Many factors including mineral nutrient levels modify the response of plants to fluoride<sup>2</sup>. Research on the effect of fluoride on plants was mostly concentrated with reference to the gaseous form, hydrogen fluoride. Sensitivity of plants to fluoride injury is related to their nutritional status and the level of various mineral nutrients in the tissue<sup>3,4</sup>. Weinstein and Alscher-Hermann<sup>5</sup> reviewed the relationship between fluoride and divalent cations. Loss of chlorophyll in green plants as a general visible symptom of toxic effect of fluoride, in fluoride fumigated plants has been reported<sup>6-8</sup>. Leaf area was significantly reduced by sodium fluoridesprayed on Hordeum vulgare and Zea mays<sup>9</sup>. Present experiment was set up to find out the effects of soil applied fluoride and other mineral nutrients on the leaf area and chlorohyll pigments and injury symptoms in radish plants.

Radish (*Raphanus sativus*) Var Arka Nishant, was used as the experimental material. Plants were raised in 30x30cm clay pots with known amount of red soil and farm yard manure. Initially for the first four weeks, all the plants were supplied with one liter of deionised glass-distilled water per pot per day until the harvest except on the day when plants were given mineral treatments. Thirty day-old plants were treated with one liter of Hoagland's nutrient solution. (Basal medium) along with sodium fluoride (1.5mM) once in a week for four weeks.

For the extraction and estimation of chloroplast pigments, the known amount of freshly harvested leaf material was cut into small pieces and immersed in test tubes containing 5ml of dimethylformamide

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Treatment	Leaf Area	Chlorophylls (mg/g Fr, wt.)			
		A	b	a/b	Total
BM-F	527	1.459	0.391	3.731	1.851
BM+F	527	1.415	0.336	4.211	1.751
BM+Ca+F	515	1.335	0.347	3.845	1.697
BM-Ca+F	491	1.192	0.304	3.920	1.496
BM+Mg+F	492	0.968	0.221	4.380	1.189
BM-Mg+F	427	0.904	0.173	5.220	1.077
BM+P+F	544	1.200	0.257	4.669	1.457
BM-P+F	500	1.200	0.258	4.651	1.458

Table 1. Effect of NaF on leaf area and chlorophyll content of Radish leaves.

BM = Basal Hoagland's nutrient medium (Calcium =  $5 \times 10^{3}$  M, Magnesium =  $2 \times 10^{3}$  M, Phosphorous  $1 \times 10^{4}$  M); + F = Sodium fluoride 1.5mM; -F=without fluoride; +Ca, +Mg, and +P indicates Calcium  $10 \times 10^{3}$  M, Magnesium  $4 \times 10^{-3}$  M and phosphorus  $2 \times 10^{-4}$  M content in the basal medium. -Ca, -Mg and -P refers to their deletion from basal medium. Values are mean of 5 replicates.

at  $4^{\circ}$  C for 48 hrs in the dark. Absorption of chlorophyll pigments was recorded with Schimadzu U.V. - VIS spectrophotometer. Content of chlorophylls were calculated by the equations of Moran<sup>10</sup>. Leaf area was measured with the help of leaf area meter.

Fluoride toxicity symptoms were observed after twenty four hours of fluoride (1.5mM) treatment. Characterstic fluoride toxicity symptoms observed in mature radish leaves, were loss of chlorophylls. Initially leaves appeared dull grey-green with water soaked discoloration of tissues and the loss of turgidity along the leaf tips and margins. Later, the water soaked areas turned light brown and became necrotic after 48 hours.

With the supply of calcium, magnesium and phosphorus at  $5x10^{-3}$ M,  $2x10^{-3}$  M and  $1x10^{-4}$  M respectively and at higher concentrations, fluoride toxicity symptoms were not observed, instead the

plants looked healthy with dark green leaves. Injury was more prominent in the absence of phosphorus but to a lesser extent without calcium or magnesium. More injury in the absence of phosphorus even at higher concentrations of calcium and magnesium indicate that not only calcium and magnesium but also phosphorus are required for protection of tissues from injury. A similar role of calcium in mitigating foliar injury caused by fluoride has been stressed<sup>5,11,12</sup>. Protective role of calcium on membrane integrity may be the mechanism of protection against fluoride injury.

Leaf area was reduced in fluoride treated plants as compared to control. In contrast calcium at  $5 \times 10^{-3}$  M, magnesium at  $2 \times 10^{-3}$  M and phosphorous at  $1 \times 10^{-4}$ M caused an increase in leaf area even in the presence of fluoride. Sodium fluoride caused more pronounced reduction in leaf area in the absence of calcium, magnesium and phosphorus. At higher concentration of calcium, magnesium and phosphorus the leaf was increased.

Sodium fluoride reduced the content of total chlorophylls, chlorophyll-a and chlorophyll-b as compared to control. This observation was more prominent in the absence of calcium and magnesium. At higher concentrations of calcium and magnesium, the content of chlorophylls increased. Calcium at  $5x10^{-3}$  M reduced the toxic effectof fluoride by increasing the chlorophyll-a and total chlorophylls and for chlorophyll-b, calcium ( $10x10^{-3}$ ) was much better. Magnesium at  $2x10^{-3}$  M reduced the toxic effect of fluoride.

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