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Effect of Graded Doses of Fluoride on Soil Microbial Activity, Growth and Yield of Okra (*Abelmoschus esculentus* L.) grown in Alfisols

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A field experiment was conducted at Department of Soil Science & Agricultural Chemistry, College of Agriculture, OUAT, Bhubaneswar in the year 2015 and 2016 to study the impact of different doses of fluoride on growth and yield of okra along with soil microbial activity like microbial biomass carbon (MBC), microbial biomass nitrogen (MBN) and dehydrogenase activity (DHA) in the post harvest soil. Graded doses of fluoride i.e. No fluoride, 100, 150, 200, 250 and 300 ppm F in form of hydrofluoric acid (HF) were applied as foliar spray. The highest fruit yield (34.5 q ha^{-1}) of okra was recorded with T_1 (No fluoride), whereas, the lowest yield (21.5 q ha^{-1}) was observed with T_6 (foliar spray of F @ 300 ppm as HF). The fruit yield decreased significantly with increasing doses of fluoride. The decrease in yield was the highest (37.7%) with T_6 (foliar spray of F @ 300 ppm as HF) over control where as lowest value of 23.5% was observed with T_2. Significantly highest total chlorophyll content (4.5 mg g^{-1} fresh leaf) was observed with T_1 which decreased to the lowest value of 2.3 mg g^{-1} fresh leaf (T_6) due to increasing doses of fluoride. Soil microbial carbon increased up to 30 days after sowing (DAS) and decreased thereafter up to 42 DAS irrespective of fluoride doses. Except control, microbial biomass nitrogen (MBN) increased with increased doses of F up to 150 ppm in all the growth stages except zero DAS. However, MBN value decreased in all the growth stages except zero DAS with increased dose of F from 150 ppm to 300 ppm. The soil dehydrogenase activity was increased due to application of graded doses of F irrespective of growth stages.

***(Key words**: Dehydrogenase, Fluoride, Microbial biomass carbon, Microbial biomass nitrogen, Okra, Yield)*

Of the elements present in the earth's crust, only 17 elements are known to be essential to all plants and 12 elements have been proven to be potentially beneficial in trace amounts. These include Silver (Ag), Cerium (Ce), Chromium (Cr), Fluorine (F), Iodine (I), Lanthanum (La), Rubidium (Rb), Tin (Sn), Strontium (Sr), Titanium (Ti), Vanadium (V) and Tungsten (W) (Pilon-Smits *et al.,* 2009). So, F is not an essential element for plant metabolism but is a beneficial element both to plant and human health as well. Moderate amounts of fluoride intake is the effective way of reducing dental caries among children and adult. F exposure is not limited only through drinking water but also through foods and beverages (Battaleb-Looie *et al.,* 2013; Malde *et al.,* 2011). Fluoride uptake by plant tissues from uncontaminated soils rarely exceed 30 mg kg^{1} dry mass, where as many plants are quite sensitive to F. It has been reported (Elloumi *et al*., 2005) that due to increase in F content, plant suffers from some physiological changes such as stunted growth, chlorosis, leaf necrosis and leaf tip burning. The plants get affected not only by anthropogenic fluoride sources such as emission from brick kiln industries, aluminum smelters, phosphatic fertilizer industries but also from soil through roots (Jha *et al.,* 2013). The fluoride uptake by plants is dependent on various parameters such as pH, activity of F in soil solution and plant species. Once the fluoride is absorbed through the leaves, it gets translocated to different plant parts via xylematic flow and accumulates there. The visible symptoms of F toxicity such as tip burning and death of the plant was reported in *Allium cepa* grown in highly contaminated soils having >400 NaF mg kg^{-1} soil (Jha *et al*., 2013). Kumar *et al.* (2013) reported the reduction of yield attributing characters, as well as, yield of wheat due to application of higher doses of NaF (100 and 200 ppm).

The principal sources of F available for human consumption are drinking water, foods like milk, fish, meat, tea, fruits, vegetable and cereals (Saravanan *et al*., 2008). Among different foods, vegetables particularly okra (*Abelmoschus esculentus*) is consumed in different forms of cooking in our country. However, uninterrupted uptake of F rich vegetables like okra for longer duration causes dental fluorosis and skeletal fluorosis (Murray *et al*., 1991). Okra is one of the most commonly grown green vegetable in tropical and subtropical India

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responding well to the added nutrients in soil. Information regarding the effect of foliar application of fluoride on growth and yield of okra is scanty. There are no reports in the literature about the effect of graded doses of fluoride on okra crop grown in *Alfisols*. Thus, the present study was designed to generate information regarding the effect of grades doses of F on growth and yield of okra, as well as, microbial activity in soil.

MATERIAL AND METHODS

A field experiment was conducted during *Rabi* 2015-16 and 2016-17 in the Department of Soil Science and Agricultural Chemistry, OUAT, Bhubaneswar, located at 20° 15.967′ N latitude and 80° 48.696′ E longitude and at an altitude of 164 feet above mean sea level. The experiment was conducted in a randomized block design (RBD) with six treatments and four replications. The treatments comprised of T_1 - Control (No fluoride); T_2 - Foliar spray of F @ 100 ppm as Hydrogen Fluoride (HF); T_3 - Foliar spray of F @ 150 ppm as HF; T_4 - Foliar spray of F @ 200 ppm as HF; T_5- Foliar spray of F @ 250 ppm as HF; T_6 - Foliar spray of F @ 300 ppm as HF. The seeds of okra cv. BO-2 (Utkal Gaurav) were sown at a spacing of 45 cm x 30 cm in plots with gross size 6 m^2 (3.0 m x 2.0 m). Full doses of P_2O_5 and K_2O and 50% of N (@ 60:30:30 kg N : P_2O_5 : K_2O per ha^{-1} were applied as basal and rest 50% N was applied at 30 days after sowing. The graded doses of fluoride were prepared from analytical reagent grade of HF. F was applied twice in entire crop growth period in form of foliar spray *i.e.* first at 15 days after sowing (DAS) and second at 30 DAS. The plant height, fruit length, fruit dry weight, chlorophyll content, fruit yield and fluoride uptake from each plot were recorded. The soil samples (0-15 cm) were collected, processed and analysed to determine the physico-chemical properties as per the standard procedures (Page *et al.,* 1982). Microbial biomass carbon (MBC), microbial biomass nitrogen (MBN) and dehydrogenase activity (DHA) of post harvest soil were determined following standard methods as described by Wu *et al.* (1990), Tabatabai (1994) and Casida *et al.* (1964), respectively. The total F in the tissue parts of okra was determined by treating the dried ground samples with 0.1 N perchloric acid and F concentration was measured with the help of ORION 5 star series ion analyzer, using Ion Selective Electrode (ISE). The collected experimental data were analysed statistically (Panse and Sukhatme, 1985).

RESULTS AND DISCUSSION

The soil of the experimental site was sandy clay loam (Sand - 60.8%, Silt - 23.4% and Clay - 15.8%), slightly acidic (pH - 6.16), low in available N (203.8 kg ha^{-1}) and available P (12.9 kg ha^{-1}), where as medium in available K (217.3 kg ha^{-1}) with non-hazardous salt content (EC - 0.25 d Sm^{-1}) medium in organic carbon (5.9 g kg^{-1}) and fluoride content was 0.21 ppm (Table1).

Yield attributing characters

The highest total chlorophyll content of 4.5 mg g^{-1} fresh leaf of okra was recorded (Table 2) with T_1 (No fluoride) where as the lowest value of 2.3 mg g^{-1} fresh leaf was observed with T_6 (foliar spray of F @ 300 ppm as HF). Chlorophyll content of fresh leaf reduced with increased doses of F. However, the chlorophyll content of leaf was significantly different in the F treated plots (T_2, T_3, T_4, T_5 and T_6). The minimum reduction in chlorophyll content was observed to be 24.4% over control due to foliar application of 100 ppm F and the maximum reduction of 48.9% was observed with foliar application of F @ 250 ppm as HF. The present result corroborated with the results of Kumar *et al*. (2013) who reported reduction in chlorophyll content of wheat plant due to addition of graded doses of F as NaF.

Plant height of okra at different growth stages did not differ significantly with application of graded doses of F, though a numerical decrease in plant height was observed with in concentration of F, however it increased with increase in growth stages (Table 2). Significant variation in fruit length of okra was observed in different

Table 1. *Initial soil characteristics of the experimental site*

Parameters	Value
Sand (%)	60.8
Silt (%)	23.4
Clay (%)	15.8
Texture	Sandy loam
B.D. (Mg m^{-3})	1.59
pH	6.16
EC (dS m^{-1})	0.25
Organic Carbon (g kg^{-1})	5.9
Available N (kg ha^{-1})	203.84
Available P (Bray's-I) (kg ha^{-1})	12.85
Available K (kg ha^{-1})	217.28
Fluoride Content (ppm)	0.21
Dehydrogenase activity (mg TPF g^{-1} soils day^{-1})	13.42
MBC (mg g^{-1} dry soil)	147.25
Microbial biomass nitrogen (MBN) (mg g^{-1} dry soil)	76.28

Table 2. *Yield and yield attributing characters of okra as influenced by graded doses of fluoride application (mean value of two years)*

Treatment Details	Chlorophyll content (mg g^{-1} fresh leaf)	Plant height (cm)			Fruit length (cm)	Fruit dry weight (g $fruit^{-1}$)	Fruit yield (q ha^{-1})	Per cent decrease in yield over control	F uptake by fruit (kg ha^{-1})
		30 DAS*	45 DAS	60 DAS					
T_1: Control (No Fluoride)	4.5	34.0	44.2	61.8	18.6	16.7	34.5	—	15.5
T_2: Foliar spray of F @ 100 ppm as HF	3.4	33.6	44.0	60.7	16.2	15.2	26.4	23.5	15.8
T_3: Foliar spray of F @ 150 ppm as HF	3.1	32.9	43.2	61.0	16.0	14.7	25.1	27.2	16.3
T_4: Foliar spray of F @ 200 ppm as HF	2.8	32.7	42.1	60.7	16.0	14.1	22.9	33.6	17.0
T_5: Foliar spray of F @ 250 ppm as HF	2.3	32.1	41.8	58.6	15.8	13.9	22.4	35.1	18.2
T_6: Foliar spray of F @ 300 ppm as HF	2.3	31.8	41.0	58.7	14.7	13.0	21.5	37.7	18.9
SEm (±)	0.12	0.68	0.95	0.96	0.58	0.33	0.79		0.51
CD (P = 0.05)	0.36	NS	NS	NS	1.77	0.99	2.39		1.56

*DAS: Days after sowing

treatments with the highest fruit length of 18.6 cm obtained with T_1 (No fluoride), where as the lowest value of 14.7 cm was recorded with T_6 (Foliar spay of F @ 300 ppm as HF). The fruit length under different fluoride applied treatments was statistically at par. However, the length of the fruits decreased numerically with increasing doses of F. From the result (Table 2) it was observed that the dry weight per fruit varied significantly among the treatments with the highest of 16.7 g obtained in T_1 and the lowest value of 13.0 g was recorded in T_6. Similar results were reported by Kumar *et al*. (2013).

Fruit yield

Significantly highest fruit yield (Table 2) of 34.5 q ha^{-1} was observed with T_1, where as the lowest value of 21.5 q ha^{-1} was observed with T_6. The fruit yield of okra with different fluoride treated plots reduced slightly with increased doses of F. A reduction in fruit yield by 23.5% over control was recorded with 100 ppm F applied treatment and the reduction was increased upto 37.7% with foliar application of 300 ppm F. It was also observed that reduction of yield in wheat was increased with application of increased doses of fluoride (Kumar *et al*., 2013; Dutta *et al*., 2012).

Fluoride uptake by fruit

Data pertaining to F uptake by fruit is presented in Table 2. Results indicated that significantly highest fluoride uptake (18.9 kg ha^{-1}) was observed with T_6, where as lowest value (15.5 kg ha^{-1}) was observed in T_1. It was observed that F uptake by fruit increased due to addition of increase doses of F in form of HF. F uptake by okra at T_1 was statistically at par with the F content due to application of F @ 200 ppm as HF. F uptake of T_1 was significantly lowest than that of T_6.

Microbial activities

Soil samples were collected from different treatments within 24 hours of F application to determine MBC, MBN and enzyme activity. Result pertaining to MBC (Table 3) revealed that it increased with increase in crop growth irrespective of treatments. The lowest MBC was noticed in the treatment where no F was applied. It was observed that, the rate of stimulation was increased with foliar application of F @ 100 ppm to 300 ppm. So far as crop growth period is concerned, stimulation of MBC was more pronounced at 15 and 30 days due to foliar application of fluoride. At 30 DAS the rate of stimulation was more as compared to 15 DAS. There after both inhibition and stimulation effect of microbial biomass carbon to F application in soil was recorded. Impacts of fluoride application with different doses on microbial biomass nitrogen (MBN) are given in Table 4. It was observed that, MBN increased with increase in growth stages of okra crop up to 30 DAS, thereafter a decrease was noted at all concentration except 100 ppm F.

Table 3. *Effect of graded doses of fluoride on soil microbial biomass carbon at different growth stages of okra crop*

Treatment Details	MBC (mg g^{-1} dry soil) at different DAS (mean value of two years)				
	0	15	21	30	42
T_1: Control (No Fluoride)	143.9	148.4	163.5	165.5	167.0
T_2: Foliar spray of F @ 100 ppm as HF	140.5	155.5	160.5	169.3	164.2
T_3: Foliar spray of F @ 150 ppm as HF	142.7	157.0	158.7	172.7	157.7
T_4: Foliar spray of F @ 200 ppm as HF	144.7	160.2	156.5	178.7	155.0
T_5: Foliar spray of F @ 250 ppm as HF	141.1	163.9	155.2	179.6	155.3
T_6: Foliar spray of F @ 300 ppm as HF	144.9	164.6	155.6	182.6	158.2
SEm (±)	2.29	3.70	6.47	3.08	3.56
CD (P = 0.05)	NS	NS	NS	3.53	NS

Table 4. *Effect of graded doses of fluoride on soil microbial biomass nitrogen at different growth stages of okra crop*

Treatment Details	MBN (mg g^{-1} dry soil) at different DAS (mean value of two years)				
	0	15	21	30	42
T_1: Control (No Fluoride)	75.9	76.8	77.1	81.5	84.1
T_2: Foliar spray of F @ 100 ppm as HF	77.5	84.2	81.3	89.3	90.5
T_3: Foliar spray of F @ 150 ppm as HF	75.8	103.5	101.9	106.4	101.1
T_4: Foliar spray of F @ 200 ppm as HF	78.4	98.2	95.3	101.6	94.2
T_5: Foliar spray of F @ 250 ppm as HF	79.4	91.4	94.9	97.8	90.9
T_6: Foliar spray of F @ 300 ppm as HF	76.6	88.1	93.1	94.6	86.5
SEm (±)	1.45	1.01	0.67	0.49	1.45
CD (P = 0.05)	NS	3.05	2.03	1.47	4.37

Table 5. *Effect of graded doses of fluoride on soil dehydrogenase activity at different growth stages of okra crop*

Treatment Details	DHA (mg TPF g^{-1} soil day^{-1}) at different DAS (mean value of two years)				
	0	15	21	30	42
T_1: Control (No Fluoride)	13.9	14.2	14.2	15.1	14.41
T_2: Foliar spray of F @ 100 ppm as HF	13.8	15.2	14.3	16.0	14.77
T_3: Foliar spray of F @ 150 ppm as HF	13.8	15.6	14.8	16.8	15.30
T_4: Foliar spray of F @ 200 ppm as HF	14.2	16.2	15.0	17.2	15.98
T_5: Foliar spray of F @ 250 ppm as HF	14.4	16.8	16.0	17.8	16.52
T_6: Foliar spray of F @ 300 ppm as HF	14.0	17.7	16.5	19.2	17.49
SEm (±)	0.71	0.63	0.44	0.69	0.49
CD (P = 0.05)	NS	1.90	1.33	2.10	1.48

Analysis of soil samples collected from F treated plots at different growth stages indicated a significant stimulation effect on MBN. However, the highest rate of stimulation was observed in treatments receiving F @ 150 ppm and there after the rate of stimulation decreased. It might be due to toxic effect of F on microbial activity in soil beyond 150 ppm. Dehydrogenase enzymes are involved in the oxidation and reduction reactions in soil (Bromfield, 1954) which reflects the metabolic activity of the soil (Salazar *et al*., 2011). Results pertaining to DHA as influenced by fluoride application are presented in Table 5. The stimulation of dehydrogenase activity (expressed as μg TPF formed g^{-1} soil day^{-1}) by fluoride application might be due to release of more enzymes from the dead and living anaerobic microorganisms as the enzymes are both extracellular and intracellular in nature. This result reveals a positive relationship of doses of F application with dehydrogenase activity.

From the present investigation it was concluded that higher concentration of fluoride application decreased crop growth parameters and yield of okra. The application of F @ 100 ppm to 300 ppm as HF stimulated the dehydrogenase activity in soils where okra was grown. Graded doses of F as HF stimulated significantly the accumulation of MBN in soil. Fluoride uptake by fruits was increased due to application of graded doses of fluoride.

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