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Comparative assessment of fluoride tolerance in two genotypes of *Zea mays*

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Abstract
The present study compares fluoride tolerance of two genotypes of *Zea mays* (maize) viz. Kanchan-25 and PHM-1 under fluoride stress. The basis of differential fluoride tolerance in maize genotypes was characterized by analyzing fluoride accumulation pattern, growth ratio and three common antioxidative responses viz., catalase, peroxidase, and glutathione peroxidase enzyme activity. Prolonged fluoride exposure influences various enzymatic activities and affects the overall metabolic processes in plants. Levels of fluoride were found higher in plants of Kanchan-25 (114 µg/g) as compared to PHM-1 (97 µg/g). Two enzymes; catalase and glutathione peroxidase decreased with increasing fluoride stress whereas peroxidase activity increased. This change was more prominently observed in Kanchan-25 variety. Hence Kanchan-25 variety is more sensitive to fluoride stress and should not be grown in fluoride contaminated regions by farmers.

Keywords: Catalase, fluoride accumulation, glutathione peroxidase, peroxidase, *Zea mays*

Introduction
Maize is a Kharif crop which is sown just before the onset of monsoon and is harvested after retreat of the monsoon. It belongs to the family Poaceae. In India, four states; Madhya Pradesh, Andhra Pradesh, Karnataka and Rajasthan contribute more than 50% of maize production. In north-west Rajasthan, fluoride level is high in ground water and soil, due to fluoride-containing minerals and excessive use of diammonium phosphate (DAP) fertilizer on crop fields that releases fluoride into the environment and contaminates the soil [1]. The symptoms of fluoride injury in plants may be acute or chronic which is dependent on the fluoride concentration as well as the period and frequency of the fluoride exposure [2]. Fluoride reduces germination by lowering the enzymatic activity and growth by slowing the rate of cellular division and expansion. Fluoride induced oxidative stress emerged as a key mechanism underlying various toxic effects associated with fluoride exposure causing increased production of reactive oxygen species (ROS) such as superoxide radicals that may damage membrane lipids, cause enzyme inactivation, and increases breakage of DNA strands [3]. It is also known that living systems have evolved an intracellular enzymatic defence system for protection against ROS [4]. Among these, catalase, peroxidase, and glutathione peroxidase are the key enzymes.

Material and Methods
Experimental design
All the chemicals and reagents used were of analytical grade. Surface sterilized seeds of two varieties of *Zea mays*; Kanchan-25 and Pratap Hybrid Maize-1 were grown in soilrite treated with different concentrations of fluoride, viz. 100mg/kg, 200mg/kg and 300mg/kg. One pot without fluoride treatment served as control for both varieties. The plants were watered regularly for proper germination and growth of seedlings. Leaves were harvested after one month for experimental work.

Growth measurements
Growth ratio (GR) was calculated using the following relationship [5]:
GR= [Plant biomass with fluoride]/ [Plant biomass without fluoride] ×100
Determination of Total fluoride Accumulation in Soilrite and Leaf Tissue
Fluoride accumulation analysis of soilrite and leaf samples was done by coupled plasma method. The samples of leaves and soilrite were sent to Shri Ram Laboratories, Ghaziabad, India for the same.

Quantitative Determination of Bioaccumulation Factor
Bioaccumulation Factor (BF) was calculated as follows [6]:
BF= [fluoride concentration in leaves]/ [fluoride concentration in soilrite]

Determination of Catalase Activity
The catalase activity was estimated by the method of Claiborne et al., [7]. 1 gm of tissue sample was taken and grounded in pestle mortar with phosphate buffer at low temperature (4 °C) and then the sample was centrifuged. The supernatant was collected in the tube. In the residue, phosphate buffer was added and extraction was repeated. Combined supernatant was used for the assay. 3 ml H_2O_2-PO_4 was taken and to it, 0.01-0.04 ml of sample was added and final volume was made to 3ml. This was read against a control cuvette containing enzyme solution as in the experiment cuvette but containing H_2O_2 free PO_4 buffer at 240nm. At required time for a decrease in absorbance from 0.45-0.40 was used for calculation. 17/t units in the assay mixture, where t is time required for the decrease in absorbance.

Determination of Peroxidase Activity
Peroxidase activity was estimated as per Reddy et al., [8]. 1 gm of fresh plant tissue was extracted in 3 ml of 0.1M phosphate buffer (pH 7) by grinding in a pre-cold mortar and pestle. Homogenate was centrifuged at 18000 rpm at 5 ^{0}C for 15 minutes. The supernatant was used as enzyme source within 2 - 4 hours. Pipette out 3ml buffer solution, 0.05ml guaiacol solution, 0.1 ml of enzyme extract and 0.03 ml of H_2O_2 in a cuvette. It was mixed well and cuvette was placed in the spectrophotometer. The required time in minutes (Δt) to increase the absorbance by 0.05 was calculated using stop watch. Since the extinction coefficient of guaiacol dehydrogenation product at 436 nm under the conditions specified is 6.39/micromole (μM), the enzyme activity per ml of extract is calculated as:
Enzyme units/ml = activity $3.18\times0.1\times1000/6.39\times1\times\Delta t\times0.1= 500/\Delta t$, where Δt is the change in time.

Determination of glutathione peroxidase
It was estimated by following the method of Mohandas et al., [9].1 gm of leaf sample was taken and homogenized in 5 fold volume of phosphate buffer (pH 9.5). It was then centrifuged at 7000 rpm for 15 minutes. The supernatant was collected. This process repeated once or twice by adding phosphate buffer in the residue. Test tubes were taken and in all the tubes 1.44 ml of phosphate buffer, 0.1 ml of EDTA, 0.1 ml NADPH, and 0.01 H_2O_2 were added. Then 0.1ml of sample was added to all the test tubes except 'blank'. The volume was made up to 2 ml and reading was taken at 340nm.
Glutathione peroxidase activity=$A_{340}\times$volume of reaction mixture/$6.22\times10^6\times$ volume of sample

Results

Effect of fluoride on plant growth
The effect of various fluoride concentrations on shoots and root length in *Zea mays* seedling are shown in terms of growth ratio (GR). The GR of *Zea mays* was evaluated at 3 different concentrations of fluoride viz.100mg/kg, 200mg/kg and 300mg/kg soilrite. The results showed that Growth Ratio has an inverse correlation with fluoride concentration. The GR value gradually declined from 1.0 in control to 0.80 in 300mg/kg fluoride concentration. As we can see from the graph (Fig 1) that the GR decreased more in Kanchan-25 as compared to PHM-1variety. This showed that the PHM-1 variety possessed healthier plants as compared to Kanchan-25 as the fluoride concentration increased.

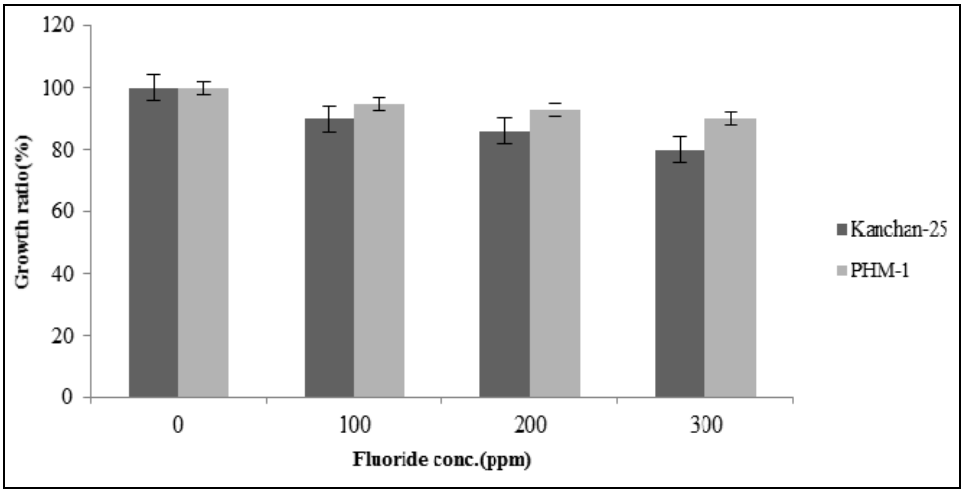

Fig 1: Effect of Fluoride on Growth Ratio of two different varieties of *Zea mays*

Fluoride Accumulation in Soilrite and Plant Leaves
The remaining fluoride in soilrite and fluoride accumulated in leaves of two varieties of *Zea mays* are shown in Fig 2 (a, b). From the figure, it is inferred that the levels of fluoride increased gradually with increase in fluoride treatment. For example, amount of fluoride accumulated in leaves increased from 46.4% at 200μg fluoride g^{-1} to 60% at 300 μg fluoride g^{-1} in Kanchan-25. Similarly, in PHM-1, it was observed that the increase in percentage was from 41.2% to 63.9%. The remaining fluoride content in the soilrite sample increased from 48% to 56% in Kanchan-25 and 30.5% to 32% in PHM-1 with increase from 200 gm fluoride kg^{-1} to 300 gm fluoride kg^{-1} respectively. Overall Kanchan-25 variety accumulated more fluoride in their leaf tissue as compared to PHM-1 variety. This can be attributed to the fact that the Kanchan-25 was procured from the low fluoride content region (Pantnagar, India) and could not adapt to the fluoride rich environment whereas PHM-1 variety belongs to Rajasthan, India and is adapted to high fluoride in soil medium.

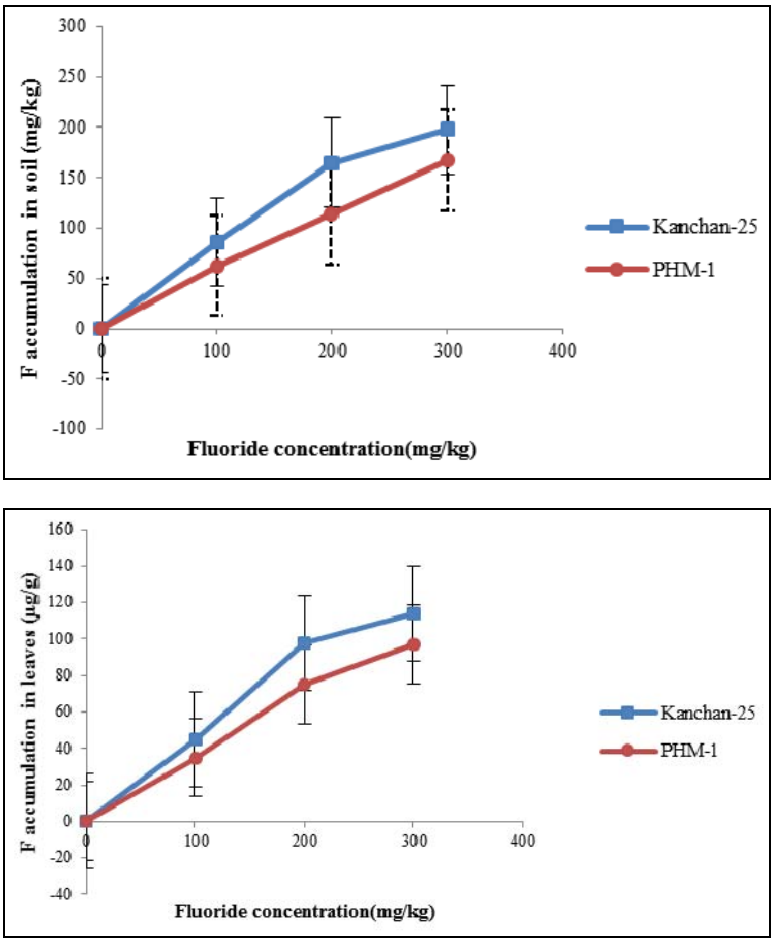

Fig 2: Accumulation of Fluoride in (a) Soilrite and (b) Leaves under F stress

Bioaccumulation Factor

In both varieties, bioaccumulation factor (BF) increased till 200mg/kg of fluoride concentration as compared to control and then decreased at 300mg/kg of fluoride concentration. Bioaccumulation factor of fluoride ranges from 0.15-0.59 suggesting very less accumulation of fluoride from soil (Table 1). In our experiment, BF of fluoride was < 1, which suggested that *Zea mays* could be an excluder of fluoride [10].

Table 1: Bioaccumulation factor in two varieties of *Zea mays* in response to increasing fluoride concentration in soilrite

Fluoride Conc. (mg/kg)	Kanchan-25	PHM-1
Control	0.15	0.15
100	0.52	0.55
200	0.59	0.65
300	0.57	0.58

Effect on Root Length and Shoot Length

As expected shoot and root length decreased with increase in concentration of fluoride [11]. It was found that in Kanchan-25 the highest fluoride concentration (300mg/kg) decreased shoot length by 31.51% whereas in PHM-1 it was 20.20% as compared to control indicating the differential sensitivity (Table 2). Reduction in root length followed the same trend as shoot length. The length of root and shoot decreased more in Kanchan-25 as compared to PHM-1 variety. This may be because PHM-1 is more tolerant to fluoride stress as compared to that of Kanchan-25.

Table 2: Shoot length and root length of Fluoride in leaves and soilrite under fluoride stress

Fluoride Concentration (mg/kg)	Kanchan-25(cm)		PHM-1(cm)	
	Shoot length(cm)	Root length(cm)	Shoot length(cm)	Root length(cm)
Control	10 ± 1.0	43 ± 0.8	11 ± 1.37	39 ± 0.9
100	9.0 ± 0.9	33 ± 0.6	10.7 ± 1.35	34 ± 0.8
200	8.4 ± 0.7	27 ± 0.3	9.6± 1.30	28 ± 0.8
300	7.6 ± 0.5	21 ± 0.2	8.0± 1.29	22 ± 0.6

Effect of fluoride on Catalase Activity

Fig 3 indicates that the catalase activities decreased in both varieties in fluoride exposed *Zea mays* plants relative to controls. The catalase activity in Kanchan-25 decreased from 566.6 unit/gm/fresh wt. (in control) to 531.3, 515.1 and 472.2 unit/gm/fresh wt. in 100 mg/kg, 200mg/kg and 300 mg/kg of fluoride concentration. In PHM-1, the activity of the enzyme decreased from 680 unit/gm/fresh wt. (control) to 566.6 unit/gm/fresh wt. (300 mg/kg F) which was recorded as decrease in activity to about 20% with respect to control.

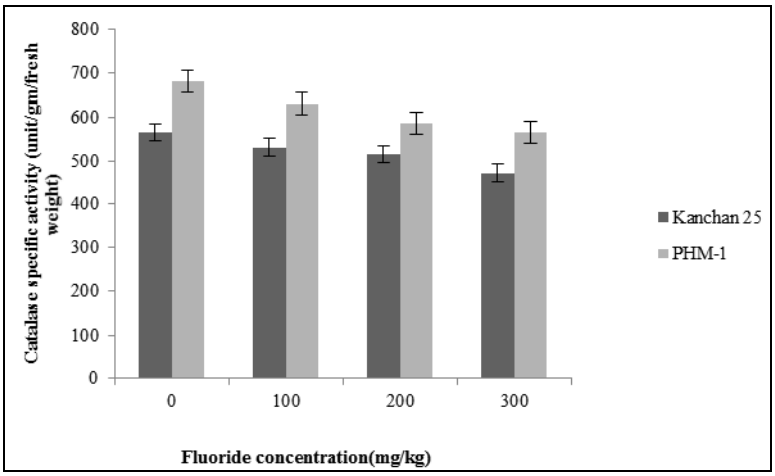

Fig 3: Effect of fluoride stress on catalase activity

Effect of fluoride on Peroxidase Activity

In this study also, a stress intensity dependent increase in peroxidase activity in both varieties was observed (Fig 4). In Kanchan-25 the activity of peroxidase increased to about 16.73% with respect to control whereas in PHM-1 it was 14.28 % increase as compared to control.

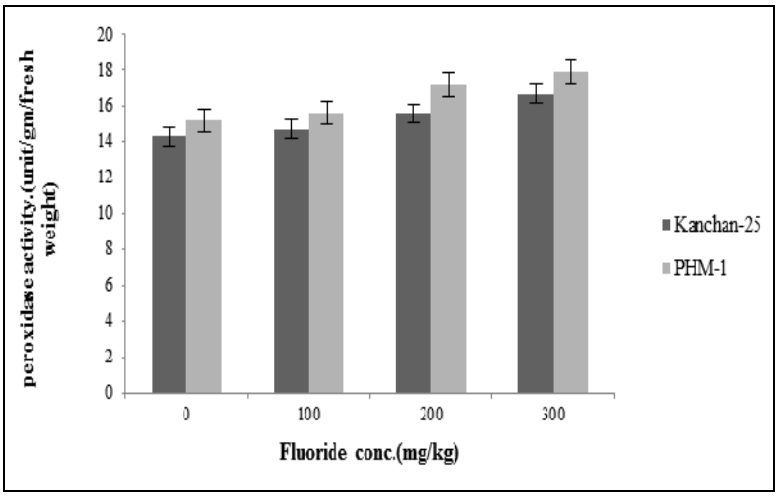

Fig 4: Effect of fluoride stress on peroxidase activity

Effect of fluoride on Glutathione Peroxidase Activity

The activity of glutathione peroxidases decreased significantly from 1.22×10^{-6} to 5.38×10^{-7} unit/gm/fresh wt. in Kanchan-25 and from 1.08×10^{-4} to 7.95×10^{-7} unit/mg/fresh wt. in PHM-1variety (Fig 5).

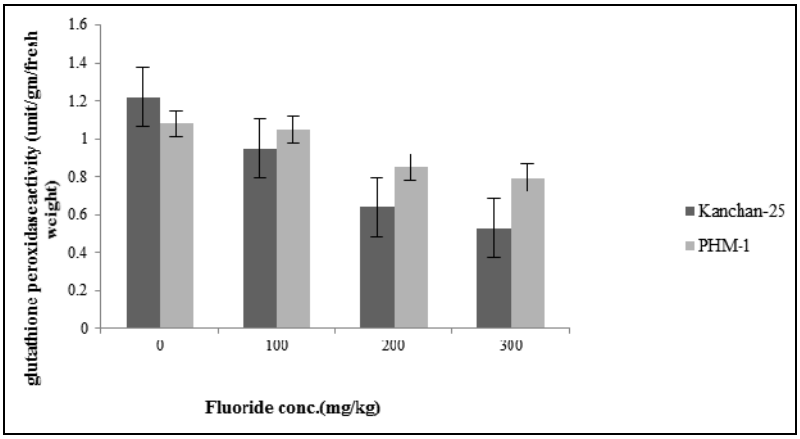

Fig 5: Effect of fluoride stress on glutathione peroxidase activity

The reduction was more in Kanchan-25 (56.6%) as compared to the PHM-1(26.3%). Similar observations were studied by other researchers in which GPX activity decreased but no significant reason was reported yet [12].

Discussion

The results show that the growth ratio was declined with the effect of fluoride. Similar results were obtained in case of wheat (*Triticum aestivum*), Bengal gram (*Cicer arietinum L.*), mustard (*Brassica juncea*) and tomato (*Lycopersicon esculentum*) where there is reported decrease in the growth of roots and shoots with increased concentration of fluoride [13]. The decrease in GR is due to the reason that biomass of the plants decreased as the fluoride concentration increased. This decrease in the growth ratio showed decline in healthy plants.

Bioaccumulation factor was first increased then decreased as compared with control in the present investigation. Bioaccumulation factors increase with increasing soil fluoride concentrations in other researches that were in similar pattern to our result [4].

The root and shoot length decreased in the two varieties of *Zea mays*. Shoot length and root length decreases because of unbalanced nutrient uptake by the plants in the presence of fluoride [14].

Fluoride exposure increases the generation of anion superoxide(O^{2-}) [15, 16]; enhanced O^{2-} concentration and its downstream consequences like hydrogen peroxide, peroxynitrite, hydroxyl radicals appear significantly important in mediating fluoride's effects. Moreover, fluoride increased NO generation [17, 18, 19] and it will react with superoxide to make peroxynitrite, and with thiols and metal centres in proteins to form nitrosyl adducts. It has also been shown interference with disulfide-bond formation and resulting in the accumulation of misfolded proteins within the endoplasmic reticulum (ER) causing ER stress and ROS production.

Extremes of fluoride compounds are known to cause oxidative damage to plants either directly or indirectly by triggering an increased level of production of ROS [1]. These ROS include superoxide radical, hydroxyl (OH^-) and hydrogen peroxide (H_2O_2) [20] and products during membrane linked electron transport activities as well as by a number of metabolic activities [21]. Our results are in conformity with other researches who found that catalase activity is inhibited by fluoride [22, 23, 24]. Researchers concluded that catalase constitutes the iron atoms which have hydroxyl groups that may be replaced by low molecular weight anions, in sufficient concentrations, such that catalase is inhibited.

Peroxidase activity increased significantly in both varieties with increasing fluoride concentrations. The present study indicated that an enhancement in the activity of peroxidase suggest that this enzyme serves as an intrinsic defence tool to resist fluoride induced oxidative damage in *Zea mays* [25]. There are reports which provide evidence that fluoride stress increased the enzyme activities such as catalase, guaiacol peroxidase [26] in drought tolerant as well as in drought sensitive rice cultivars. The activity of peroxidase increased in water stressed seed leaves [27].

Glutathione peroxidase (GPX), like APX, detoxifies H_2O_2 to H_2O, but uses GSH directly as the reducing agent. The regeneration of GSH is made possible by the reduction of GSSG by glutathione reductase (GR), closing the GPX cycle [28]. In general, GR activity increases in plants under oxidative stress. This has been observed in *Raphanus sativus* [29], *Crotalaria juncea* [30], *Beta vulgaris* and *Beta maritime* [31] especially in the leaves. This tendency, however, was not confirmed by our experiment. In our experiment, the GR activity decreases significantly. Similar observations were studied by other researchers in which GPX activity decreased but no significant reason was reported yet [12].

Conclusion

The present study concludes that an increased concentration of fluoride in soilrite inhibits the morphological and biochemical parameters of seedlings with respect to change in seed germination, shoot/root length, antioxidative enzyme activities in both the varieties. However, these changes were less pronounced in PHM-1(Rajasthan) variety as compared Kanchan-25 variety. As PHM-1 variety is more tolerant to fluoride stress, we can conclude that Kanchan-25 variety should not be grown in fluoride rich region as this may induce alternations in metabolism resulting in the reduction in crop field. Further, Kanchan-25 variety accumulated more fluoride in leaf tissues as compared to PHM-1. Leaves of *Zea mays* are generally used as fodder for animals and there is a risk that through animals the excessive fluoride may ultimately reach human beings causing health-related issues [32].

References

1. Bhargava D, Bhardwaj N. Effect of sodium fluoride on seed germination and seedling growth of *Triticum aestivum* Var. Raj. 4083. J Phytol. 2010; 2(4).
2. Choubisa SL. Fluoride toxicosis in immature herbivorous domestic animals living in low fluoride water endemic areas of Rajasthan, India: an observational survey. Fluoride. 2013; 46(1):19-24.
3. Baunthiyal M, Ranghar S. Physiological and biochemical responses of plants under fluoride stress: an overview. Fluoride. 2014; 47(4):287-93.
4. Saini P, Khan S, Baunthiyal M, Sharma V. Organ-wise accumulation of fluoride in *Prosopis juliflora* and its potential for phytoremediation of fluoride contaminated soil. Chemosphere. 2012; 89(5):633-5.
5. Baker AJ. Metal tolerance. New phytol. 1987; 106(s1):93-111.
6. Zhao FJ, Lombi E, McGrath SP. Assessing the potential for zinc and cadmium phytoremediation with the hyperaccumulator *Thlaspi caerulescens*. Plant soil. 2003; 249(1):37-43.
7. Claiborne AL. Catalase activity. CRC handbook of methods for oxygen radical research. 1985; 1:283-4.
8. Reddy KP, Subhani SM, Khan PA, Kumar KB. Effect of light and benzyladenine on dark-treated growing rice (*Oryza sativa*) leaves II. Changes in peroxidase activity. Plant cell Physiol. 1985; 26(6):987-94.
9. Mohandas J, Marshall JJ, Duggin GG, Horvath JS, Tiller DJ. Differential distribution of glutathione and glutathione-related enzymes in rabbit kidney: possible implications in analgesic nephropathy. Biochem Pharmacol. 1984; 33(11):1801-7.
10. Chakrabarti S, Patra KP. Effect of sodium fluoride on seed germination, seedling growth and biochemistry of paddy (*Oryza sativa* L.). Asian J Exp Biol Sci 2013; 4(4):540-4.
11. Rezvani M, Zaefarian F. Bioaccumulation and translocation factors of cadmium and lead in'Aeluropus littoralis'. Australian Journal of Agricultural Engineering. 2011; 2(4):114.
12. Vestena S, Cambraia J, Ribeiro C, Oliveira JA, Oliva MA. Cadmium-induced oxidative stress and antioxidative enzyme response in water hyacinth and salvinia. Braz J Plant Physiol. 2011; 23(2):131-9.
13. Pant S, Pant P, Bhiravamurthy PV. Effects of fluoride on early root and shoot growth of typical crop plants of India. Fluoride. 2008; 41(1):57.
14. Gadi BR, Pooja V, Ram A. Influence of NaF on seed

germination, membrane stability and some Biochemicals content in Vigna seedlings. Journal of Chemical, Biological and Physical Sciences (JCBPS). 2012; 2(3):1371.
15. Garcia-Montalvo EA, Reyes-Perez H, Del Razo LM. Fluoride exposure impairs glucose tolerance via decreased insulin expression and oxidative stress. Toxicol. 2009; 263(2):75-83.
16. Izquierdo-Vega JA, Sanchez-Gutierrez M, Del Razo LM. Decreased in vitro fertility in male rats exposed to fluoride-induced oxidative stress damage and mitochondrial transmembrane potential loss. Toxicol Appl Pharmacol. 2008; 230(3):352-7.
17. Liu G, Chai C, Cui L. Fluoride causing abnormally elevated serum nitric oxide levels in chicks. Environ Toxicology Pharmacol. 2003; 13(3):199-204.
18. Hassan HA, Yousef MI. Mitigating effects of antioxidant properties of black berry juice on sodium fluoride induced hepatotoxicity and oxidative stress in rats. Food Chem Toxicol. 2009; 47(9):2332-7.
19. Sireli M, Bulbul A. The effect of acute fluoride poisoning on nitric oxide and methemoglobin formation in the Guinea pig. Turk J Vet Anim Sci. 2004; 28(3):591-5.
20. Stevens DP, McLaughlin MJ, Alston AM. Phytotoxicity of the fluoride ion and its uptake from solution culture by Avena sativa and Lycopersicon esculentum. Plant Soil. 1998; 200(2):119-29.
21. Singh S, Singh J, Singh N. Studies on the impact of fluoride toxicity on growth parameters of Raphanus sativus L. Indian J Sci Res. 2013; 4(1):61
22. Ali MB, Hahn EJ, Paek KY. Effects of temperature on oxidative stress defense systems, lipid peroxidation and lipoxygenase activity in Phalaenopsis. Plant Physiol Biochem. 2005; 43(3):213-23.
23. Beers Jr RF. Equilibrium Inhibition of the Catalase–Hydrogen Peroxide System during the Steady State. J Phys Chem. 1955; 59(1):25-30.
24. Cho UH, Seo NH. Oxidative stress in *Arabidopsis thaliana* exposed to cadmium is due to hydrogen peroxide accumulation. Plant Sci. 2005; 168(1):113-20.
25. Csiszár J, Szabó M, Erdei L, Márton L, Horváth F, Tari I. Auxin autotrophic tobacco callus tissues resist oxidative stress: the importance of glutathione S-transferase and glutathione peroxidase activities in auxin heterotrophic and autotrophic calli. J Plant Physiol. 2004; 161(6):691-9.
26. El-baky A, Hanaa H, Amal A, Hussein MM. Influence of salinity on lipid peroxidation, antioxidant enzymes and electrophoretic patterns of protein and isoenzymes in leaves of some onion cultivars. Asian J Plant Sci. 2003; 2(8):633-8.
27. Pelter GQ, Mittelstadt R, Leib BG, Redulla CA. Effects of water stress at specific growth stages on onion bulb yield and quality. Agr Water Manag. 2004; 68(2):107-15.
28. Apel K, Hirt H. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol. 2004; 55:373-99.
29. Vitória AP, Lea PJ, Azevedo RA. Antioxidant enzymes responses to cadmium in radish tissues. Phytochemistry. 2001; 57(5):701-10.
30. Pereira GJ, Molina SM, Lea PJ, Azevedo RA. Activity of antioxidant enzymes in response to cadmium in Crotalaria juncea. Plant Soil. 2002; 239(1):123-32.
31. Bor M, Özdemir F, Türkan I. The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet Beta vulgaris L. and wild beet Beta maritima L. Plant Sci. 2003; 164(1):77-84.
32. Choubisa SL, Choubisa L, Choubisa D. Osteo-dental fluorosis in relation to nutritional status, living habits, and occupation in rural tribal areas of Rajasthan, India. Fluoride. 2009; 42(3):210.