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Effects of fluoride on germination, early growth and antioxidant enzyme activities of legume plant species *Prosopis juliflora*

Poonam Saini, Suphiya Khan, Mamta Baunthiyal and Vinay Sharma*

Department of Bioscience and Biotechnology, Banasthali University, Banasthali Vidyapith-304 022, India

**Corresponding Author email : vinaysharma30@yahoo.co.uk*

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Abstract

Prosopis juliflora (Mimosoideae) is a fast growing and drought resistant tree of semi-arid region of India where fluoride (F) toxicity is a common problem. In the present investigations this species was fluoride tested to check their capacity as bioindicator plant and its efficiency to accumulate. To achieve this aim, *P. juliflora* seedlings grown in hydroponic culture containing different concentrations of F were analyzed for germination percentage together with some biochemical parameters viz, antioxidant enzyme activities, total chlorophyll and accumulation of F in different plant parts. After 15 days of treatment, root growth ($r = -0.928$, $p<0.01$), shoot growth ($r = -0.976$, $p<0.01$), vigor index ($r = -0.984$, $p<0.01$) were in decreasing trend with increasing concentration of NaF. Both catalase (3.2 folds) and peroxidase (2.7 folds) enzymes activity increased with increase in F concentration. Plant accumulated larger portion of the F in the roots (1024.63 µg g^{-1} d.wt.) followed by shoot (492.30 µg g^{-1} d.wt.). As *P. juliflora* did not show any morphological changes (marginal and tip chlorosis of leaf portions, necrosis and together these features are referred to as leaf "tip-burn") therefore, this species may be used as suitable bioindicator species for potentially F affected areas. Further, higher accumulation of F in roots indicates that *P. juliflora* is a suitable species for the removal of F in phytoremediation purposes.

Key words

Fluoride toxicity, Antioxidants, *Prosopis juliflora, Bioindicator*

Introduction

Phytotoxicity due to fluoride (F) is one of the severe ecological problems in the world. Several reports suggests that, F affect the growth of plants and soils (Horner and Bell, 1995; Bhargava and Bhardwaj, 2010). Such an environment is not totally devoid of flora and fauna, as a number of species have adopted to tolerate increased level of F. Presence of plants in these contaminated soils necessarily implies that they are tolerant to toxic levels of F and can accumulate them to a high cellular concentration. Several plant species are well known indicators of metal pollution in soil (Al-Qahtani, 2012). Honey mesquite (*Prosopis juliflora*), is also an indicator species of salinity, alkalinity and fluorine in waters in areas where fluorosis is an endemic disease (Rashid and Abbas, 2011). *P. juliflora* is a highly esteemed fuel wood source in several tropical countries like India, a valued tree for shade, timber and forage. It is widely populated in arid and semi arid regions of Rajasthan, India, the region which is highly endemic to F (Yadav *et al*., 2009). It is proved to be the only exotic species capable of growing in a wide variety of soils and climatic conditions. Moreover, it is an ideal species for afforestation and helps in the reclamation of waste lands, stabilizing, shifting sand which obstructs the railroad (Kumar *et al.,* 2005).

The phytotoxicity of F depends on its concentration in the atmosphere, duration of exposure and sensitivity of the plant species (Weinstein and Davison, 2004). Fluorine and many fluorides, viz sodium and potassium fluorides (NaF/KF) are extremely poisonous. High concentrations of

these compounds can occur in acid soils, which are contaminated with pollutants coming from the atmosphere (Fornasiero, 2001). The importance of seed germination in plant growth is widely recognized, and the effects of F toxicity on it have been studied by various researchers (Stanley *et al.,* 2002**;** Sabal *et al.,* 2006). Certain physiological processes are known to be markedly affected by F including seed germination, early root and shoot growth, chlorosis, leaf tip burn and leaf necrosis (Weinstein and Davison, 2004). Under optimal conditions, many metabolic processes occurring in plants produce active oxygen species which, besides their deleterious potential may act as signaling molecules (Cristina *et al.,* 2008). Soil salinity increases the catalase and peroxidase activity among tolerant and sensitive varieties of plant (Vaidyanathan *et al.,* 2003). The relationship between salinity and antioxidants indicate that O^-_2 radical and H_2O_2 could play an important role in the mechanism of adaptation (Li, 2009; Chookhampaeng, 2011). Peroxidase (POX) is widely distributed in all higher plants and protects cells against the destructive influence of H_2O_2 by catalysing its decomposition through oxidation of phenolic substrates (Lin and Kao, 2002). Catalase (CAT) is present in the peroxisomes of nearly all aerobic cells and is virtually absent from chloroplast (Vellosillo *et al*., 2010). It can protect the cell from H_2O_2 by catalyzing its decomposition into O_2 and H_2O (Foyer and Noctor, 2000).

In the present investigations, this legume species is tested to check their capacity to use as suitable bioindicator plant and their potential use in the reclamation of F contaminated soils. To achieve this aim, the study was done to assess the effect of NaF on the germination, early root and shoot growth, moisture percentage, vigor index, antioxidant enzyme activities (peroxidase and catalase), total chlorophyll content and accumulation of F in different plant parts of *P. juliflora* seedlings.

Materials and Methods

Plant material and growth conditions : Seeds of *P. juliflora* were procured from Central Arid Zone Research Institute (CAZRI), Jodhpur, India. Surface sterilized and imbibed seeds were germinated on wet filter paper in petri plates. After 7 days, the germinated seeds were grown hydroponically in Hoagland nutrient solution in petridishes at different NaF concentration. Stock solution was prepared by dissolving 221 mg NaF in 1 l of deionized water (100 mg l^{-1}). Appropriate dilutions were made to give 10, 20, 30, 40 and 50 mg l^{-1} NaF concentration. Control experiments were also set up in identical manner with deionised water devoid of NaF. Throughout, the germinating seeds were maintained in a growth chamber at a temperature 30±2^{0}C, light intensity of 1000 lux with 14 hrs photoperiod and 70% relative humidity. Plantlets were harvested after the 15th day of F exposure. Vigor index was calculated as per equation of Anderson and Abdul- Baki (1973).

Enzymes assay : One g of fresh mass of plantlets was ground with a pestle in an ice-cold mortar with 10 ml of 50 mM sodium phosphate buffer (pH = 7.0). The homogenates were filtered through 4 layers of cheese cloth and then centrifuged at 4°C for 10 min at 10000 rpm. The supernatants were used for the assays of enzyme activities. CAT activity (EC 1.11.1.6) was determined by measuring the change of absorbance at 240 nm that accompanied the consumption of H_2O_2 (Luck, 1974). CAT activity was defined as the amount required to decompose 1 nmol H_2O_2 min mg^{-1} $fresh^{-1}$ weight. POD activity (EC 1.11.1.7) was determined as oxidation of guaiacol by H_2O_2 (Putter, 1974). The absorbance at 420 nm was measured. One unit of POD activity was defined as the amount required to decompose 1 µmol guaiacol min^{-1} mg^{-1} f. wt.

Estimation of total chlorophyll: Total chlorophyll content in *Prosopis juliflora* leaves were carried out spectrophoto metrically by the method of Arnon (1949).

Determination of fluoride: The total F content in different parts of *P. juliflora* was determined by using the method of alkali fusion-ion selective technique (McQuaker and Gurney, 1977). After 15 days of treatment, roots/shoots and plantlets were harvested for studying parameters various morphological such as percent germination, root length, shoot length, and biochemical parameters such as antioxidant enzyme activities, chlorophyll content and F accumulation.

Standard deviation (SD) and Spearman's correlation were calculated by statistical software package (SPSS 17.0) to examine the differences between each treatment and the level of statistical significance was set at $p \leq 0.01$ of the experimental data.

Results and Discussion

The effects of F on plants are complex because they are involved with biochemical reaction (Kumar and Rani, 2011). There also appear to be a general reduction in growth and reduced seed germination. Seed germination, moisture percentage and vigor index (Table 1) showed a decreasing trend in germination percentage with increasing concentration of NaF. As shown in Table 1, under control conditions 76% germination had occurred, whereas at 50 mg l^{-1} NaF, the germination decreased to 73%. Differences observed in percentage germination exhibited inverse relationship with increased

Table 1 : Effect of NaF on germination, moisture percentage, root length, shoot length and vigor index in *Prosopis juliflora* seedlings after 15 days of treatment

Concentration (mg l^{-1})	Germination (%)	Moisture (%)	Root length (cm)	Shoot length (cm)	Vigor index
Control	76.2 ± 0.14	76.7 ± 0.09	7.8 ± 0.12	6.2 ± 0.21	1066.8
10	75.8 ± 0.24	75.2 ± 0.04	7.2 ± 0.64	5.8 ± 0.34	985.40
20	75.2 ± 0.31	74.6 ± 0.12	7.4 ± 0.34	5.5 ± 0.41	970.08
30	75.1 ± 0.54	73.1 ± 0.14	7.1 ± 0.76	5.3 ± 0.23	931.24
40	74.4 ± 0.21	72.6 ± 0.06	6.8 ± 0.03	5.2 ± 0.17	892.80
50	73.0 ± 0.26	71.4 ± 0.08	6.7 ± 0.18	5.0 ± 0.25	854.10

Values are mean of three replicates ± SD of three samples

fluoride concentration in *Solanum melongena* due to its toxic effects (Siddhu *et al.,* 2008). Moisture percentage and vigor index also showed a decreasing trend with increasing F concentration.

Similar results were reported in *Cyamopsis tetragonoloba* (Sabal *et al.,* 2006), *Oryza sativa* (Gupta *et al.,* 2009) and *Triticum aestivum* (Bhargava and Bhardwaj, 2010). This has been attributed to reduction of metabolic activity in presence of fluoride as the latter acts as a metabolic inhibitor. The increasing concentrations of NaF showed phytotoxic effects on physiology and biochemical parameters of seedling growth. F might affect some developmental processes in germinating cereals. Weinstein (2004) suggested that NaF might inhibit carbohydrate metabolism of germinating seedlings. In addition, at 50 mg l^{-1} the moisture content in the leaves was found to be 71.4% which suggests that increasing level of NaF causes reduction in elongation of root and shoot length and thus also reduction in their moisture percentage and vigor index. Correlations with increasing concentrations of NaF were significant in case of germination percentage ($r=-0.954$, $p<0.01$), moisture percentage ($r = -0.992$ $p<0.01$) and vigor index ($r = -0.984$, $p<0.01$) in *P. juliflora.*

In the present study, after 15 days of F treatment, decreasing trend in the growth of roots and shoots with increasing concentration of NaF was found. The correlation analysis has confirmed a decreasing trend in growth of roots and shoots with increasing concentration of NaF in all the seedlings. Correlations with increasing concentrations of NaF were significant in case of root ($r = -0.928$, $p<0.01$) and shoot ($r = -0.976$, $p<0.01$) in *P. juliflora* plant. As seen in Table 1, the average root and shoot length decreased with increasing F concentration. In this study, the shoot system was affected more by higher concentrations of NaF in comparison to roots. Fluoride causes reduction in root length and shoot length due to unbalanced nutrient uptake by seedlings in presence of fluoride (Sabal *et al.,* 2006).

The hydrogen peroxide is toxic for plants as it acts both as an oxidant as well as a reductant. The hyperactivity of POD under metal stress indicated the scavenging activity of H_2O_2 generated through activity of photo respiration in plant cells. Therefore, an increase in POD activity prevents plants from toxic effects of H_2O_2 (Ali *et al.,* 2003). During the present study, enhancement of POD activity suggests its role in constant detoxification of H_2O_2 in F toxicity. The higher activity of antioxidant enzymes might be consequences of the strategies adapted by plant for its survival under stress imposed by F. Peroxidase activity increased with increase in F concentration. However, highest increase was observed at 40 mg l^{-1} F concentration where a maximum of 2.3 fold increase in activity was seen. Therefore, there was a direct correlation between F concentration and peroxidase activity ($r = 0.64$, $p<0.01$).

Catalase scavenges H_2O_2 by breaking it down directly to form water and oxygen. Catalase activity increased steadily with increase in F concentration, at 50 mg l^{-1} the increase was 3.2 folds. There was a significant correlation ($r = 0.969$, $p<0.01$) between increase in CAT activity and NaF concentration. Similar trends in increase in CAT activity were also obtained with increase in salinity levels in *Triticum aestivum* (Heidari, 2009). The higher CAT activity indicates that F tolerant lines had better ability to scavenge H_2O_2. From the results, it is evident that *Prosopis juliflora* treated with different concentrations of F showed an increase in CAT and POD activites. Antioxidants are well known to play a prominent role in the defense against free radicals in plants. Although H_2O_2 takes part in several important functions in plant cells such as cell wall lignification, protein cross linking, and signal transduction (Low and Merida, 1996), control of H_2O_2 build-up is essential to prevent oxidative damage to membranes and proteins. Catalase scavenges H_2O_2 by breaking it down directly to form water and oxygen while peroxidase decomposes H_2O_2 by oxidation of phenolic compounds. The increased activities of catalase and peroxidase in *P. juliflora* suggest that the plant depend on these antioxidative enzymes for elimination of H_2O_2 under

F stress. As seen in (Fig. 1), the increase in CAT and POD is strongly correlated with F ion concentration. This can be compared with an earlier report on cadmium effect on enzyme activity in *Colocassia esculentum* (Mandakini *et al.,* 2005). El-baky *et al.* (2003) reported that salt stress increased the enzyme activities such as catalase, guiacol peroxidase, ascorbic acid oxidase, polyphenol oxidase. Antioxidants are well known to play a prominent role in defense against free radicals in plants. Fluoride toxicity causes oxidative stress in plant leaves and the antioxidant enzymes appear to play a key role in counteracting the oxidative stress in plants. This is understable as, unlike iron, fluoride is not an oxido-reducing non metal, the oxidative stress induced by fluoride in plants appear to be an indirect effect of fluoride toxicity leading to production of reactive oxygen species with simultaneous increase in tissue levels of antioxidant enzymes (Kumar *et al.,* 2009).

One of the early symptoms of fluoride damage in plants is a loss of chlorophyll, which seems to be related to the destruction of chloroplasts. As showed in (Fig. 2), the total chlorophyll content of the leaves was significantly decreased with increasing nutrient F concentration. The results obtained in this study is in agreement by those of Jaleel *et al*. (2008). Reduction in chlorophyll concentrations is due to the break down of chlorophyll during stress or due to inhibition of incorporation of δ-aminolevulinic acid into chlorophyll synthetic pathway (Ali, 2004; Bhargava and Bhardwaj, 2010). The mechanisms by which fluoride depressed apparent photosynthesis are not clear, but suggested mechanisms of fluoride effects include enzymatic inhibitions, loss of sub-cellular organization and granulation of chloroplasts (Kumar and Rao, 2008).

The F treated *P. juliflora* seedlings accumulated 1024.63 µg g^{-1} d. wt. in roots 492.30 µg g^{-1} d. wt. F in shoots. Thus, there was higher accumulation of F in roots than shoots in hydroponics grown plants (roots > shoot) (Fig. 3). In the present work, higher accumulation of the F was observed in the roots of *P. juliflora.* Earlier, Jha *et al.* (2009) (*Allium cepa* L.) reported greater accumulation of F in roots than shoots. The higher accumulation of F occurs in roots, could be due to the fact that the soluble fluoride fraction in soil is taken up passively by roots as the non-ionic form at low pH, which is more readily taken up by cell membranes (Horner and Bell, 1995).

Overall, the study concludes that fluoride had significant impact on seed germination and seedling growth of *P. juliflora*. It adversely affected various growth parameters, which reduced the plant general fitness prior to or in the absence of the appearance of any visible injury. Thus, this legume species may be used as a suitable

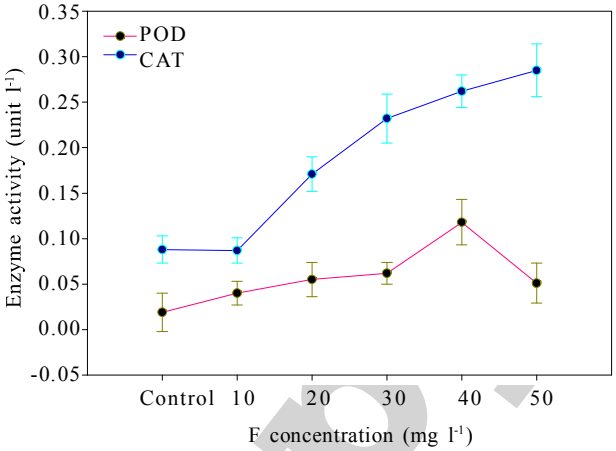

Fig. 1 : Activities of POD and CAT in *Prosopis juliflora* after 15 days of NaF treatment. Values are mean of three replicates ± SD

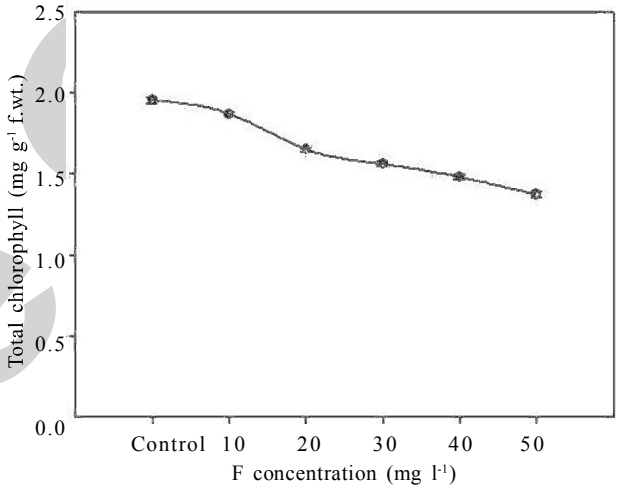

Fig. 2 : Total chlorophyll content in *Prosopis juliflora* after 15 days of NaF treatment. Values are mean of three replicates ±SD

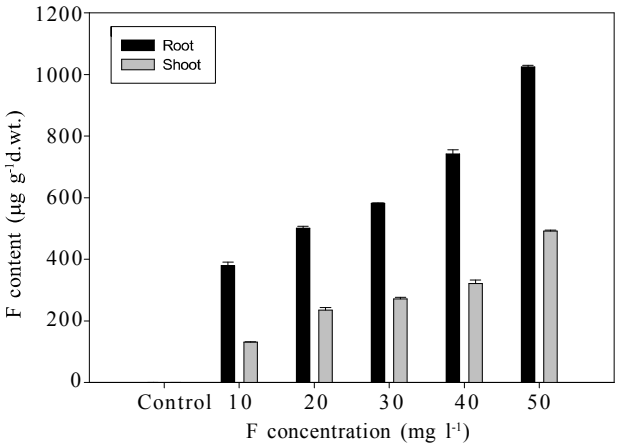

Fig. 3 : F content in roots and shoots of *Prosopis juliflora*, after 15 days NaF treatment. Values are mean of three replicates ± SD

bioindicator for potentially F affected areas, in absence of necrosis. Higher accumulation of F was found in roots than shoots in hydroponics grown seedling. Higher antioxidant activities with increase in F concentrations suggest a role in imparting tolerance to fluoride stress in *P. juliflora*. Further study on this plant will suggest a view of its ability to hyperaccumulate F and its possible use for phytoremediation purposes.

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