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Effect of Fluoride Stress on Phenolics of Medicinally Important Plant *Simarouba glauca* DC

KEYWORDS: Fluoride, Polyphenol, Flavonoids, Anthocyanin and Simarouba glauca

Mali V.V.	Bhoite A.S.	D.K.Gaikwad
Department of Botany, Shivaji University, Kolhapur, 416004 (MS) India.	Department of Botany, Shivaji University, Kolhapur, 416004 (MS) India.	Department of Botany, Shivaji University, Kolhapur, 416004 (MS) India.

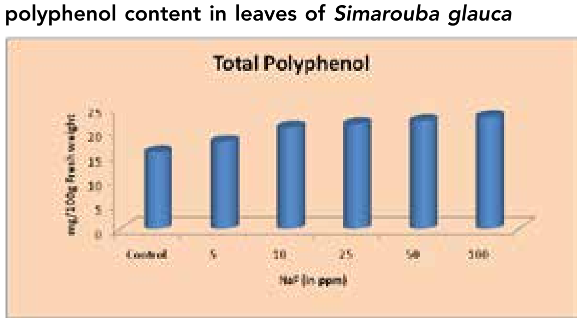
ABSTRACT *The sporadic incidence of high fluoride content in ground water reserve has been reported from various parts of world. The abnormal level of fluoride in phreatic groundwater causes serious health hazards to the human beings. Hence, it is essential to screen the plants for reclamation of such contaminated waters. The present paper reports the influence of various concentrations of fluoride on plant growth and metabolism. It was noticed that, the seedlings of Simarouba glauca shows tolerance to various concentrations of fluoride (5ppm, 10ppm, 25ppm, 50ppm, 100ppm.). The stable growth of Simarouba glauca has been reflected changes in secondary metabolites. It was reported that the total polyphenols, anthocyanins and flavonoid contents were increased in response to fluoride stress. This might be due to induction of secondary metabolites under stress condition which will be beneficial for induction of fluoride stress tolerance. Thus, the induction of secondary metabolites due to stress will improve the bioactive potential of this medicinally important plant.*

Introduction:
Fluoride is found in soil, water, air and plants with varying concentrations but is non-essential for normal growth of plants (Weinstein and Davison 2004). Fluoride occurs in the earth surface in very low amount but it act as environmental pollutant (Jacobsan *et al*.1966). Plant as well as animal shows the fluoride toxicity and act as potent metabolic inhibitor.Polyphenols are the aromatic compounds containing one or more phenol units. These are broadly divided into different catagories such as flavonoids, tannins, phenylpropanoids, hydroxyl cinnamate, esters, benzoic acid derivatives, lignins etc. According to Croteau *et al*. (2000), most but not all the plant phenolics are products of phenyl propanoid metabolism. These secondary metabolite primarily from products of the Shikimic acid pathway. Flavonoids are one of the major groups of phenolic compounds present in the plants. Anthocyanins are the pigmented flavonoids present in the plants which impart red, pink purple and blue colors to different plant parts. These compounds play a significant role in antioxidant metabolism and eliminates reactive oxygen species. On the other hand *Simarouba glauca* is medicinally important oil yielding plant tolerating variety of environmental conditions. Gaikwad and Pawar (2012) noticed 0.1 to 29 ppm fluoride content in the aquifers from sindhudurg district during post monsoon season while surface saline water of the rivers of sindhudurg district contains 4 to 1104ppm fluorides during post-monsoon season. They concluded that high fluoride content of river water is due to fishery industries located near the banks. Hence, an attempt has been made to study the effect of fluoride stress on Polyphenolic compounds.

Material and Methods:
The effect of fluoride stress on one year old seedlings of *Simarouba glauca* DC were studied by applying 5ppm, 10ppm, 25ppm, 50ppm, 100 ppm sodium fluoride treatment twice in a weak alternating with watering for a period of 40 days. The leaves from each treatment were randomly selected and washed throughly with water and blotted to surface dry. The total polyphenols , flavonoids and anthocyanin were estimated following the methods of Ragazzi and Veronese (1973), Luximon-Ramma *et al.* (2002) and Murray and Hackett (1991) respectively and the results are expressed in terms of mg/100g fresh weight.

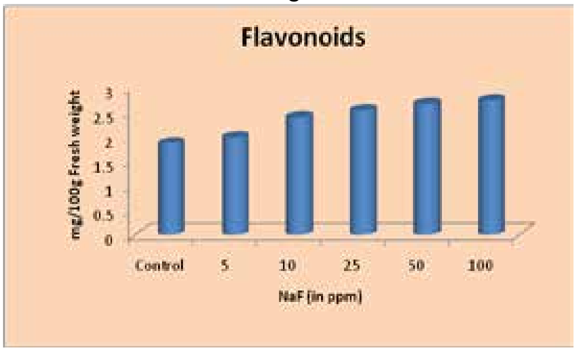
Result and Discussion:
The total polyphenols in leaves of *S. glauca* increases significantly with increasing NaF treatments (5ppm, 10ppm, 25ppm, 50ppm, 100ppm.).(Fig. No. 1). Polyphenols are known to play variety of roles including tolerance to biotic and abiotic stresses (Chalker-Scott, 2002; Wahid and Ghazanfar, 2006 and Wahid, 2007). Some workers reported changes in phenolic compounds in response to salinity stress. The level of phenolic acids such as p-coumaric acid, chlorogenic acid, caffeic acid and gallic acid was increased under saline conditions in the lentil (Amarowicz *et al*., 2009 and Giannakoula *et al*., 2012). However, moderate salinity may cause an increase in phenolic acids in plants. Li and Ni (2009) reported decline in polyphenols with increasing fluoride treatments in leaves of *Camellia sinensis.* In the present study increase in the content of total polyphenol under fluoride stress was observed. This increase in phenolic content clearly indicates a stimulation of secondary metabolism due to fluoride stress and probable involvement of the phenolic compounds in the defense against oxidative stress developing due to fluoride stress.

Fig. No. 1 Effect of sodium fluoride stress on Total polyphenol content in leaves of *Simarouba glauca*



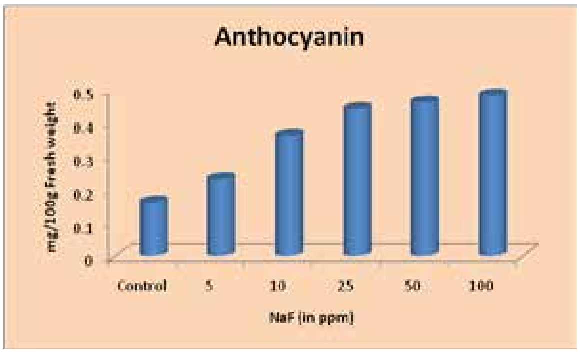
The flavonoid content increased considerably in leaves of *Simarouba glauca* with increasing exposure to sodium fluoride. (Fig. No. 2). Ali and Abbas (2003) studied effect of salt stress (50 and 100 mM NaCl) on flavonoid content of shoots and roots of barley. They noticed significant increase in flavonoid content of roots and shoots of barley in response to salt stress. Total flavonoid contents of the *Paulownia* clones (TF 01 and EF 02) were increased significantly with increasing salt stress (100 and 200 mg/L NaCl treatment). Rezazadeh *et al.* (2012) studied effect of increasing salinity on the flavonoid contents of the *Cynara scolymus* under field conditions and in the pot culture experiments. They found an increase in flavonoid contents in the field conditions upto 6.45 dSm^{-1} salinity then there was decline in flavonoid content (29 dSm^{-1}). The flavonoid level is increased due to fluoride stress which may contribute to the antioxidant metabolism in fluoride stressed leaf tissue of *Simarouba glauca.*

Fig. No. 2 Effect of sodium fluoride on flavonoid content in leaves of *Simarouba glauca*



The anthocyanins were to noticeably increased in response to fluoride stress in leaves of *Simarouba glauca*. (Fig. No. 3). Eryilmaz (2006) reported increasing salt stress enhanced the total anthocyanin content of tomato and red cabbage seedlings. A long term salt treatment increased the anthocyanin contents of the Strawberry plant (Keutgen and Pawelizik, 2008).In the present study we noticed increase in anthocyanin content under fluoride stress. Thus the protective role of anthocyanin under fluoride stress conditions is apparent in leaves of *Simarouba glauca*.

Fig. No.3 Effect of sodium fluoride on anthocyanin content in leaves of *Simarouba glauca*



Phenols are responsible for the antioxidant activity of many plants (AL-Fartosy, 2011). Polyphenols were positively/ strongly correlated with antioxidant potential in various antioxidant assays (DPPH radical scavenging assay, FRAP assay, Lipid peroxidation assay, H_2O_2 radical scavenging assay, •OH radical scavenging assay) reported by different workers (Dudonne *et al.*, 2009, and Kiessoun *et al.*, 2010). Yen *et al.* (1993) noticed that phenolic compounds are effective donors of Hydrogen atom or electron donors, from the phenolic structures, showing its redox property which enables them to act as potent antioxidants. and scavenge variety of free radicals including oxy-radicals such as hydroxyl radicals (Rafat Hussain *et al.*, 1989), superoxide anion radicals (Afanaslev *et al.*, 1989), lipid peroxyl radicals (Magali *et al.*, 2008).The plant membranes are susceptible to stress causing oxidation of membrane lipids which leads to leaky membranes exhibiting susceptible nature of plants to stresses. In the present study increased level of polyphenolics is evident due to fluoride stress. This increased level of phenolics might be responsible for scavenging of free radicals produced during fluoride stress, which will be helpful for induction of fluoride stress tolerance of this tree species. The indication of secondary metabolites under stress condition improve the medicinal and bioactive potential and to protect the plants from oxidative damage. Further the seedlings of *Simarouba glauca* might be used for plantation in fluoride affected areas to avoid abnormal level of fluorides in phreatic ground water.

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