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STUDIES ON EFFECT OF SODIUM FLUORIDE ON SEED GERMINATION, SEEDLING GROWTH AND PHOTOSYNTHETIC PIGMENTS IN RAUVOLFIA TETRAPHYLLA, L.

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Fluoride could be a toxic substance present within the air, water, soil, and Industrial growth, moreover as human activities, are accountable for increasing its level within the environment that inhibit plant growth and productivity. In view of this, present study was undertaken to analyze the results of salt Sodium fluoride (NaF) on seed germination, seedling growth, and photosynthetic pigments in *Rauvolfia tetraphylla*, L. using (0.0, 0.5, 1.0, 1.5 2.0, 2.5 and 3.0 mg/L) NaF concentrations. At the tip of fortnight treatment period, reduction in germination parameters (i.e. germination %, emergence %, and emergence rate and vigor index) were found more pronounced in *Rauvolfia tetraphylla*. The identical trend was also observed for mobilization efficiency (ME) which decreased in R. tetraphylla seedlings at higher concentrations. During early seedling growth, the basis and shoot length, and biomass of *R. tetraphylla* seedlings were compromised with increasing NaF level. However, in contrast to *R. tetraphylla*, root and shoot length were stimulated at 1.0mg/L NaF concentration and 1.5 and 2.5 mM NaF stimulated root and shoot biomass in *R. tetraphylla*. Higher NaF levels turned inhibitory to length and biomass of root and shoot tissues in *R. tetraphylla*. Estimation of photosynthetic pigments in *R. tetraphylla* revealed a rise in total chlorophyll, chlorophyll and b, and carotenoids at (1.5 and 2.0) mg/L over control while the synthesis of these pigments was reduced within the case of *R. tetraphylla* with increasing NaF concentrations. This study revealed *R. tetraphylla* to be more liable to the toxic nature of fluoride (F).

Keywords: *R. tetraphylla*, NaF, Seed germination, Seedling height. Seedling weight and photosynthetic pigments

Introduction:

To most plants, fluoride (F) is phytotoxic through altering a series of metabolic pathways.1-3 Fluoride are often deposited into the soil from several anthropogenic sources, both directly through phosphate fertilizers or indirectly through atmospheric pollution from industrial activities and therefore the burning of fossil fuels. (Arnesen 1998). Effects from the soil, F is absorbed by plant roots then transported via xylematic flow to the transpiratory organs, mainly the leaves, where it can accumulate with adverse effects that are described within the literature. (Klumpp 1996 and Davison 1998). The speed at which symptoms appear depends on many environmental factors, like the sort and concentration of pollutants, distance from the emission source, length of exposure, and environmental condition.

Fluoride (F) is an anion of the halogen family and therefore the 13th most abundant element of the layer and occurs at about 0.3 gkg–1 of the earth's crust. Fluorides are naturally occurred within the type of salt or fluoride in rocks, coal, clay, and soil and released into the environment through the weathering of minerals, emissions from volcanic ash, and marine aerosols. (Tylenda 2011). In water, inorganic fluorides usually remain in solution (as fluoride ions) under conditions of relatively low pH and hardness. Though, F is taken into account an absolutely non– essential element for plants, (Kabata–Pendias 2001). Its presence in soil, air and water causes alterations in physiological, biochemical and structural activities in plants, (Jha *et. al.,* 2009) sometimes even without showing any visible symptoms of injury. (Jha et. al., 2009) reported that the order of retention of fluoride in onion was found to root> shoot > bulb. Certain plant species are observed to be injured as a results of the accumulations of excessive fluoride from the atmosphere. The annual global release of fluoride from volcanic sources through passive degassing and eruptions range from 60 to 6,000 kilotons. From which approximately 10% could also be introduced directly into the stratosphere. (Symonds *et.al.,* 1998). Fluoride can even be deposited into the soil from several anthropogenic sources i.e. production of phosphate fertilizers, pesticides (such as sulfuryl fluoride), detergents, bricks, tiles and ceramics, and atmospheric pollution from industrial activities (used in aluminum production and as a flux within the steel and optical fiber industries) and burning of fossil fuels. (Elloumi and Abdallah 2005). Among thermostable fluoropolymer plastics, polytetrafluoroethylene (PTFE) may be a very common/useful fluorine-containing plastic sold and used domestically as cooking utensils because of its heat resistance and nonstick properties. Gaseous fluoride enters into the plant leaves through stomata whereas soil fluoride enters through absorption by roots and subsequently translocated into the shoot. Certain plant species accumulate F in their roots and shoot at higher concentrations up to 4000 µgg–1 F without showing any signs of toxicity. (Baunthiyal and Ranghar, 2015). However, most of the opposite plants show signs of toxicity at relatively much lower F concentrations. F affects plant growth and development by interfering with several metabolic pathways related to photosynthesis, respiration, protein synthesis, carbohydrate metabolism, and nucleotide synthesis. (Barbier *et. al.,* 2010 and Yadu *et. al.,* 2016). Several studies are reported on F contamination of soil and its effects on different plant species6 including cereals (Kaur and Duffus 1989) and vegetables. Chandra *et.al.* 2012).

However, there's little information available on the effects of F on the germination and early growth characteristics of commonly grown crop plants by farmers. The importance of seed germination in plant growth is widely known and its study has been used as a model for investigating elemental toxicity. *Rauvolfia tetraphylla*. L. plant is employed for the current investigations.

The experiments were conducted at the Department of Botany, University College of Science, Osmania University, and Hyderabad. Hence, the target of this study was to analyze and measure the results of F on the germination, early growth characteristics of seedlings and content of photosynthetic pigments in *R. tetraphylla* a medicinal Important Plant.

Material and Methods:

Certified seeds of *Rauvolfia tetraphylla*, L were sterilized with 0.1% (w/V) of Mercuric chloride solution for 5 minutes followed by thorough repeated rinsing with distilled water. For germination studies, 100 surface sterilized seeds were sown in 120 mm diameter Petri dishes containing equal volume of sterilized sand.

Each sand– filled Petri dish was added with 80 ml of 0 (distilled water, control), 0.5,1.0, 1.5,2.0, 2.5 and 3.0 mg/L Sodium fluoride solution prepared from a 20.0 mg/L stock solution. Counts were made on each day for seedlings emerging above the sand mix to in order to estimate % of Seed Germination rate. The experiments were terminated on 30th day and the seeds/seedlings were used for estimating final percent seed germination, root and shoot length, root and shoot biomass and content of photosynthetic pigments (Total chlorophyll, Chlorophyll a, chlorophyll b and carotenoids). Fluoride treatments to the six month old uniform sized plants were given as sodium fluoride (NaF) solution to the roots at weekly intervals for 8 weeks, during the month of June every year.

Plants were harvested after fluoride treatment and their fresh weight was recorded. For determination of dry weight, the plants were air-dried and kept in hot air oven at 80 ^{0}C for 48 hours or till the samples exhibited constant weights. Germination percentage in each fluoride treatment was recorded on 10th day after sowing according to following formula:

Seeds Germination Percentage = Number of Germinated seeds/ Total Number of Seeds X 100

For estimation of chlorophylls (chl) and Carotenoids, 30th day old Petri dish grown seedlings were harvested separately and 100 mg leaf tissue from randomly chosen seedlings was placed in 2 ml dimethyl sulfoxide (DMSO) liquid in dark for overnight period according to Hiscox and Israelstam GF (1979) for pigment extraction. The extract was centrifuged at 5000 rpm for 5 minutes and then the absorbance (O.D.) of leaf extract (supernatant) was recorded at 480, 510, 645 and 663 nm using spectrophotometer. The pigment value of total Chl, Chl a, Chl b and carotenoids were estimated according to Anon (1949) and (Lichtenthaler and Wellburn (1983). The actual pigment content (mg/g FW) was computed as Pigment value X V/1000 X 1/W, where V is the volume of DMSO extract (in ml) and W is the weight of the leaf tissue used (in g).

Result and discussion:

The effects of NaF on various germination percentage and seedling growth are summarized in (Table- 1). Data of the clearly indicate that *R. tetraphylla* treated with various levels of NaF exhibited a marked reduction in % germination and Seedling growth (Height and Weight). Maximum (100%) germination was recorded in case of control and minimum (78 and 70%, respectively) at (2.5 and 3.0mg/L) NaF level. These observations on germination behavior are in conformity with the findings of (Singh *et. al.*, 2013) and (Iram *et al.*, 2016) in case of *Raphanus sativus* L. and *Abelmoschus esculentus* L., respectively, who reported the inhibition of root and shoot elongation and biomass production by sodium fluoride treatments. (Shaddad *et al.*,1989) also observed adverse effect of NaF on seed germination and seedling growth in *Zea mays* L., *Helianthus annus* L. and *Vicia faba* L. during exposure to varying levels of $CdCl_2$, NaF and 2,4– DNP individually.

Seed germination is an energy driven developmental process and requires rapid hydrolysis of reserve food materials along with high rate of respiration. Fresh weight and dry weight decreased monotonically in both the test crops with increasing fluoride concentration due to reduction of metabolic activity in presence of fluoride because germination is a one kind of metabolism and fluoride acts as a metabolic inhibitor. Yu (1996) exposed Mung bean seeds to 10.0 mM NaF and recorded F– induced inhibition of ATPase and 5'– nucleotidase during germination which turned to be correlated with lowered amylase and lipase activity.

The effect of NaF was found to vary among different plant species with respect to their roots and shoots development (Table 2). In the present study, the root and shoot growth (in terms of length) of *R. tetraphylla*, revealed inhibitory effect at even at lowest NaF concentration tested (i.e. 3.0 mg/L) as compared to control. The degree of inhibition of length was much higher in root in comparison to shoot. The data on root and shoot biomass almost followed the trend observed with length parameter but a drastic decrease in root biomass was recorded during increase from 2.5 to 3.0 mg/L NaF. Fluoride causes reduction in root length and shoots length due to imbalanced nutrient uptake by seedlings. (Sabal *et.al*, 2006). These findings are in conformity with the study of Singh *et al.* 2013) and Iram *et al.*, (2016) on *Raphanus sativus* L. and *Abelmoschus esculentus* L., respectively wherein they reported the inhibition of root and shoot elongation and biomass by sodium fluoride treatments. Such a reduction of biomass with increasing F concentration has also been earlier reported by Jha *et al*. 2009).

Table -1 Effect of different Sodium fluoride concentrations on seed germination and Seedling Growth in *R. tetraphylla*

Concentration of NaF mg/L	% of Germination	Length in C.M ± (SE)*			Fresh Weight in GM ± (SE)*		
		Shoot	Root	Root Shoot Ratio	Shoot	Root	Root Shoot Ratio
Control	100	14.04± 0.22	16.62± 0.43	0.84± 0.32	180.00± 0.42	200.0 ± 0.42	0.90± 0.32
0.5	95	13.04± 0.02	16.42± 0.32	0.74± 0.22	168.0± 0.42	190.0± 0.46	0.88± 0.52
1.0	90	10.04± 0.32	13.00± 0.52	0.77± 0.23	150.0± 0.32	177.0± 0.32	0.84± 0.32
1.5	87	9.00± 0.22	13.75± 0.22	0.65± 0.34	140.0± 0.62	185.0± 0.44	0.75± 0.62
2.0	80	8.00± 0.23	12,65± 0.52	0.63± 0.34	130.0± 0.32	180.0± 0.23	0.74± 0.32
2.5	78	6.45± 0.52	10.05± 0.22	0.58± 0.23	120.0± 0.62	180.0± 0.32	0.66± 0.52
3.0	70	5.75± 0.22	11.05± 0.32	0/57± 0.56	110.0± 0.32	170.0± 0.32	0.64± 0.42

*** Mean ± Standard Error**

Table-2 Changes in different photosynthetic pigments in *R. tetraphylla* seedlings under different Sodium fluoride

Concentration of NaFmg/L	Total Chl* (mg/g) ± (SE)*	Chl a* (mg/g) ± (SE)*	Chl b* (mg/g± (SE)*	Carotenoids* (mg/g) ± (SE)*	Chl a: Chl b ratio± (SE)*	Total chl : carotenoid ratio± (SE)*
0.5	2.78± 0.32	2.40± 0.42	0.38± 0.12	1.105± 0.32	6.31± 0.32	5.71± 0.32
1.0	2.80± 0.43	2.30± 0.32	0.40± 0.32	1.143± 0.42	5.75± 0.22	5,03± 0.22
1.5	3.00± 0.32	2.60± 0.52	0.30± 0.44	1.160± 0.22	8.60± 0.42	7.41± 0.52
2.0	2.65± 0.34	2.83± 0.32	0.82± 0.32	1.180± 0.32	3.45± 0.32	2.90± 0.32
2.5	2.40± 0.32	2.87± 0.62	0.73± 0.12	1.843± 0.32	3.93± 0.22	2.13± 0.22
3.0	2.30± 0.52	2.00± 0.22	0.80± 0.33	1.673± 0.22	2.54± 0.52	1.51± 0.12

*** Mean ± Standard Error**

However, 1.0 mg/L NaF level showed stimulatory effects on both (length and biomass) the parameters of root and shoot in case of *R. tetraphylla*. Further increase in NaF concentration beyond 1.0 mg/L led to reduction in length and biomass of root and shoot in barley as was found in case of *R. tetraphylla* on comparable F concentrations. Thus, *R. tetraphylla* showed some tolerance towards NaF at lower (1.0 mg/L) concentration. The degree of reduction in length and biomass of root and shoot was lesser in *R. tetraphylla*. This differential response of the root and shoot developments in presence of NaF in the test plants of the present study can be attributed to the taxonomic differences amongst the *R. tetraphylla* plant species.

In a study with fluoride applied (aerially on leaves and systemically via roots) *Vicia faba* plants, the ability of roots to accumulate higher amount of F than that of the shoot system was noted, which may well explain as to why in our study we found the roots of *R. tetraphylla* were relatively more tolerant than the shoots at 1.0 and 2.5 mg/L NaF concentrations, respectively. (Davies *et.al.*, 1998).

This fact also conforms to previous observation of relatively high uptake and accumulation of F in both grass and legume species and May account for higher phytotoxicity to root tissues Changes in the content of photosynthetic pigments in seedlings obtained on various NaF treatments are summarized in (Table-2). In case of *R. tetraphylla* treatment with 1.0 and 1.5 mg/L NaF showed stimulation in total chl (2.80 and 3.00 mg/g, respectively) over the control.

This increase of chlorophyll in presence of F is an exception observation and differs from majority of previous studies in range of species6 including another cultivar (Anuradha) of *C. arietinum*. (Datta *et.al.*, 2012) In a study on *Triticum aestivum*, Tomar et al., (Tomar and Aery 2000) reported steady increase in the length of root and shoot and chlorophyll contents by 20 and 40 g/ ml NaF. The present observations in *C. arietinum* cv. Azad conform to results of (Tomar and Aery 2000) and such may be due to genotype– specific response to fluoride stress. Carotenoid content was also stimulated at (1.0 and 2.5 mg/L) NaF and reached maximum (1.105 mg/g) at (2.5 mg/L) NaF whereas it decreased at subsequent higher levels of NaF and was recorded minimum (1.843 mg/g, lesser than control) at 2.0 mg/L concentration Increase in NaF to (2.5 and 3.0mg/L) caused a rapid decrease in total chlorophyll and as well as in chlorophyll a, chlorophyll b and carotenoids. Comparison of chl a: chl b ratio revealed that at (1.0 and 2.5) mg/L NaF chlorophyll changes were mediated mainly through chlorophyll b whereas at (2.5 and 3.0mg/L). Chlorophyll changes were mediated through both chlorophyll a and b. Total chl carotenoid ratio varied within a narrow range in *R. tetraphylla* and did not indicate a clear increasing/decreasing pattern. On the contrary to the *R. tetraphylla*,

This decrease may be either due to inhibition of chlorophyll biosynthesis (as high F was found to reduce the availability of Fe2+ ions which are essential for chlorophyll synthesis), or due to enhanced breakdown of chlorophyll during fluoride stress. Carotenoids are accessory pigments in photosynthetic systems and protect chlorophyll against oxidative stress.

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