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Fluoride induces morphological change in Fenugreek (*Trigonella foenum-graecum* L) during germination and early seedling

UPSANA BURGOHAIN[1], AND BHABEN CHOWARDHARA[1]*

[1]Department of Botany, Arunachal University of Studies, Namsai (Arunachal Pradesh), India-792103

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ABSTRACT

The goal of this study was to assess the toxicological effects of exposing popular variety of fenugreek [Trigonella foenum-graecum L.] to fluoride during the germination and early seedling stages. In January 2022, the experiment was carried out at Arunachal University of Studies, Namsai. Surface sterilized seeds of uniform size were set for germination in petri-plates over cotton beds, treated with fluoride solutions of varied strengths (0.0, 2.0, 4.0 and 6.0 mM), made from a stock solution of sodium fluoride (50.0mM). The results showed that fluoride might cause considerable phytotoxicity during germination and seedling growth. Fluoride administration resulted 13 to 43.10% suppression of germination from 2.0mM to 6.0mM. Similar results were also found in germination index, germination energy, relative germination rate and seedling vigor index which were significantly reduced by 43.1, 43.1, 34.0, and 84.0% in 150.0 mM, respectively. Fluoride also had significant detrimental impact on seedling length (71.78%) in the intact seven days with increasing doses of fluoride.

Keywords: Fluoride, germination, seedling vigour index, growth

INTRODUCTION

In today's world, increasing environmental pollution with harmful xenobiotic chemicals is a big issue. Fluoride (F) released into the environment as a result of numerous anthropogenic activities such as industries, mines, agricultural areas, and various home activities is slowly becoming a threat to environmental safety (Hong *et al.* 2016). According to Bhattacharya and Samal (2018), the level of F in soil in some parts of India, such as Bankura and Purulia (West Bengal) and Newai Tehsil (Rajasthan), is between 55–399 and 50 180 mg kg^{-1} of soil, respectively, whereas the level of F in water is between 0.8–1.3 and 0.3–9.8 mg L^{-1}, which is significantly higher than the World Health Organization's recommended level of 1.5 mg L^{-1} (WHO). The widespread accumulation of F ions in plant biomass is caused by the use of polluted water for agriculture irrigation. Some reports mentioned that irrigating crops with water with 7.4–14 mg L^{-1} F resulted in F accumulation in plants such as spinach (26 mg kg^{-1} fresh weight) and fenugreek (16 mg kg^{-1} fresh weight). Rice and other water-intensive crops absorb F ions from the environment via the chloride channels CLC1 and CLC2 (Banerjee *et al.* 2019a), resulting in the formation of reactive oxygen species (ROS) such as H_2O_2 via the Mehler pathway (Hong *et al.* 2016). The pH of the surrounding media is another crucial factor that influences F uptake from the soil.The accumulation of F in the tissues (leaf, steam, and roots) of tea plants was highest at pH 5.5which can be explained by the fact that lower pH promotes the dissociation of HF and NaF, increasing the level of F ions in the surrounding areas, which are readily absorbed by the plants. Chlorophyll deterioration, membrane damage, and electrolyte leaks are all caused by an oxidative burst in the tissues (Singh *et al.* 2021). Other harmful metabolites such as malondialdehyde (MDA) and methylglyoxal are also increased when ROS is produced (MG).

Seed germination parameters, growth, development, mineral nutritional status, photosynthesis, respiration, metabolic activity, yield and yield characteristics, and other morphological, physiological, and biochemical processes are all influenced by fluoride toxicity (Sahariya *et al.*, 2021; Sodani *et al.*, 2021). When comparing fluoride-treated and control plants, researchers have found that the fluoride-treated plants have significantly lower growth and related parameters, such as seedling germination percentage, roots and shoot length, plant height, and fresh and dry biomass Singh *et al.*, 2013; Sodani, 2018. The flowering annual legume fenugreek (*Trigonella foenum-graecum*

Corresponding authors email: bhaben1986@gmail.com

L.) leaves and seeds are used for a variety of applications in different parts of the world, including medical purposes, food preparation, natural stored grain protection, and scent. Extended spells of dryness, as well as inappropriately high irrigation, are key crop production restrictions in semiarid and arid regions. Environmental stressors such as drought, salt, and cold have an impact on fenugreek yield. The objective of this study was to study the impact of fluoride on germination and early seedling stages of fenugreek plants.

MATERIALS AND METHODS

The local common cultivars of fenugreek surface sterilized seeds were subjected to germination in a petri-plate (Borosil, 9.0 cm in diameter) with cotton embedding and treated with a F solution (NaF salt). Three F concentrations used were: 2.0 mM, 4.0 mM and 6.0 mM excluding control. Twenty seeds were inserted on each plate throughout the experiment, and 10.0 ml of solution was poured into the petri-plates. Plants were cultivated in a growth chamber with a photon flux density of 52 mol m^{-2} s^{-1} (PAR), a mean day and night temperature of 22/14±3 °C, and a relative humidity of 62°±5 % under white light. In this experiment, total twelve treatment combinations were randomly repeated three times in 36 petri plates. Seven days after sowing petri-plates were evaluated for final germination percentage (FGP), germination index (GI), seedling vigor index (SVI), relative germination rate, relative injury index and germination energy as well as seedling length, using the protocols provided by Li (2008), Abdul-Baki and Anderson (1973), and Moulick *et al.* (2016), all in triplicates. SPSS 21 (Windows version) software was used to calculate standard error (mean ± SE) and correlation analysis. Furthermore, the difference between the various treatments was assessed using a two-way ANOVA and a post-hoc Tukey's HSD (honest significant difference) test at the 0.05 level of significance.

RESULTS AND DISCUSSION

Seed germination is an important stage in the life cycle of any plant, and it is greatly influenced by a range of environmental conditions (Seneviratne *et al.* 2017). Under heavy metals stress, seed germination is suppressed due to a lack of necessary/sufficient protective arrangements throughout this stage of the plant's life cycle (germination). Plants come into contact with the external environment for the first time at this point (during germination). If a stressor (biotic or abiotic, such as F) is present, germination and seedling growth (early embryonic stage) are more likely to be inhibited (Liu *et al.* 2012a, b).

Seed germination: In the control without fluoride containing plate, the rate of germination in fenugreek seed was 100% under control environment where different concentrations of fluoride containing plates significantly reduced as increased the concentrations was 13.7%, 27.5% and 43.1% in 2.0mM, 4.0mM and 6.0mM treatments after 7 DAS, respectively (Table 1). Similar result also found in other plants under fluoride stress in *Cicer arietinum* cv. Anuradha (Datta *et al.* 2012), *Cicer arietinum* L., *Helianthus nnus* L. and *Vicia faba* L. (Shaddad *et al.* 2018).

Germination Index: The result was clearly showed that the germination index was significantly reduced with increase the concentration of fluoride in fenugreek as compared with control, respectively. 13.7%, 27.5% and 43.1% of germination index was enormous reduced in fenugreek as 2.0, 4.0 and 6.0mM of fluoride respectively (Table 1). Similar results were also found in others plants under different abiotic stresses such as *Limonium sinense* kuntze,*Glycine soja* sjeb and *Sorghum sudanense* Stapf under salinity stress (Li, 2008).

Germination Energy: In this parameter, it was found that the germination energy was gradually reduced ($p < 0.001$) with increasing the doses of fluoride as compared with control at 7 DAS. The germination energy was reduced by 13.7%, 27.5% and 43.1% in 2.0, 4.0 and 6.0mM respectively in fenugreek (Table 1). Similar trends also found in other plants under different stresses condition like *Limonium sinense* kuntze, *Glycine soja* sjeb and *Sorghum sudanense* Stapf under salinity stress (Li, 2008).

Table 1: Consequences of F toxicity on germination, seedling growth, germination energy, relative injury index, germination index and seedling vigor index (intactseedlings) at 7 DAS (days after sowing)

Fluoride conc.(mM)	Final germination % (FGP)	Germination index (GI)	Germination energy (GE)	Relative germination rate (RGR)	Relative injury index (RII)	Seedling vigour index (SVI)
Control	19.33±0.33	2.76±0.04	0.96±0.01	-	-	123.94±5.25
2.0	16.66±0.33*	2.38±0.04*	0.83±0.01*	0.86±0.03***	0.13±0.03*	77.25±12.40**
4.0	14.0±0.57***	2.0±0.08***	0.7±0.02***	0.72±0.03***	0.27±0.03***	48.82±3.95***
6.0	11.0±0.57***	1.57±0.08***	0.55±0.02***	0.57±0.03***	0.43±0.03***	19.80±0.85***
Source of variation			*F*- value			
F stress	57.45***	57.45***	57.45***	186.08***	44.23***	39.81***

*# Values refer to the mean value (n=3) followed by *, **, and *** indicate that values weresignificant at p< 0.005, 0.01 and 0.001 levels respectively*

Relative germination rate: In fenugreek under fluoride stress showed that it had direct impact on relative germination rate as day by day with compared with control. The relative germination rate of 16.0% and 34.0% was significantly reduced (*p <0.001*)in 4.0 and 6.0 mM respectively as compare with control (Table 1). Li (2008) was found that same kind of result in *Limonium sinense* kuntze, *Glycine soja* sjeb and *Sorghum sudanense* Stapf under salinity stress.

Relative injury index (RII): The inhibition of germination rate as a result the directly effect of relative injury index in fenugreek as compared with control. The relative injury index was significantly (*p <0.001*) (by 16.0% and 34.0%) reduced as the doses increased (4.0 and 6.0mM) as compared with control (Table 1). Similar kind of the result also observed in other plants such as *Cicer arietinum* cv. Anuradha under fluoride (Datta *et al.* 2012), *Brassica juncea* (L.) czern and Coss under Zn and Cd stress (Chowardhara *et al.*2020).

Seedling vigour index: The seedling vigour index (SVI) has recently been utilized as a phytotoxicity tool to assess how heavy metal affects seedling growth. Seedling vigour is a measurement of the amount of damage that occurs as viability falls, and the damage accumulates in seeds until they are unable to germinate and die. In our result showed that the fluoride concentrations gradually increased with significantly reduced the seedling vigour index (SVI) in fenugreek at 7DAS. The seedling vigour index was significantly (*p<0.001*) decreased (by 37.6%, 60.6% and 84.0%) in 2.0, 4.0 and 6.0mM of fluoride as compared with control (Table 1). This kind of result was also found in *Cicer arietinum* L. cv. Anuradha (Datta *et al.* 2012).

Seedling growth: The impact of seedling growth under fluoride stress condition was significantly (*p<0.001*) hamper as compared with control. The result showed that the doses (2.0, 4.0, and 6.0mM) of fluoride increased as consequence the growth of seedlings was significantly reduced (28.0%, 45.6% and 71.7%) as compared with control (Table 2). Fluoride inhibited the growth in other plants such as *Oryza sativa* (Banerjee *et al.* 2019b; Singh *et al.* 2021).

Table 2: Consequences of F toxicity on seedling growth, fresh weight, turgor weight, dry weight and relative water content (intact seedlings) at 7 DAS (days after sowing)

Fluoride conc. (mM)	Seedling length (cm)	Fresh weight (gm)	Turgor weight (gm)	Dry Weight (gm)	Relative water content (gm)
Control	6.40±0.17	0.90±0.09	1.55±0.23	0.15±0.02	
2.0	4.61±0.66*	0.77±0.11^{ns}	1.16±0.39^{ns}	0.09±0.01^{ns}	0.75±0.16
4.0	3.48±0.19**	0.53±0.02^{ns}	0.78±0.21^{ns}	0.08±0.01^{ns}	0.72±0.12^{ns}
6.0	1.80±0.09***	0.33±0.07**	0.46±0.14^{ns}	0.07±0.02^{ns}	0.53±0.03^{ns}
Source of Variation		*F*-value			
F stress	28.39***	8.76***	3.19***	2.80***	0.80***

*# Values refer to the mean value (n=3) followed by *, **, and *** indicate that values weresignificant at p< 0.005, 0.01 and 0.001 levels respectively*

Biomass: Result showed that fluoride induced negative impact on biomass in fenugreek seedlings as compared with control. The fresh weight of fenugreek seedlings was gradually declined (12.9%, 39.9% and 62.0%) with increased the concentrations of fluoride (2.0, 4.0, and 6.0mM) as compared with control, respectively (Table 2). Similar trends also found in turgor weight and dry weight (25.4%, 49.7%, 70.3% and 36.7%, 47.3%, 49.9%) as the concentrations of fluoride increased as 2.0, 4.0 and 6.0mM as compared with control (Table 2), respectively. Singh *et al.* (2021) reported similar results in *Vigna radiata* and *Vigna mungo*.

Table 3: Correlation coefficients among FGP, GI, GE, RGR, RI, SVI, SL, FW, TW, DW and RWC

Parameters	FGP	GI	GE	RGR	RII	SVI	SL	FW	TW	DW	RWC
FGP	1	0.955***	0.967***	-0.48^{ns}	-0.99***	0.96***	0.95***	0.88***	0.78***	0.62***	-0.54^{ns}
GI		1	0.97***	-0.48^{ns}	0.9***	0.95***	0.95***	0.88***	0.78*	0.62*	-0.54^{ns}
GE			1	-0.48^{ns}	-0.9***	0.96***	0.95***	0.88***	0.78*	0.62*	-0.54^{ns}
RGR				1	0.47^{ns}	-0.59*	-0.52^{ns}	-0.34^{ns}	-0.36^{ns}	-0.62*	0.34^{ns}
RII					1	-0.96***	-0.96***	-0.90***	-0.81**	-0.57^{ns}	0.56^{ns}
SVI						1	0.99***	0.87***	0.79*	0.62*	-0.52^{ns}
SL							1	0.89***	0.80***	0.59*	-0.49^{ns}
FW								1	0.91***	0.46^{ns}	-0.58*
TW									1	0.21^{ns}	-0.79*
DW										1	-0.12^{ns}
RWC											1

**p< 0.05, 0.01, 0.001, ^{ns} not significant*

Relative water content (RWC): The results showed that the relative water content decreased significantly (*p<0.001*) in fenugreek treated plants compared to the control plants. In comparison to the control plants, the RWC decreased by 2.6% and 31.16% in 4.0 and 6.0mM treated plants, respectively (Table 2). Similar result was also observed in *Oryza sativa* L. (Banerjee *et al.* 2019a).The correlation analysis clearly showed that all parameters had direct impact on plants under fluoride stress (Table 3). The gradually increasing the concentrations of fluoride with increasing the effects on the plants. The final germination percentage direct significantly (*p<0.001*) positive correlated with germination index, germination energy, seedling vigour index, seedling length, and biomass of fenugreek (Table 3) where negative correlated with relative germination rate, relative injury index and relative water content (Table 3). Similar trends were also observed in GI. The completely negative correlation was observed in relative injury index (RII) with SVI, SL, FW, DW, TW except RWC which means the relative injury index increased in seedlings as consequence the negative effect on fenugreek seedling growth (Table 3) as well as similar fashion result were also found in seedling vigour index (SVI). The significantly (*p<0.001*) positive correlation had found in seedling length (SL), fresh weight (FW), dry weight (DW), turgor weight (TW) except insignificant (*p>0.5*) negative correlation found in relative water content (Table 3) among these parameters.

It may be concluded that increased fluoride concentrations in the range of 2.0mM to 6.0mM resulted in lower germination percentage. Fluoride concentrations rise, resulted in shorter whole seedling length as well as less fresh and dry materials in the seedlings. However, higher levels of Fluoride had a greater impact on whole seedling length.

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