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Effect of fluoride on wheat (*Triticum aestivum* L.) seed imbibition, germination and seedling growth

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Abstract
Germination paper roll test experiments were conducted to study the deleterious impacts of fluoride at concentrations of 0 (T_1), 100 (T_2), 200 (T_3) and 300 (T_4) ppm on seed germination and morphological parameters of wheat (*Triticum aestivum* L.) variety HUW-234. It is observed that across increasing concentration of F the normal imbibition by seeds was restricted. This in turn evidently decreased seed germination percentage. Increased fluoride concentrations also reduced root and shoot lengths as well as root and shoot dry matter, with the critical observation of change in the ratio of root-to-shoot weight and the magnitude increased with increasing fluoride content. It can be concluded that increased fluoride content in germination media resulted in a decrease in seed germination and seedling growth and associated morphological parameters in HUW 234 cultivar of wheat, popular cultivar in the eastern parts of Uttar Pradesh.

Keywords: Wheat (*Triticum aestivum*), fluoride, germination, imbibition

1. Introduction
Fluoride is necessary for optimal plant development in small amounts, but at higher concentrations, it may harm both plants and the environment (Gao *et al.*, 2012) [8]. Fluoride (F) occurs naturally in the environment, and its total amount in the crust is estimated to be 0.077 per cent (Cai *et al.*, 2017) [5]. In the hierarchy of environmental toxins, fluoride is ranked fifth among the contaminants that contribute to environmental pollution (Pelc *et al.*, 2020) [15]. The largest source of fluoride contamination in the environment is industrial activity, the manufacturing of artificial fertilizers, and the release of fluoride compounds into the atmosphere as dust and gas from aluminum smelters (Gautam and Bhardwaj, 2010) [9]. Fluorosis, a disease caused by F toxicity in human, has been detected in Sonbhadra, Mirzapur, Unnao, Agra, Mathura and their surrounding districts in Uttar Pradesh (Kumar *et al.*, 2008) [10]. Wheat (*Tricitum aestivum* L.) is the world's second-largest crop after rice, accounting for 761.5 million tonnes harvested in 2019 (Lethin *et al.*, 2020) [12]. It is an important source of food for the majority of the world's population and produces significant yields. Wheat also rich in carbohydrates, protein, fiber, minerals, and B vitamins (Kumar *et al.*, 2011) [11]. One of the most essential factors or even the foremost input for getting higher yield in any agricultural crop is "seed" with good germination and seedling vigour. It is the start of the first developmental phase of a plant's life cycle, which is followed by the seedling's growth (Wolny *et al.*, 2018) [23].Seed quality evaluation for viability and vigour is, therefore, an indispensable step to assess the crop stand in the field. Germination studies conducted in laboratory conditions helps in assessing seed quality and its performance both under favorable and stress conditions (Sodani, 2018; Wolny *et al.*, 2018) [19, 23] and gives representative results under the similar stress environments in the field.
Fluoride toxicity affects seed germination parameters, growth, development, mineral nutritional status, photosynthesis, respiration, metabolic activity, yield and yield characteristics and other morphological, physiological and biochemical processes (Chae *et al.*, 2018; Sahariya *et al.*, 2021; Sodani *et al.*, 2021) [6, 17, 20]. Comparing fluoride-treated and control plants, the fluoride-treated plants are reported to exhibit markedly reduced growth and related parameters, including seedling germination percentage, roots and shoot length, plant height, and fresh and dry biomass (Singh *et al.*, 2013; Sodani, 2018, Sodani *et al.*, 2018) [18, 19, 21].

2. Materials and Methods
Present study was conducted during *rabi* (winter season) 2019-20 and 2020-21using wheat variety HUW-234 (Malviya Wheat 234) which is widely grown in India's North Eastern Plains

Zone. The experiment was carried out at the Tissue Analysis Laboratory of the Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, using seeds obtained from the Department of Genetics and Plant Breeding. The data recorded during the two years of the experiment are pooled and presented here. Data were analyzed with standard statistical methods for factorial complete randomized design.

2.1 Parameters for germination

(a) Seed Inbibition rate

This experiment was conducted in *Petri dishes* lined with filter paper and moistened with 0 (T_1), 100 ppm (T_2), 200 ppm (T_3) and 300 ppm (T_4) fluoride prepared by dissolving required amounts of sodium fluoride (NaF) in distilled water. The experiment was conducted in twelve replications for each treatment and stage. Twenty five healthy seeds were weighed for initial dry (W_1), and placed at with uniform spacing on filter paper in *Petri dishes* and soaked with 0 (T_1), 100 ppm (T_2), 200 ppm (T_3) and 300 ppm (T_4) fluoride NaF solutions. Seeds were removed after 24 and 48 hours, surface water was blot dried gently and weighed (W_2) for calculating the amount of water imbibed (imbibition) on weight basis. Seed imbition for the initial 24 and 48 hours were expressed as per cent water absorbed by seeds and calculated (Rahman *et al*., 2008) [16] as follows:

$$\text{Seed imbibition (\% on fresh weight basis of seed)} = \frac{W_2 - W_1}{W_1} \times 100$$

(b) Germination Paper Roll Test

A germination paper of size (44×30 cm) was taken and placed on a sheet of butter paper of same size such that the lowest 4 cm of the germination paper was not covered by the butter paper. Germination paper sheets were thoroughly saturated by 0 (T_1), 100 (T_2), 200 (T_3) or 300 (T_4) ppm fluoride solutions prepared by dissolving NaF. Sheet saturated with distilled water served as control. Seeds were evenly placed 15 cm above the bottom line on germination paper sheets. Another saturated germination paper of the same size was placed over it. The butter paper was folded inside and rolled on top of the germination paper. Rolls were placed in a 500 mL beaker containing the same concentration of fluoride solution such that the bottom section of the roll that was not covered with butter paper remained dipped inside the solution. At room temperature, seeds were allowed to germinate and observations were recorded at 3, 6 and 9 days after germination by unrolling the sheets.

(c) Germination percentage (%)

The number of germinated seeds was recorded stating at 3, 6 and 9 days after initiation of germination. A seed was considered to be germinated when 2 mm of radicle had emerged. Germination percentage was calculated (Association of Official Seed Analysis; 1983) [3] as follows:

Germination percentage = [Number of seeds germinated/ Total number of seeds tested] × 100

Morphological parameters

(a) Root length (cm)

The maximum root length from the base of the shoot to the longest root tip was measured with the help of scale at 3, 6 and 9 days after germination.

(b) Shoot length (cm)

The maximum shoot length from the root and shoot junction to tip of the shoot was measured with the help of meter scale at 3, 6 and 9 days after germination.

(c) Root and shoot fresh and dry weight (mg five seedlings^{-1})

Root and shoot were excised, surface water was bloted to remove adhered water and weighed for fresh weight. After taking fresh weight they were placed in an oven (NSW-142) at 105°C for 5 minutes than at 65°C till constant weight was achieved. Dry weight was taken at 3, 6 and 9 days after germination.

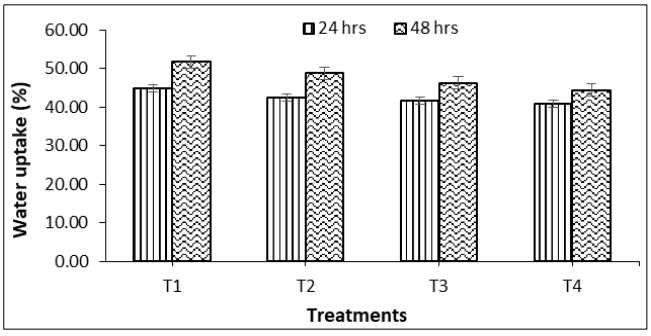
(d) Root: Shoot ratio (dry weight basis)

Dry weights of root and shoot, as recorded above, were used to calculate the root/shoot ratio for each treatment.

3. Results

3.1 Seed imbibition

The percent water intake reduced marginally as the fluoride concentration in the germination medium increased and differences between treatments were highly significant. Water absorptions by seeds in T_1, T_2, T_3 and T_4 treatments were 44.93, 42.51, 41.75, and 40.95% after 24 hours and 51.81, 48.90, 46.35, and 44.45% after 48 hours, respectively (Fig 1).



*T_1, T_2, T_3, and T_4 represent 0, 100, 200, and 300 ppm fluoride, respectively

Fig 1: Effect of different concentrations of sodium fluoride on seed imbibition (water uptake %) after 24hrs and 48hrs of treatment in wheat genotype HUW-234.

3.2 Germination percentage

The T_1 (control) treatment registered the maximum mean germination percentage (87.61%), whereas in T_2, T_3 and T_4 treatments it decreased to 68.61, 58.88 and 42%, respectively. It was evident that when the fluoride levels in the germination media increased, the germination percentage concomitantly reduced (Table 1).

Table 1: Effect of different concentrations of fluoride on germination percentage in wheat genotype HUW-234 at 3, 6 and 9 days after germination

S. No.	Treatments*	Stage (days after sowing) **						Mean	
		3		6		9			
1.	T_1	67.83	(55.43)	95.00	(77.29)	100.00	(89.39)	87.61	(74.04)
2.	T_2	49.33	(44.60)	76.66	(61.09)	79.83	(63.33)	68.61	(56.34)
3.	T_3	41.00	(39.79)	65.33	(53.91)	70.33	(56.99)	58.88	(50.23)
4.	T_4	29.66	(32.97)	46.50	(42.98)	49.83	(44.89)	42.00	(40.28)
	Mean	46.95	(43.20)	70.87	(58.82)	75.00	(63.65)		
		S.Em±		CD (5%)					
Stage (S)		0.56		1.64					
Treatment (T)		0.64		1.91					
S × T		1.12		3.29					

*T_1, T_2, T_3, and T_4 represent 0, 100, 200, and 300 ppm fluoride, respectively.
**Values in parentheses indicate angular transformed values.

3.3 Shoot length (cm)

In Table 2, data analysis indicated that mean shoot length increased considerably as the germination period advanced, however, mean treatment values decreased significantly when fluoride level in the germination medium increased. The interaction mean values were greater under T_1 (control) treatment at all stages of observation and reduced significantly with increased fluoride content in the germination medium (Table 2).

Table 2: Effect of different concentrations of fluoride on shoot and root length (cm) in wheat genotype HUW-234 at 3, 6 and 9 days after germination

S. No.	Treatment*	Shoot length				Root length			
		Stage (days after sowing)			Mean	Stage (days after sowing)			Mean
		3	6	9		3	6	9	
1.	T_1	1.56	5.15	9.95	5.55	3.80	10.94	15.12	9.95
2.	T_2	1.16	4.70	9.19	5.01	2.81	8.39	11.33	7.51
3.	T_3	0.99	4.18	8.49	4.56	2.37	7.20	8.34	5.96
4.	T_4	0.70	3.86	7.38	3.98	2.02	5.82	7.07	4.97
	Mean	1.11	4.47	8.75		2.75	8.08	10.46	
		S.Em±	CD (5%)			S.Em±		CD (5%)	
Stage (S)		0.05	0.16			0.04		0.14	
Treatment (T)		0.06	0.19			0.05		0.16	
S × T		0.09	0.28			0.09		0.28	

*T_1, T_2, T_3, and T_4 represent 0, 100, 200, and 300 ppm fluoride, respectively.

3.4 Root length (cm)

Results of the data analysis showed that root length significantly increased with germination time, while treatment mean values decreased significantly when fluoride levels in the germination medium increased.

The interaction mean values were greater under T_1 (control) treatment at all stages of observation and reduced significantly with increased fluoride content in germination medium (Table 2).

3.5 Fresh weight of shoot (mg 5 seedlings^{-1})

Shoot fresh weight increased as germination progressed (Table 3); however, it decreased when fluoride concentrations in germination medium increased. At all stages of observation, stage (S) × treatment (T) interaction data revealed significantly higher shoot fresh weight under T_1 (control) treatment, which decreased significantly and proportionately with increased fluoride concentration at each stage (Table 3).

Table 3: Effect of different concentrations of fluoride on fresh weight and dry weight (mg 5 seedlings^{-1}) of shoot in wheat genotype HUW-234 at 3, 6 and 9 days after germination

S. No.	Treatment*	Shoot fresh weight				Shoot dry weight			
		Stage (days after sowing)			Mean	Stage (days after sowing)			Mean
		3	6	9		3	6	9	
1.	T_1	53.50	324.21	554.81	310.84	8.28	35.76	58.50	34.19
2.	T_2	35.56	254.69	436.28	242.17	5.44	28.13	50.49	28.02
3.	T_3	33.49	206.19	327.24	188.97	4.51	26.47	48.35	26.45
4.	T_4	26.40	175.53	291.06	164.33	3.44	19.51	35.25	19.40
	Mean	37.23	240.15	402.34		5.42	27.47	48.15	
		S.Em±	CD (5%)			S.Em±		CD (5%)	
Stage (S)		0.82	2.39			0.26		0.78	
Treatment (T)		0.94	2.76			0.31		0.90	
S × T		1.63	4.79			0.53		1.55	

*T_1, T_2, T_3, and T_4 represent 0, 100, 200, and 300 ppm fluoride, respectively.

3.6 Dry weight of shoot (mg 5 seedlings^{-1})

Extended germination period increased mean shoot dry weight, however, a higher fluoride level in the germination medium reduced it (Table 3).

At all phases of observation, stage (S) × treatment (T) interaction data show significantly higher shoot dry weight under T_1 (control) treatment, which declined significantly and proportionately with increased fluoride concentration at each stage (Table 3).

3.7 Fresh weight of root (mg 5 seedlings^{-1})

Significant differences were found in terms of the stage (S), treatment (T) and S× T. Increased germination period resulted in significantly higher mean values for root fresh weight (Table 4). Increasing the fluoride level in the germination medium resulted in a considerable reduction in mean root fresh weight. T_3 and T_4 treatments showed a significant reduction in comparison to T_1. Seedlings in the T_1 (control) treatment had significantly higher root fresh weight than those in the other treatments at all stages (Table 4).

Table 4: Effect of different concentrations of fluoride on fresh weight and dry weight (mg 5 seedling^{-1}) of root in wheat genotype HUW-234 at 3, 6 and 9 days after germination

S. No.	Treatment*	Root fresh weight				Dry weight of root			
		Stage (days after sowing)			Mean	Stage (days after sowing)			Mean
		3	6	9		3	6	9	
1.	T_1	76.01	305.35	615.72	332.36	9.18	33.28	44.39	28.95
2.	T_2	62.84	257.89	548.84	289.86	7.34	22.06	27.48	18.96
3.	T_3	52.78	243.33	513.52	269.88	6.98	18.37	23.45	16.27
4.	T_4	43.82	170.82	367.44	194.03	4.93	11.86	14.92	10.58
	Mean	58.86	244.34	511.38		7.11	21.39	27.56	
		S.Em±		CD (5%)		S.Em±		CD (5%)	
Stage (S)		0.63		1.84		0.27		0.80	
Treatment (T)		0.72		2.12		0.32		0.93	
S × T		1.25		3.67		0.55		1.60	

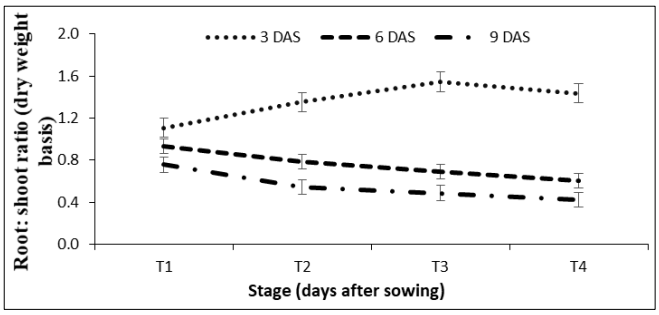
*T_1, T_2, T_3, and T_4 represent 0, 100, 200, and 300 ppm fluoride, respectively.

3.8 Dry weight of root (mg 5 seedlings^{-1})

With the increase in germination period mean root dry weight values increased considerably with significant differences between stage (S), treatment (T) and S × T (Table 4). When the fluoride concentration in the germination medium was increased, the mean root dry weight decreased significantly. T_1 (control) seedlings showed significantly higher root dry weight than the other treatments at all stages (Table 4).

3.9 Root: Shoot ratio (dry weight basis)

The root shoot ratio on dry weight basis decreased significantly in T_4 when comparison with T_1, T_2 and T_3. Fluoride concentration increased, it was higher in the T_1 and T_3 treatments than in the T_2 treatment and decreased in the T_4 treatment. There were significant differences in terms of the stage (S), treatment (T) and S × T (Fig.2).



*T_1, T_2, T_3, and T_4 represent 0, 100, 200, and 300 ppm fluoride, respectively

Fig 2: Effect of different concentrations of fluoride on root: shoot ratio (dry weight basis) in wheat genotype HUW-234 at 3, 6 and 9 days after germination

4. Discussion

Germination occurs following recovered metabolic activity, both physiologically and biochemically. Then, for remobilization of the seed's reserve components, a large number of particular metabolic activities involving hydrolytic enzyme activity take place. When seeds initiate metabolic activities and grow, they require the conducive internal and external conditions. Alterations in these circumstances, such as those generated by toxic compounds like F, trigger changes in the seed's metabolism (Montagnolli *et al.*, 2017) [13].

High levels of F have negative impacts on seed germination and early seedling development phases, which are physiologically complex processes. Plants reveal decreased seed germination when exposed to an overabundance of F. Reduced water intake, slowed cell division and decrease in metabolic activity associated with these phases might all lead to germination failure. Similar observations were recorded for F-treated mung (Gadi *et al.*, 2021) [7] and wheat seedlings (Aske *et al.*, 2014) [2].

Plant germination and seedling growth are crucial for their survival and sustainability (Ahmad *et al.*, 2009) [1]. Poor germination and seedling growth lead to poor crop growth and yield. As a result of F toxicity in soils, wheat germination is reduced (Tomar and Aery, 2000) [22]. An increased concentration of fluoride in the germination medium significantly impacted seed moisture uptake after 24 hours of imbibition (Figure 1). As the reduction in seed imbibition was observed even at lower F levels (100 ppm) therefore, it is concluded that F had a deleterious effect on seed imbibition. A similar finding was observed with Yadu *et al.* (2017) [24].

It had been noted that as compared to control F treatment inhibited all germination-related parameters *viz*. germination percentage (Table 1), shoot and root length (Table 2) and fresh and dry weights of seedlings (Table 3). Research has shown that high levels of fluoride in wheat germination medium adversely affect germination and other seedling characteristics (Kumar *et al.*, 2011; Sodani, 2018: Sodani *et al.*, 2018: Sodani *et al.*, 2021) [11, 19, 20, 21]. With increased F concentration seedling growth (shoot length, root length and vigor index) and seed germination were reduced, particularly at 100 ppm and higher levels of F. This study, therefore,

indicated that fluoride in germination medium even at 100 ppm had a negative impact on germination and germination-related parameters.

In citrus seedlings, a close link was discovered between nutritional deficiencies and growth responses under F stress (Brewer, 1960) [4]. F has also been reported to restrict growth by lowering DNA synthesis, which leads to lower RNA and protein synthesis, leading in reduced cell division and elongation. Excessive availability of F has been shown to suppress the ATP synthase enzyme in higher plants, altering energy metabolism and therewith the plant metabolic processes and growth (Panda, 2015) [14].

Plants treated with different concentrations of F, exhibited significant reductions in shoot length, root length (Table 2). These findings were similar to the results observed by several other workers (Pelc *et al*., 2017; Chae *et al*., 2018; Sodani, 2018: Sodani *et al.*, 2021; Sahariya *et al.*, 2021) [6, 15, 17, 19, 20]. Fluoride toxicity also reduced fresh and dried weights of seedlings (Table 2) and other germination parameters. These findings were also in close conformity with others who reported similar observations (Sodani, 2018; Pelc *et al.*, 2020; Sodani *et al.*, 2021) [15, 19, 20]. Fluoride concentrations as low as 100 ppm were enough to cause substantial reductions in seedling growth parameters, while 300 parts per million was highly toxic (Sodani, 2018: Sodani *et al.*, 2021) [19, 20]. Because the root: shoot ratio improved marginally under fluoride levels of 100 to 200 ppm (Figure. 2) before declining at 300 ppm, therefore, it is inferred that up to 200 ppm, fluoride allocated more dry matter to roots, but at 300 ppm and above, it assigned the more dry matter to the shoot. In other words; at higher levels suppresses root growth more than shoot growth. Singh *et al.* (2013) [18] also reported similar results.

5. Conclusion

Present study concluded that increased fluoride concentrations in the range of 100 to 300 ppm decreased imbibition of germinating wheat seeds resulting in reduced germination percent. Increased fluoride concentrations resulted in reduced root and shoot lengths, fresh and dry matter in the shoot and root. Nevertheless, higher levels of F affect root growth more than shoot growth as evident by root: shoot weight ratio. It is suggested that the mechanisms that allow the fluoride to affect the distribution of dry matter between root and shoot and its influence on germination-related parameters should be investigated.

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