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Studies on the impact of elevated concentrations of fluoride in soil on morphological parameters of wheat (*Triticum aestivum* L.)

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Abstract
Effect of six levels of fluoride *viz*.0 (T_1), 50 (T_2), 100 (T_3), 200 (T_4), 250 (T_5) and 300 (T_6) mg fluoride kg^{-1} soil on morphological parameter of wheat genotype HUW-234 was studied under pot culture during 2016-17 and 2017-18. Observations were recorded at 30 days interval. Elevated concentrations of fluoride significantly reduce shoot dry matter, leaf area, relative growth rate (RGR) and net assimilation rate (NAR) while plant height at 50 mg fluoride kg^{-1} soil was marginal stimulated but at higher concentration it declined. It is concluded that elevated fluoride level in soil causes reduction in plant growth and development primarily by reducing photosynthetic area and NAR.

Keywords: Fluoride, morphology, leaf area, RGR, NAR

Introduction
Wheat (*Triticum aestivum* L.) is an important staple cereal crop throughout the world. Wheat is the second most important cereal crop next to rice. In India, during 2016-17, wheat was grown over an area of 30.42 million hectare with production of 98.51 million tonnes (Anonymous 2018) [1]. Fluoride (F^-) is the anion form of fluorine. Fluorine is an element of halogen family. Organic and inorganic fluorine compounds are sometimes called fluoride (Greenwood *et al.* 1997) [4]. It is reported that present of sodium fluoride in soil caused reduction in dry matter and leaf area pn wheat (Shadad *et al*. 1989) [8]. Rathore (1992) [7] found that various concentrations of sodium fluoride (5-500 ppm) cause significant reduction in leaf area, net assimilation rate and relative growth rate in *Hordeum vulgare* and *Zea mays*. both crop plants. *Hordeum vulgare* could not tolerate a fluoride application beyond 100 ppm while *Zea mays* tolerated up to 500 ppm. Sodium fluoride toxicity is also reported to reduce plant height and leaf area per plant in two varieties of barley *viz.* DL-69 and K-24 and in two varieties of wheat *viz.* HD-2009 and PBW-226 (Singh *et al*.2014) [10].

Materials and methods
This experiment was conducted during *rabi* 2016-17 and 2017-18 in pots (diameter 25 and height 25 cm) filled with 7.50 kg pulverized dry soil collected from the field and mixed with FYM (4:1). Pots used in this experiment were without hole at bottom. Soil was supplemented with N, P and K @ 120:60:40 kg ha^{-1}, respectively. 50% N was applied as basal and rest in two equal split doses at 21 days after sowing (DAS) and at boot stage.

Imposing fluoride treatment and growing plants
Pots were divided into 6 sets and supplemented with 0 (T_1), 50 (T_2), 100(T_3), 200(T_4), 250(T_5) and 300 (T_6) mg fluoride kg^{-1} soil. NaF was used as a salt to supply fluoride. Amount of fluoride required to attain desired level of fluoride in each pot was calculated. It was dissolved in 1.5L water and full volume was applied to respective pot. Pots were left for 5 days to equilibrate and by that time soil moisture content was suitable for sowing. Sowing was done on November 23 and December 5 during 2016-17 and 2017 18, respectively. After 7 days of sowing, thinning was done to maintain 5 plants of uniform growth in each pot.
Fluoride treatments were again repeated at 25, 50, 75 and 100 days after sowing (DAS) by irrigating pots to saturation level with the solution of the same concentration of F. Observations were recorded at 30, 60, 90 days after sowing (DAS) and harvest stages.

Morphological parameters
Observations were recorded in three replications from three randomly selected pots. For each replication three plants from a pot were selected.

Mean value of such three plants was considered as one replication in each treatment at a particular stage.

(a) Plant height (cm)

Height of the selected plants was measured from the surface of soil in pots up to the growing tip of the main stem with meter scale and expressed in cm. It was recorded at 30, 60, 90 DAS and harvesting stage.

(b) Dry weight (g plant^{-1})

Three plants from each replication were taken at an interval 30 days from 30 DAS till harvest and oven dried at105°C for 1h followed by at 65°C till constant weight. Plant dry weight was expressed as g plant^{-1}.

(c) Leaf area (cm^2 plant^{-1})

All leaves (lamina portion) from individual plants were excised. Their maximum lengths and breadths were measured. Leaf area of individual leaf was calculated as:

Leaf area (cm^2) = [Maximum length × Maximum breadth] × 0.66

Areas of all leaves of a plant were summed to find out leaf area plant^{-1}.

Factor 0.66 was derived by taking leaves of different sizes, determining their area by paper weighing method and finding out relationship between their [maximum length × maximum breadth] and area.

(d) Relative growth rate (RGR)

It was calculated by using the formula given by Blackman (1919) and expressed as mg mg^{-1} 30 days^{-1}. It was measured between 0-30, 30-60 and 60-90 DAS.

$$RGR = \frac{Log_e W_2 - Log_e W_1}{T_2 - T_1}$$

Where,

W_1= Dry weight of the plant (mg) at time T_1

W_2= Dry weight of the plant (mg) at time T_2

(e) Net assimilation rate (NAR)

It is rate of dry matter increase per unit of leaf area per unit time (Radford, 1967) and expressed as mg cm^{-2} 30 days^{-1} and calculated as follows:

$$NAR = \frac{(W_2 - W_1)}{(T_2 - T_1)} \times \frac{(Log_e A_2 - Log_e A_1)}{(A_2 - A_1)}$$

Where,

A_1 and W_1= Leaf area (cm^2) and dry weight of the plant (mg), respectively, at time T_1

A_2 and W_2= Leaf area (cm^2) and dry weight of the plant (mg), respectively, at time T_2

Individual plant weigtht and leaf area plant^{-1} was used to calculated this parameter. It was measured between 0-30, 30-60 and 60-90 DAS.

Result

1. Plant height (cm plant^{-1})

Height of plants grown in pots at elevated concentration of fluoride in soil was measured at 30, 60, 90 days after sowing and at harvest during 2016-17 and 2017-18. Differences were found to be significant with respect to stage (S), treatment (T) and S × T (Table 1). During 2016-17, as expected, with advancement in days after sowing (DAS) plant height increased. The increment was relatively higher between 60-90 DAS. When mean values for different treatments were compared; it decreased with increased level of fluoride in the root zone. Mean plant height did not differ significantly between T_1 and T_2 treatments, but with further increase in fluoride level in soil; it decreased significantly at all stages. When S × T interactions were examined, at a particular stage, with increased fluoride level plant height under T_1 and T_2 treatment did not differ significantly. However, as compared to T_1, plants under other treatments had significantly lower plant height. Similar trend was observed during 2017-18.

Table 1: Effect of different concentrations of fluoride on plant height (cm plant^{-1}) in wheat genotype HUW-234 at 30, 60, 90 and at harvest during *rabi* 2016-17 and 2017-18

S. No.	Treatments*	Year									
		2016-17				Mean	2017-18				Mean
		Stage (days after sowing)					Stage (days after sowing)				
		30	60	90	At harvest		30	60	90	At harvest	
1.	T_1	33.00	58.67	68.33	73.00	53.33	36.33	60.33	70.00	74.67	55.56
2.	T_2	33.67	59.00	70.67	73.67	54.44	37.00	61.00	72.67	75.67	56.89
3.	T_3	32.00	47.67	66.00	65.33	48.56	34.00	49.67	68.00	67.33	50.56
4.	T_4	31.67	41.00	61.33	63.00	44.67	33.67	43.00	63.33	65.33	46.67
5.	T_5	29.33	39.33	55.00	62.33	41.22	31.33	41.33	57.00	64.33	43.22
6.	T_6	28.33	38.33	48.00	54.67	38.22	30.33	40.33	50.00	58.00	40.22
	Mean	31.33	47.33	61.56	65.33		33.78	49.28	63.50	67.56	
			SEm±	CD (5%)				SEm±	CD (5%)		
Stage (S)			0.37	1.04				0.38	1.08		
Treatment (T)			0.45	1.28				0.46	1.32		
S × T			0.90	2.55				0.93	2.64		

*T_1, T_2, T_3, T_4, T_5, and T_6 represent 0, 50, 100, 200, 250 and 300 mg fluoride kg^{-1} soil, respectively.

2. Shoot dry matter (g plant^{-1})

Shoot dry weight was quantified at 30, 60, 90 days after sowing and at harvest during 2016-17 and 2017-18. Differences were significant with respect to stage (S), treatment (T) and S × T (Table 2). During 2016-17, with advancement in days after sowing (DAS) mean values for dry matter plant^{-1} increased. Mean treatment values decreased with increased level of fluoride in the root zone. At all stages of observation; the maximum dry matter was recorded in plants under T_1 (control) treatment, i. e., 0.36, 2.14, 4.08 and 3.50 g plant^{-1} at 30, 60, 90 DAS and harvest stages, respectively. It was evident that as compared to 90 DAS shoot dry weight plant^{-1} decreased at harvest stage. Similar pattern was found in during 2017-18.

Table 2: Effect of different concentrations of fluoride on shoot dry matter (g plant^{-1}) in wheat genotype HUW-234 at 30, 60, 90 and 120 days after sowing during *rabi* 2016-17 and 2017-18

S. No.	Treatments*	Year									
		2016-17				Mean	2017-18				Mean
		Stage (days after sowing)					Stage (days after sowing)				
		30	60	90	At harvest		30	60	90	At harvest	
1.	T_1	0.36	2.14	4.08	3.50	2.20	0.37	2.21	4.26	3.71	2.28
2.	T_2	0.33	2.04	3.68	2.89	2.02	0.34	2.06	4.05	3.48	2.15
3.	T_3	0.29	1.84	3.46	2.77	1.86	0.29	1.89	3.75	2.93	1.98
4.	T_4	0.28	1.77	3.36	2.60	1.80	0.28	1.86	3.64	2.77	1.92
5.	T_5	0.24	1.75	3.04	2.27	1.68	0.25	1.75	3.47	2.47	1.82
6.	T_6	0.15	1.35	2.74	1.92	1.41	0.16	1.43	3.02	2.01	1.54
	Mean	0.27	1.82	3.39	2.66		0.28	1.87	3.70	2.89	
			SEm±	CD (5%)				SEm±	CD (5%)		
Stage (S)			0.02	0.05				0.02	0.06		
Treatment (T)			0.02	0.06				0.03	0.07		
S × T			0.04	0.11				0.05	0.15		

*T_1, T_2, T_3, T_4, T_5, and T_6 represent 0, 50, 100, 200, 250 and 300 mg fluoride kg^{-1} soil, respectively.

3. Leaf area (cm^{-2} plant^{-1})

Leaf area was measured at 30, 60, 90 days after sowing during 2016-17 and 2017-18. Differences were significant with respect to stage (S), treatment (T) and S × T (Table 3). During 2016-17, at 30, 60 and 90 DAS the mean value of leaf area plant^{-1} was the maximum in plants under T_1 (control) treatment, i. e., 68.02, 188.33 and 225 cm^{-2} plant^{-1}, respectively, which at different stages gradually declined with increase in fluoride concentration in soil. As compared to 30 and 60 days stages leaf area was the maximum at 90 DAS in plants under respective treatments. Almost similar trend was recorded in 2017-18 and the mean differences were analogous.

Table 3: Effect of different concentrations of fluoride on leaf area (cm^{-2} plant^{-1}) in wheat genotype HUW-234 at 30, 60 and 90 days after sowing during *rabi* 2016-17 and 2017-18

S. No.	Treatment*	Year							
		2016-17			Mean	2017-18			Mean
		Stage (days after sowing)				Stage (days after sowing)			
		30	60	90		30	60	90	
1.	T_1	68.02	188.33	225.00	160.45	69.18	192.00	230.00	163.73
2.	T_2	66.18	182.00	214.67	154.28	66.68	186.33	218.00	157.00
3.	T_3	62.16	175.33	193.67	143.72	62.76	176.67	196.00	145.14
4.	T_4	55.57	162.00	182.67	133.41	56.69	164.00	184.00	134.90
5.	T_5	50.51	150.00	175.00	125.17	51.45	152.00	176.33	126.59
6.	T_6	45.66	142.67	163.00	117.11	46.91	143.67	163.00	117.86
	Mean	58.02	166.72	192.33		58.94	169.11	194.56	
			SEm±	CD (5%)			SEm±	CD (5%)	
Stage (S)			0.69	1.98			0.49	1.41	
Treatment (T)			0.97	2.80			0.70	2.00	
S × T			1.69	4.84			1.21	3.46	

*T_1, T_2, T_3, T_4, T_5, and T_6 represent 0, 50, 100, 200, 250 and 300 mg fluoride kg^{-1} soil, respectively.

4. Relative growth rate (mg mg^{-1} 30 days^{-1})

Relative growth rate (RGR) was calculated between 0-30, 30-60 and 60-90 days after sowing (DAS) during 2016-17 and 2017-18 and presented in Table 4 differences were found to be significant with respect to stage (S), treatment (T) and S × T. During 2016-17, as compared to 0-30 DAS, mean value for RGR decreased markedly between 30-60 and 60-90 DAS. Mean RGR for T_1 was 2.77mg mg^{-1}30 days^{-1}, which declined successively with increased fluoride concentration in soil to 2.74, 2.72, 2.71, 2.67 and 2.64 mg mg^{-1} 30 days^{-1}under T_2, T_3, T_4, T_5 and T_6 treatments, respectively. Between 0-30 day stages, as compared to T_1, RGR decreased progressively up to T_6 treatment, while between 30-60 day stages it increased up to T_6 treatment. Between 60-90 day stages it decreased up to T_5 treatment level then increased. Experiment conducted during second year also showed similar trend but between 30-60 and 60-90 day stages, as compared to T_1, RGR increased progressively up to T_6 treatment (Table 4).

Table 4: Effect of different concentrations of fluoride on relative growth rate (mg mg^{-1} 30 days^{-1}) in wheat genotype HUW-234 between 0-30, 30-60 and 60-90 days after sowing during *Rabi* 2016-17 and 2017-18

S. No.	Treatment*	Year							
		2016-17			Mean	2017-18			Mean
		Stage (days after sowing)				Stage (days after sowing)			
		0-30	30-60	60-90		0-30	30-60	60-90	
1.	T_1	5.89	1.78	0.65	2.77	5.91	1.79	0.66	2.79
2.	T_2	5.80	1.82	0.59	2.74	5.81	1.82	0.67	2.77
3.	T_3	5.66	1.86	0.63	2.72	5.67	1.87	0.69	2.74
4.	T_4	5.62	1.86	0.64	2.71	5.64	1.89	0.67	2.73

5.	T_5	5.49	1.98	0.55	2.67	5.51	1.95	0.69	2.72
6.	T_6	4.99	2.22	0.71	2.64	5.04	2.22	0.75	2.67
	Mean	5.57	1.92	0.63		5.59	1.92	0.69	
			SEm±	CD (5%)			SEm±	CD (5%)	
Stage (S)			0.01	0.01			0.01	0.01	
Treatment (T)			0.01	0.02			0.01	0.02	
S × T			0.01	0.03			0.01	0.03	

*T_1, T_2, T_3, T_4, T_5, and T_6 represent 0, 50, 100, 200, 250 and 300 mg fluoride kg^{-1} soil, respectively.

5. Net assimilation rate (mg cm^{-2}30 $days^{-1}$)

Net assimilation rate (NAR) was studied between 0-30, 30-60 and 60-90 days after sowing (DAS) during 2016-17 and 2017-18 and presented in (Fig. 1; 2). Differences were found to be significant with respect to stage (S), treatment (T) and S × T. During 2016-17, mean NAR was significantly higher between 0-30 DAS which decreased sharply and progressively between30-60 and 60-90 DAS. Treatment means also declined with increased fluoride level in root zone. T_6 level appeared to be vary deleterious than other fluoride treatments. Between 0-30 days stage NAR decreased with increased fluoride concentration while between 30-60 days stage NAR decreased up to T_4 treatment than increased at T_5 treatment and further declined at T_6 treatment. Between 60-90 DAS; with increased fluoride level NAR declined but no definite pattern was observed. Almost similar pattern was found during 2017-18 but during this year between 60-90 days stage NAR increased with fluoride concentration up to T_5 treatment and then declined at T_6 treatment.

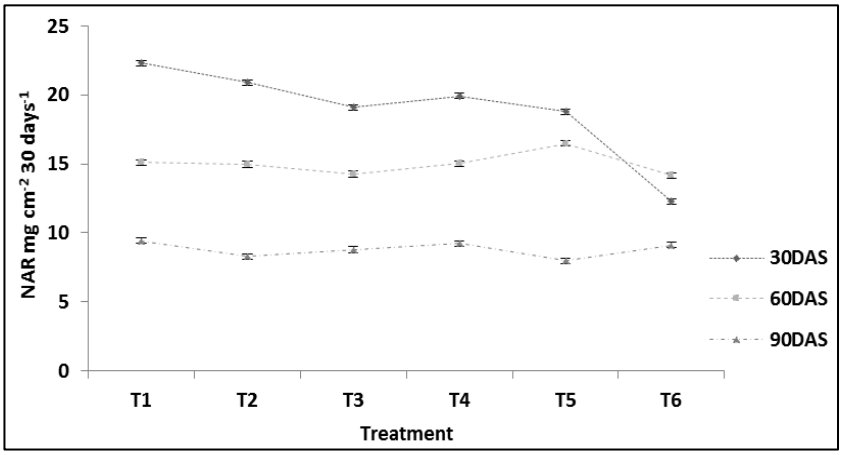

Fig 1: Effect of different concentrations of fluoride on net assimilation rate (mg cm^{-2} 30 $days^{-1}$) in wheat genotype HUW-234 between 0-30, 30-60 and 60-90 days after sowing during *rabi* 2016-17. Vertical bars on graph represent standard error ($p \leq 0.05$). *T_1, T_2, T_3, T_4, T_5, and T_6 represent 0, 50, 100, 200, 250 and 300 mg fluoride kg^{-1} soil, respectively

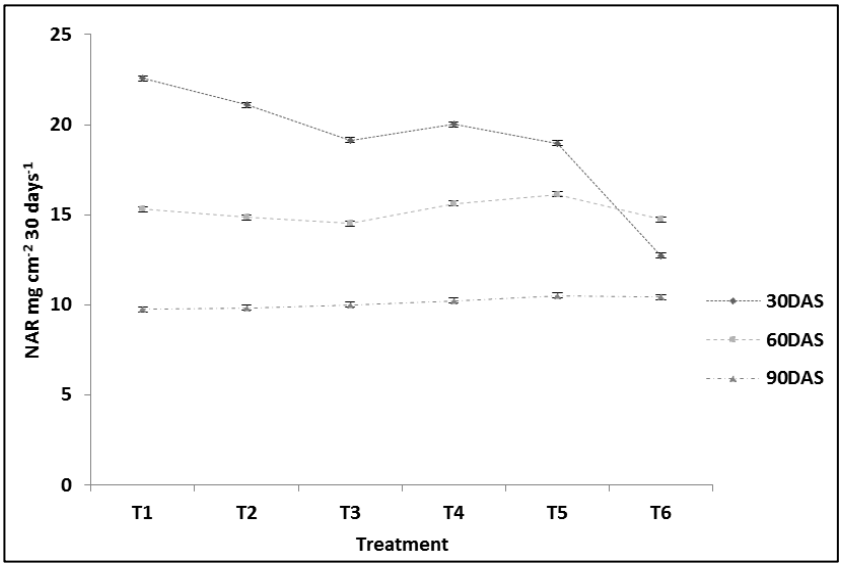

Fig 2: Effect of different concentrations of fluoride on net assimilation rate (mg cm^{-2} 30 $days^{-1}$) in wheat genotype HUW-234 between 0-30, 30-60 and 60-90 days after sowing during *rabi* 2017-18. Vertical bars on graph represent standard error ($p \leq 0.05$). T_1, T_2, T_3, T_4, T_5, and T_6 represent 0, 50, 100, 200, 250 and 300 mg fluoride kg^{-1} soil, respectively

Discussion

Fluoride toxicity is reported to reduce plant height in wheat (Singh *et al*. 2001) [9]. At 50 mg fluoride kg^{-1} soil had marginal stimulatory effect on plant height (Table 1).There is a report indicating that fluoride may have stimulatory effect on plant growth at very low concentration (Gao *et al.* 2012) [3]. However, dry matter $plant^{-1}$ (Table 2) and leaf area $plant^{-1}$ (Table 3) decreased concomitantly as the level of fluoride

increased in soil. Even 50 mg fluoride kg^{-1} soil had significantly retarding effect on these parameters and 300 mg fluoride kg^{-1} soil was highly toxic. Reports are available to indicate that higher concentrations of sodium fluoride significantly reduced leaf area, net assimilation rate and relative growth rate in maize and barley (Shadad *et al*. 1989, Rathore 1992) [7, 8]. Leaves are the major photosynthetic organ in wheat; therefore, reduction in leaf area is reflected in terms of reduction in rate of dry matter accumulation per unit of time (RGR). In the present study increased fluoride level decreased RGR (Table 4) at all stages of observation, proving that fluoride has deleterious effects on RGR of wheat. As the concentration of fluoride increased then mean NAR declined (Fig. 1; 2). It is reported that increased fluoride level causes reduction in net photosynthetic rate (Yamauchi *et al*. 1983) [12]. By affecting photosynthetic area as well as photosynthetic pigments (Justyna *et al*. 2017, Singh *et al.* 2017) [5, 11]. In another experiment it was observed that photosynthetic pigments level declined under fluoride toxicity. It is concluded that elevated fluoride level in soil causes reduction in plant growth and development primarily by reducing photosynthetic area and NAR.

Conclusion

It is concluded that the elevated concentrations of fluoride on wheat genotype HUW-234 significantly reduce shoot dry matter, leaf area, relative growth rate and net assimilation rate at various growth stages but plant height increased at 50 mg fluoride kg^{-1} soil than declined. Significant effects were obtained at 5% CD values. Control plants demonstrate best performance than plants subjected to 250-300 mg fluoride kg^{-1} soil.

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