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Effect of Fluoride Toxicity on Physiological and Biochemical Parameters of Wheat (*Triticum aestivum)*

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ABSTRACT

Wheat is the second most important cereal crop next to rice. Fluoride toxicity effects on physiological (relative water content, chlorophyll and carotenoids) and biochemical (soluble sugar, starch, free amino acid, proline and malondialdehyde (MDA) contents parameters of wheat plants from their germination to maturity and ultimately reduces the yield.A pot experiment was conducted to investigate the effect of different concentrations [0 (T_1), 50 (T_2), 100 (T_3), 200 (T_4), 250 (T_5) and 300 (T_6) mg fluoride kg^{-1} soil] of fluoride in wheat variety HUW-234. Observation was recorded at 30, 60 and 90 DAS. The results indicate that increased level of fluoride decline RWC, chlorophyll, carotenoids, starch, soluble protein, free amino acid while chlorophyll a: b ratio, soluble sugar, proline and MDA contents increased. The present study revealed that fluoride increased concentrations of soluble sugars, proline and MDA indicated that like other stresses fluoride also caused oxidative stress in wheat. Chlorophyll a : chlorophyll b ratio also increased in plant leaves exposed to fluoride. It is noted that fluoride caused more reduction in level of accessory photosynthetic pigments (chlorophyll b and carotenoids) than in the level of main photosynthetic pigments.

Key words: *Triticum aestivum, Fluoride, RWC, Lipid peroxidation, Proline, Sugar, Protein.*

Introduction

In India, many states fluoride ion (F) occurs naturally in the groundwater in varying amounts (Choubisa, 2017). In the groundwater higher concentration of fluoride is highly toxic for plants (Baunthiyal and Ranghar, 2014 and Yadhu *et al.*, 2016) and human beings (Choubisa, 2001). Reduction in photosynthetic rate is reported due to reduction in photosynthetic pigments and increased stomatal and non-stomatal limitation to photosynthesis (Cai *et al.*, 2017). Fluoride interferes with the metabolism of proteins, lipids and carbohydrates (Choubisa *et al.*, 2009; Ahmad *et al.*, 2014 and Gadi, 2016). Fluoride impedes starch biosynthesis in tubers of potato and grains of jowar but its effects on sucrose biosynthesis are either inhibitory or promontory (Viola *et al.*, 1991). In respect to fluoride tolerance genetic differences have been reported in wheat (Kumar *et al.*, 2013; Singh *et al.*, 2014 ; Alim et.al., 2017) and other crops (Singh *et al.*, 2013 and Kumar *et al.*, 2009) which have been related to differential responses of these crops/genotypes at physiological, biochemical and genetic levels (Rao *et al.*, 2013 and Pelc *et al.*, 2017). Total soluble sugar and proline contents increased along with a steady increase of fluoride concentration while the reduction in proline content in gram (Datta *et al.*, 2012). 100–200 ppm sodium fluoride (NaF) effect on the activities of amylase and transaminases in relations to the conversion of starch to free sugars and soluble protein and fluoride ion also drastically decreased

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starch content and caused accumulation of reducing and non-reducing sugars in root and shoot of wheat (Asthir and Tak, 2017). Fluoride toxicity leads to oxidative stress and occurs various changes like a decrease in chlorophyll content, changes in the level of soluble sugars and proline content in plants (Rizzu *et al.*, 2021).

Wheat (*Triticum aestivum* L.) is an important cereal next to rice. Cellular metabolism is drastically affected by fluoride toxicity in wheat because fluoride is readily absorbed by its roots and translocated to other parts of plants (Kamalluddin and Zwiazek, 2003). Hence, in the present study, we have investigated the different concentrations of fluoride change in chlorophyll, carbohydrates, amino acids and lipid peroxidation of *Triticum aestivum*.

Materials and Methods

The present pot culture investigation was conducted at Research Farm of the Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (India), during rabi 2016-17 and 2017-18. Seeds of wheat variety HUW-234, procured from the Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, B. H. U., Varanasi. Plastic pots without a hole at the bottom (diameter 25 and height 25 cm) were filled with 7.50 kg pulverized dry soil mixed with FYM. The 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 as top dressing.

To impose fluoride toxicity to the wheat plants, sodium fluoride (NaF) was added to the pots @ 50 (T_2), 100 (T_3), 200 (T_4), 250 (T_5) and 300 (T_6) mg fluoride kg^{-1} soil. Control pots with no added fluoride (T_1) were taken along for comparison. Ten wheat seeds were sown in each pot and after germination of seeds, 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). Observations pertaining to contents of relative water content, chlorophyll, carotenoids, total soluble sugar, starch, total soluble protein, free amino acids, proline and malondialdehyde (MDA) were recorded at 30, 60 and 90 days after sowing (DAS) in the uppermost fully expanded leaf/flag leaf. Relative water content (RWC), chlorophyll, carotenoids, total soluble sugar (SS) and starch content, total soluble protein content, free amino acid content were determined by Weatherly (1950), Hiscox and Israelstam (1979), Anthrone method (Dubois *et al.*,1956), Bradford method (Bradford, 1976) and Ninhydrin reagent (Yem and Cocking, 1955), respectively. Proline content was determined by the method described by Bates *et al.*, 1973. The level of membrane lipid peroxidation was determined as MDA content, according to the method of Heath and Parker 1968. Mean values were taken of three independent replications from each treatment. Analysis of variance for CRD was performed by SPSS version 20.0 software. A significant difference among treatments was determined by Duncan's multiple range test.

Results

Chlorophyll and carotenoids

In the present investigation fluoride caused significant reduction in chlorophyll a, chlorophyll b and total chlorophyll content (Table 1 & 2). It was found that as compared to 30 DAS chlorophyll a increased at 60 DAS and then declined at 90 DAS. Chlorophyll b and total chlorophyll contents also followed a

Table 1. Effect of different concentrations of fluoride on chlorophyll a and chlorophyll b content (mg g^{-1} fresh weight) in first fully expanded leaf from top of wheat (mean data of two years).

Treatments	Chlorophyll a content (mg g^{-1} FW)			Chlorophyll b content (mg g^{-1} FW)		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T_1	0.95± 0.01^{a}	1.25 ±0.03^{a}	0.94 ± 0.01^{a}	0.52± 0.01^{a}	0.75 ±0.02^{a}	0.62 ± 0.01^{a}
T_2	0.89± 0.01^{b}	1.04 ±0.04^{b}	0.89 ± 0.00^{b}	0.41± 0.01^{b}	0.70 ±0.02^{b}	0.55 ± 0.02^{b}
T_3	0.87 ±0.00^{c}	0.98 ±0.01^{c}	0.82 ±0.00^{c}	0.40 ±0.01^{bc}	0.48 ±0.01^{c}	0.51 ±0.01^{b}
T_4	0.85 ±0.01^{d}	0.95 ±0.00^{c}	0.80 ± 0.00^{d}	0.38 ±0.01^{cd}	0.47 ±0.00^{c}	0.40± 0.02^{c}
T_5	0.82 ±0.00^{e}	0.93 ±0.01^{c}	0.77 ±0.01^{e}	0.36 ±0.00^{de}	0.42 ±0.00^{d}	0.24 ±0.02^{d}
T_6	0.81 ±0.00^{e}	0.91 ±0.01^{c}	0.75 ± 0.00^{f}	0.34 ±0.00^{e}	0.33 ±0.01^{e}	0.18± 0.01^{e}

Presented data are means of three replicates with standard errors. Within each treatment, different letters indicate significant differences by Duncan's multiple range test at $P < 0.05$.

similar pattern as observed for chlorophyll a during both years. The ratio of chlorophyll a: chlorophyll b in leaves increased (Table 2), while amount of carotenoids decreased (Table 3). Increased chlorophyll a to chlorophyll b ratio and decreased carotenoids content indicated that under fluoride toxicity accessory pigments are affected more than the main pigment.

Relative water content (RWC)

Table 3 represents increased concentration of fluoride in the root zone, relative water content (RWC) in leaves declined progressively at all stages of observations. At 30, 60 and 90 DAS, the maximum relative water content was recorded in plants under T_1 (control) treatment, i. e., 75.27, 70.42 and 63.76 %, respectively.

Soluble Sugar (SS) and Starch content

Total soluble sugar content increased with increase in fluoride concentration at all stages of observation. Soluble sugar content was recorded highest at 60 DAS. It was found to be 7.98 mg g^{-1} FW at 60 DAS in the control plants and on imposing fluoride stress @ 300 mg kg^{-1} (T_6) the soluble sugar content in leaf increased to extent of 19.92 mg g^{-1} FW at the same 60 DAS. Conversely, the starch content decreased with increased fluoride concentration in the root zone at the 30, 60 and 90 DAS. The maximum starch content was recorded in control leaf amounting to 15.04 mg g^{-1} FW followed by T_2 treatment 13.33 mg g^{-1} FW at 60 DAS (Table 4).

Soluble protein and free amino acid

Table 5 represents soluble protein content and free amino acid content in wheat leaves declined progressively at all stages of observation. Maximum soluble protein content was found at 60 DAS (13.60 mg g^{-1} FW) as compare to 90 DAS (7.43 mg g^{-1} FW) and 30 DAS (5.20 mg g^{-1} FW). At 30, 60 and 90 DAS, the maximum free amino acids content was recorded in plants under T_1 (control) treatment, i. e., 5.41, 13.23 and 6.44 mg g^{-1} FW, respectively.

Proline and malondialdehyde (MDA) content

Results obtained in this study found that imposed fluoride stress in the root zone increased the level of proline and MDA content (Table 6). Under all the

Table 2. Effect of different concentrations of fluoride on total chlorophyll content and chlorophyll a: b ratio (mg g^{-1} fresh weight) in first fully expanded leaf from top of wheat (mean data of two years).

Treatments	Total chlorophyll content (mg g^{-1} FW)			Chlorophyll a : b ratio (mg g^{-1} FW)		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T_1	1.47± 0.01^{a}	2.00 ±0.02^{a}	1.56 ± 0.01^{a}	1.84 ± 0.01^{a}	1.67 ±0.07^{a}	1.52 ± 0.01^{a}
T_2	1.30± 0.01^{b}	1.74±0.02^{b}	1.44 ± 0.02^{b}	2.19± 0.03^{b}	1.49±0.00^{b}	1.63 ± 0.08^{b}
T_3	1.27±0.01^{bc}	1.46 ±0.01^{c}	1.33 ±0.01^{c}	2.18±0.05^{bc}	2.02 ±0.02^{c}	1.63 ±0.03^{b}
T_4	1.23±0.01^{cd}	1.42 ±0.00^{c}	1.20 ± 0.02^{d}	2.26 ±0.04^{cd}	2.01 ±0.01^{c}	2.01 ± 0.06^{c}
T_5	1.18±0.00^{de}	1.35 ±0.00^{d}	1.01 ±0.02^{e}	2.26 ±0.04^{de}	2.23±0.02^{d}	3.22 ±0.01^{d}
T_6	1.15 ±0.00^{e}	1.24 ±0.01^{e}	0.93 ± 0.01^{f}	2.38 ±0.02^{e}	2.76 ±0.04^{e}	4.18 ± 0.10^{e}

Presented data are means of three replicates with standard errors. Within each treatment, different letters indicate significant differences by Duncan's multiple range test at $P < 0.05$.

Table 3. Effect of different concentrations of fluoride on total carotenoids content (mg g^{-1} fresh weight) and relative water content (per cent) in first fully expanded leaf from top of wheat (mean data of two years).

Treatments	Carotenoids content (mg g^{-1} FW)			Relative water content (per cent)		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T_1	0.42± 0.01^{a}	0.67 ±0.01^{a}	0.49 ± 0.01^{a}	75.27± 0.43^{a}	70.42 ±0.69^{a}	63.76 ± 0.40^{a}
T_2	0.36± 0.01^{b}	0.62±0.00^{b}	0.44 ± 0.00^{b}	73.40± 0.53^{b}	69.96 ±0.56^{a}	61.96 ± 0.25^{b}
T_3	0.35±0.00^{b}	0.47±0.02^{c}	0.42 ±0.00^{c}	70.71 ±0.25^{c}	67.25 ±0.25^{b}	60.54 ±0.20^{c}
T_4	0.33 ±0.00^{c}	0.42 ±0.02^{d}	0.39± 0.00^{d}	68.66 ±0.56^{d}	64.78 ±0.53^{c}	58.40 ± 0.43^{d}
T_5	0.32±0.00^{cd}	0.41±0.01^{d}	0.26 ±0.00^{e}	67.25 ±0.24^{d}	62.14 ±0.43^{d}	56.43 ±0.38^{e}
T_6	0.31 ±0.00^{d}	0.31±0.01^{e}	0.18 ± 0.00^{f}	65.62 ±0.61^{e}	59.83 ±0.29^{e}	55.03 ± 0.38^{f}

Presented data are means of three replicates with standard errors. Within each treatment, different letters indicate significant differences by Duncan's multiple range test at $P < 0.05$

treatments proline and MDA content increased with the advancement of growth at all stages of observation. Increment in proline content was more under all the treatments at 60 DAS. Maximum MDA content was reported in T6 treatment 5.42 µmol g^{-1} FW at 90 DAS which shows that the maximum oxidative damage to membranes under fluoride toxicity

Discussion

In the present part of the study, results indicate that fluoride induced changes in chlorophyll content, carotenoids, relative water content, carbohydrate composition, total soluble protein, free amino acid content and lipid peroxidation in the leaves of wheat (*Triticum aestivum* L.). Reports are available to indicate that fluoride toxicity causes reduction in chlorophyll content (Pelc *et al*., 2017and Singh *et al*., 2017), causes derangement in grannal structure (Fink, 1988) and reduces stomatal opening (Fink, 1988). Chlorophyll a is main photosynthetic pigment which is directly involved in photosynthesis. Other

Table 4. Effect of different concentrations of fluoride on total soluble sugar content and starch content (mg g^{-1} fresh weight) in first fully expanded leaf from top leaf of wheat (mean data of two years).

Treatments	Total Soluble Sugar content (mg g^{-1} FW)			Starch content (mg g^{-1} FW)		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T_1	4.29 ± 0.08^{f}	7.98 ±0.41^{e}	7.13 ± 0.26^{f}	5.56 ± 0.11^{a}	15.04 ±0.31^{a}	10.60 ± 0.32^{a}
T_2	5.97± 0.34^{e}	10.68 ±0.46^{d}	9.58 ± 0.57^{e}	4.21± 0.07^{b}	13.33 ±0.18^{b}	9.04± 0.32^{b}
T_3	8.19 ±0.22^{d}	13.88 ±0.60^{c}	12.02 ±0.37^{d}	3.51 ±0.23^{c}	11.47 ±0.50^{c}	6.88 ±0.06^{c}
T_4	10.19±0.21^{c}	15.84 ±0.34^{b}	14.48 ± 0.55^{c}	2.66 ±0.12^{d}	9.90 ±0.34^{d}	5.09 ± 0.11^{d}
T_5	11.64 ±0.15^{b}	18.61 ±0.61^{a}	16.93 ±0.41^{b}	2.41 ±0.07^{d}	9.57 ±0.19^{d}	4.15 ±0.30^{e}
T_6	12.58 ±0.38^{a}	19.92 ±0.46^{a}	19.54 ± 0.59^{a}	2.00 ±0.03^{e}	8.54 ±0.08^{e}	3.37 ± 0.10^{f}

Presented data are means of three replicates with standard errors. Within each treatment, different letters indicate significant differences by Duncan's multiple range test at P <0.05

Table 5. Effect of different concentrations of fluoride on free amino acid content and soluble protein content (mg g^{-1} fresh weight) in first fully expanded leaf from top of (mean data of two years).

Treatments	Free Amino Acid content (mg g^{-1}FW)			Total Soluble Protein content (mg g^{-1}FW)		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T_1	5.41 ± 0.15^{a}	13.23 ±0.12^{a}	6.44 ± 0.20^{a}	5.20 ± 0.06^{a}	13.60 ±0.10^{a}	7.43 ± 0.08^{a}
T_2	4.36± 0.20^{b}	11.26 ±0.01^{b}	5.32 ± 0.24^{b}	4.59± 0.07^{b}	11.62 ±0.04^{b}	5.86 ± 0.13^{b}
T_3	3.48 ±0.10^{c}	10.66 ±0.21^{c}	4.55 ±0.08^{c}	4.17 ±0.11^{b}	9.79 ±0.02^{c}	5.29 ±0.19^{c}
T_4	3.23 ±0.11^{c}	9.75 ±0.16^{d}	3.97 ± 0.10^{d}	3.42 ±0.11c	9.69 ±0.16^{cd}	4.06 ± 0.04^{d}
T_5	2.71 ±0.11^{d}	9.45 ±0.26^{d}	3.51 ±0.06^{e}	2.71 ±0.25^{d}	9.38 ±0.18^{d}	4.04 ±0.13^{d}
T_6	2.23 ±0.08^{e}	7.68 ±0.24^{e}	3.24 ± 0.04^{e}	2.50 ±0.12^{d}	8.29 ±0.12^{e}	3.92 ± 0.09^{d}

Presented data are means of three replicates with standard errors. Within each treatment, different letters indicate significant differences by Duncan's multiple range test at P <0.05.

Table 6. Effect of different concentrations of fluoride on proline content (mg g^{-1} fresh weight) and malondialdehyde (MDA) content (ìmol g^{-1} fresh weight) in first fully expanded leaf from top of wheat (mean data of two years).

Treatments	Proline content (mg g^{-1} FW)			Malondialdehyde content (µmol g^{-1} FW)		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T_1	0.07 ± 0.00^{f}	0.21 ±0.00^{f}	0.44 ± 0.01^{f}	1.91± 0.02^{f}	2.09 ±0.03^{f}	3.04 ± 0.01^{f}
T_2	0.10 ± 0.00^{e}	0.32 ±0.01^{e}	0.52 ± 0.00^{e}	2.52 ± 0.02^{e}	3.00 ±0.05^{e}	3.97 ± 0.08^{e}
T_3	0.12 ±0.00^{d}	0.37 ±0.01^{d}	0.70 ±0.01^{d}	3.45 ±0.04d	3.47 ±0.02^{d}	4.35 ±0.00^{d}
T_4	0.14 ±0.00^{c}	0.51 ±0.01^{c}	0.94 ± 0.01^{c}	3.66 ±0.01^{c}	3.75 ±0.03^{c}	4.71 ± 0.01^{c}
T_5	0.17 ±0.00^{b}	0.63 ±0.01^{b}	1.18 ±0.03^{b}	3.73 ±0.02^{b}	4.07 ±0.02^{b}	5.01 ±0.03^{b}
T_6	0.20 ±0.00^{a}	0.76 ±0.03^{a}	1.30 ± 0.00^{a}	3.90 ±0.01^{a}	4.33 ±0.02^{a}	5.42 ± 0.03^{a}

Presented data are means of three replicates with standard errors. Within each treatment, different letters indicate significant differences by Duncan's multiple range test at P < 0.05.

pigments such as chlorophyll b and carotenoids act as accessory pigment. Accessory pigments absorb light energies and transfer them to chlorophyll a for photochemical act (Salisbury and Ross, 2010).

NaF, which is a salt, has property to cause osmotic as well as ionic stresses in plants. Therefore, under induced fluoride toxicity by NaF may have influence on plant water relation parameter. Literature is available to indicate the influence of fluoride stress on plant water relation parameters (Zouari *et al.*, 2016). As with increased fluoride level reduction in leaf RWC was significant, therefore, it is inferred that increased fluoride level in root zone caused reduction in availability of water to plant resulting in decreased leaf relative water content. It will be interesting to investigate if either availability of water to roots from soil or the water absorption efficiency of root, or both the processes are affected by fluoride toxicity causing reduced leaf RWC. Under stress condition changes in the level of soluble sugar, starch, free amino acid and soluble protein are well documented (Asthir and Tak, 2017 and Kim *et al.*, 2003). Role of proline and soluble sugars in the osmotic adjustment of wheat plants is also well documented (Asthir and Tak, 2017). Leaf carbohydrate composition in the form of total soluble sugars and starch were determined. Total soluble sugar content increased with increase in fluoride concentration at all stages of observation. It indicated that increased soluble sugars in the leaf of fluoride stressed plants has been primarily due to hydrolysis of starch present in leaves (Asthir and Singh, 1995). Fluoride toxicity is clearly evident in the total soluble protein and free amino acid content of wheat leaves, observing that there is a sharp decrease with higher levels of toxicity. This observation is in agreement with others that fluoride inhibiting chain elongation of polypeptides (Rao *et al.*, 2013). It is inferred that reduction in soluble protein content in leaf has been due to the lesser availability of amino acids for protein synthesis under fluoride toxicity. Reference is also available to indicate that fluoride toxicity leads to increased tissue protein content (Asthir and Tak, 2017). The level of reactive oxygen species (ROS) is increased under fluoride stress (Wang and Jiao, 1991), which is detrimental to plants by damaging biomembranes, primarily due to the peroxidation of membrane lipids. One of the ways to observe the extent of biomembrane damages is by measuring the malondialdehyde (MDA) content in tissues of plants (Bansal and Srivastava, 2012). It is reported that fluoride toxicity causes increased production of reactive oxygen species causing an increase in the level of MDA (Saini *et al.*, 2013). Mulberry genotypes with low MDA content under fluoride stress are reported to tolerate higher levels of fluoride (Kumar *et al.*, 2009). In the present investigation increased fluoride level resulted in increased MDA content in wheat leaves. Though in this investigation levels of reactive oxygen species scavenging systems were not studied but by examining MDA content, it is concluded that increased fluoride levels caused an increase in the level of reactive oxygen species resulting in increased damage of biomembranes.

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