THE EFFECT OF DIFFERENT CONCENTRATIONS OF THE FLUORIDE ION ON THE GROWTH AND NUTRITIONAL VALUE OF TWO ELITE GENOTYPES OF *TRITICUM AESTIVUM*

Huma Alim,[a] M Afaq Ahmad,[a,*] Iqbal Munir,[a] Imran Khan,[b] Ghulam Mustafa,[b] Ikram Ullah,[b] M Nauman Ahmad,[c] Hamayoon Khan,[d] Masoom Yasinzai,[b] Afia Zia,[c] Imtiaz Khan,[e] M Ishfaq Khan[e]

Peshawar and Islamabad, Pakistan

ABSTRACT: Increased pollution poses a serious threat to the crop productivity of economically important crops. One of the major sources of air pollution is the fluoride ion (F) coming from brick kilns and other industries. F accumulates in plants and interferes with various metabolic activities. The present study was conducted to find out the effect of different concentrations of F (0 [control], 10, 25, 50, and 100 ppm) on two elite genotypes of wheat, Nemat and Ghanimat. After 14 days of F treatment, data was recorded for both genotypes on the germination rate, leaf area, growth rate, net assimilation rate, total soluble protein content, and protein profiling. In comparison to the control group, all the treated plants were negatively affected but the maximum negative effect was recorded, for both genotypes, at 25 ppm for the germination rate, leaf area, growth rate, and net assimilation rate. The protein content and profile of both varieties also showed increased levels of specific proteins at 25 ppm. Our conclusion was that the Nemat genotype showed more resistance to F stress than the Ghanimat genotype.

Keywords: Ghanimat; Growth rate; Nemat; Net assimilation rate; Pakistan; Protein; *Triticum aestivum*; Wheat.

INTRODUCTION

Air pollution by the fluoride ion (F) is a serious threat to both human and agricultural life. In crops, it may cause severe injuries leading to reduced growth and yield. In animals, the consumption of F-contaminated feed and water may lead to fluorosis. F is abundant in the earth's crust, often behaves as an environmental pollutant, and is present in most soil types. The total soil F concentration is approximately 20–1,000 µg/g of soil and F is mainly associated with the soil colloid or clay fraction. F is highly phytotoxic, diffuses easily in soil, and is taken up more readily by plant roots when the soil pH is more acidic.[1,2] The F concentration in agricultural water may be increased by the high use of phosphate fertilizers and by contamination by smoke from ceramic industries and brick kilns.[3]

F may enter the plant by various routes. Aerial F enters through stomata and then translocates to the margins and tips of the leaves.[4] F from the soil and water enters through roots by the process of passive diffusion and is then transported through the xylem into the shoots by the apoplastic and symplastic pathways. The flow is

[a]Institute for Biotechnology and Genetic Engineering (IBGE), The University of Agriculture, Peshawar, Pakistan; [b]Sulaiman bin Abdullah Aba Al-Khail Centre for Interdisciplinary Research in Basic Sciences (IIU), Islamabad, Pakistan; [c]Agricultural Chemistry Department, University of Agriculture, Peshawar, Pakistan; [d]Department of Agronomy, University of Agriculture, Peshawar, Pakistan; [e]Department of Weed Science, University of Agriculture, Peshawar, Pakistan; For correspondence: M Afaq Ahmad, Institute for Biotechnology and Genetic Engineering (IBGE), The University of Agriculture, Peshawar, Pakistan; E-mail: mianafaq@aup.edu.pk

unidirectional.[5] F is mainly accumulated in the leaves of plants rather than in other parts.[6] After F accumulates in plants, symptoms may appear of necrosis at the tips and margins of the leaves, leaf notching, falling of leaves, and chlorosis.[7] Ultimately plant growth and yield are reduced.[7] High levels of F disturb all the biochemical and physiological processes in plants. At the molecular level, F toxicity causes inactivation of the enzymes required for metabolism, the generation of reactive oxygen species (ROS), and alterations in gene expression.[8]

The growing of wheat, for making bread, is likely to have been of central importance at the beginning of agriculture and wheat is now one of the most important of the small number of domesticated crops grown around the world as a food source. Pakistan is an agricultural country with wheat as the principal crop. 70% of the Pakistani population are directly dependent on wheat while another 16% have an indirect dependence.[9] Ahmad et al. noted that increased urbanisation throughout South Asia had increased the number of brick kilns that typically surrounded major cities, with Peshawar, Pakistan, having 400–450 operating throughout the year in the agricultural areas around the city.[10]

The objective of the present study was to examine, in the Nemat and Ghanimat genotypes of wheat, the effect of different levels of F on the germination rate, the leaf area, the growth rate, the net assimilation rate, the soluble protein content, and the protein profiling.

METHODOLOGY

The experiments were carried out in a green house at the Institute for Biotechnology and Genetic Engineering (IBGE) at The University of Agriculture, Peshawar, Pakistan. The two wheat varieties, Nemat and Ghanimat, were subjected, to four F treatments, in triplicate, for 14 days, with 10, 25, 50, and 100 ppm of F in distilled water. Distilled water with 0 ppm of F was used for the control groups.

Germination rate: The seeds were sterilized and then transferred to beakers containing agar, distilled water, and sodium fluoride with 10, 25, 50, and 100 ppm of F. Distilled water along with agar was used for the control groups. After 15 days the experiments was terminated. The seed germination percentage and the dry weight of the seedlings were determined.[11]

Leaf area: The leaf area of both varieties was calculated using a planimeter.[8]

Net assimilation rate (NAR): The net assimilation rate (NAR), the rate of increase of dry weight per unit of leaf weight, was calculated by the formula as defined by Watson:[12,13]

$$NAR = \frac{(\log_e L_2 - \log_e L_1)}{(L_2 - L_1)} \times \frac{(W_2 - W_1)}{(t_2 - t_1)}$$

where: NAR=net assimilation rate; L_1 and L_2= total leaf area at times t_1 and t_2; W_1 and W_2=dry weight at times t_1 and t_2; and t_1 and t_2=time 1 and time 2 so that the time interval between the observations is $t_2 - t_1$.

Relative growth rate (RGR): The relative growth rate (RGR) was calculated by the formula given by Watson: [17]

$$RGR = \frac{(\log_e W_2 - \log_e W_1)}{(t_2 - t_1)}$$

where: RGR=relative growth rate; W_1 and W_2=dry weight at times t_1 and t_2; and t_1 and t_2=time 1 and time 2 so that the time interval between the observations is $t_2 - t_1$.

Statistical analysis: The results were analysed by using one way ANOVA and the LSD test. The means and standard errors of the means were calculated by Microsoft Excel (2007).

RESULTS

Two elite genotypes of wheat, Nemat and Ghanimat, were studied for the effect of different concentrations of F on the germination rate, leaf area, net assimilation rate, growth rate, total soluble protein content, and protein profiling. Elevated levels of fluoride negatively affected both the wheat genotypes studied.

Germination rate: In the control plates with 0 ppm of F, a germination rate of 100% was observed in both wheat genotypes. At 10 ppm, a negative effect on germination was seen in both genotypes in comparison to the controls. At 25 ppm, both genotypes were significantly affected and showed further reductions in the germination rate. However, compared to the 25 ppm group, at 50 ppm, the germination rate was increased in both genotypes with further increases in the 100 ppm group (Figure 1).

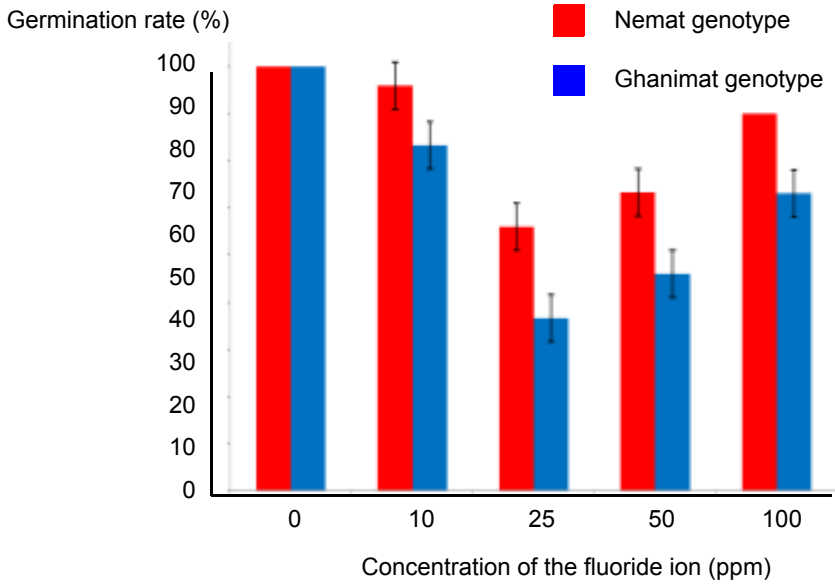

Figure 1. Germination rates (%) for the two different genotypes of wheat, Nemat and Ghanimat, with different levels of the fluoride ion (0, 10, 25, 50, and 100 ppm). Values are the mean±SEM of the experiments, which were performed in triplicate.

Leaf area: The leaf area (cm^2) for both genotypes was maximal in the control plates and was decreased at both 10 and 25 ppm of F. The leaf area recovered for both genotypes and increased at 50 ppm, compared to 25 ppm, and again at 100 ppm, compared to 50 ppm (Figure 2).

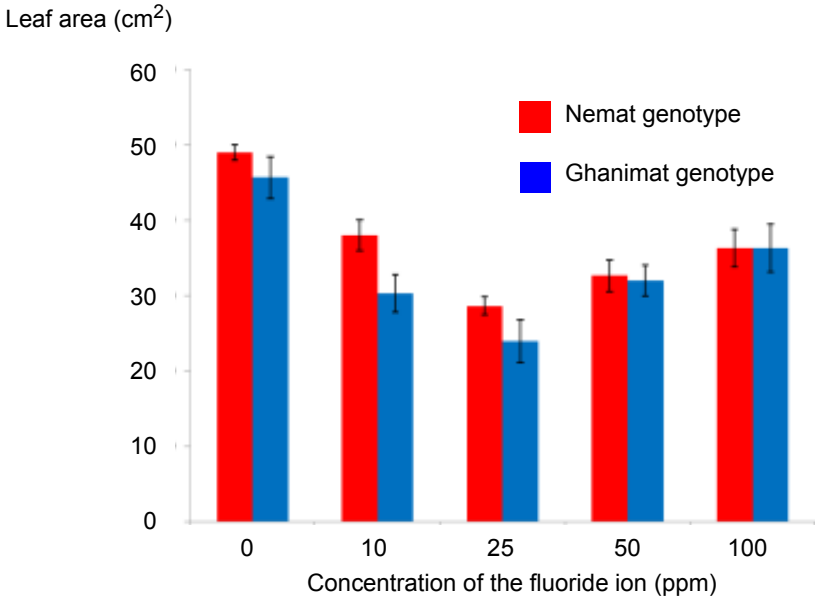

Figure 2. Leaf areas (cm^2) for the two different genotypes of wheat, Nemat and Ghanimat, with different levels of the fluoride ion (0, 10, 25, 50, and 100 ppm). Values are the mean±SEM of the experiments, which were performed in triplicate.

Growth rate: The growth rate (mg dry weight [dw]/day) for both genotypes was maximal in the control plants and decreased in the 10 ppm of F group but not to a significant extent. A significant reduction in the growth rate was found in the 25 ppm of F group. The growth rate was increased in the 50 ppm and 100 ppm of F groups compared to the 25 ppm of F group (Figure 3).

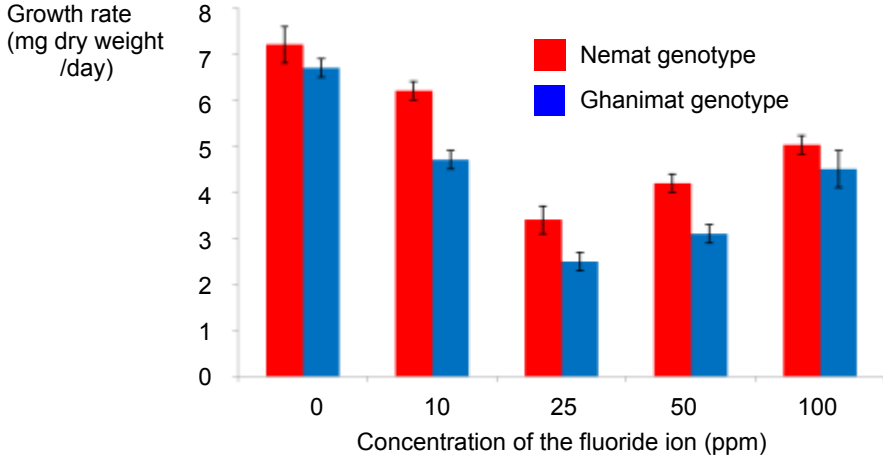

Figure 3. Growth rates (mg dry weight/day) for the two different genotypes of wheat, Nemat and Ghanimat, with different levels of the fluoride ion (0, 10, 25, 50, and 100 ppm). Values are the mean±SEM of the experiments, which were performed in triplicate.

Net assimilation rate: The net assimilation rate (mg dw/day) for both genotypes was maximal in the control group with 0 ppm of F and slightly reduced at 10 ppm of F compared to the control group. A more evident reduction was present in the 25 ppm of F group. Compared to the 25 ppm of F group, the NAR was increased in the 50 ppm of F group and further increased in the 100 ppm of F group (Figure 4).

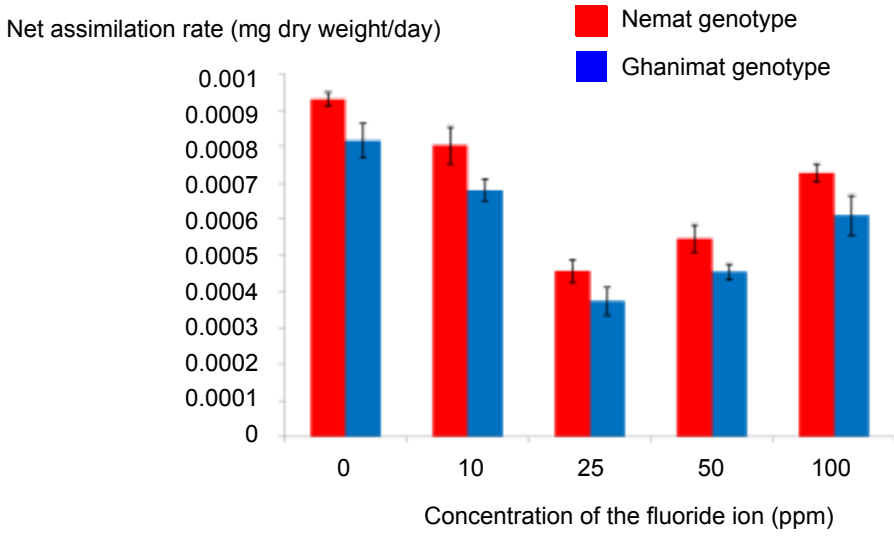

Figure 4. Net assimilation rates (mg dry weight/day) for the two different genotypes of wheat, Nemat and Ghanimat, with different levels of the fluoride ion (0, 10, 25, 50, and 100 ppm). Values are the mean±SEM of the experiments, which were performed in triplicate.

Protein profiling: The total protein contents were extracted from the genotypes and eight detectable bands were present in all samples of both genotypes (Figure 5).

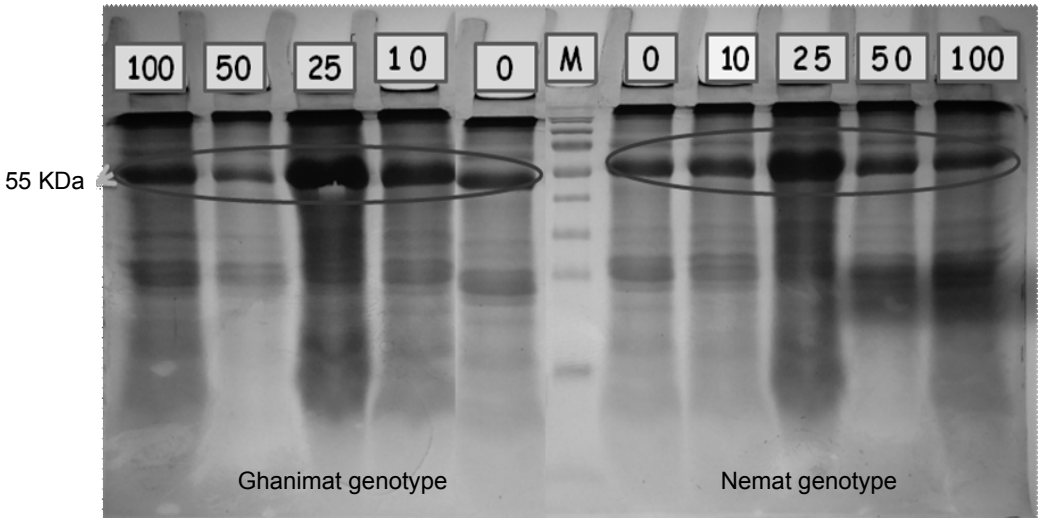

Figure 5. Protein profiling for the two different genotypes of wheat, Nemat and Ghanimat, with different levels of the fluoride ion (0,10, 25, 50, and 100 ppm) for 14 days.

The molecular sizes of the bands varied from 15 KDa to 130 KDa. The control groups showed a high intensity of proteins in both genotypes. At 10 ppm of F, the intensity of all the bands were similar to that of the control groups in both genotypes. At 25 ppm, the 55 KDa band concentrations were up-regulated in both genotypes compared to the 10 ppm group. In contrast, at 50 ppm of F, the intensity of the 55 KDa band was down-regulated in both genotypes. However, at 100 ppm all the bands in both genotypes showed an increased intensity compared to the 50 ppm of F group.

DISCUSSION

The present study showed that increased levels of F negatively affected the germination rate in both wheat genotypes, Nemat and Ghanimat. The germination rate decreased with increasing F concentrations and showed the maximum reduction at 25 ppm of F in both genotypes. This might be due to a dephosphorylation of the phytin compound. Phytin releases free phosphate and is thus a source of energy for germinating seeds. An increasing F concentration may inhibit this metabolic process. At 50 and 100 ppm there was an increase in the germination rate, as compared to the 25 ppm group, in both genotypes. This is consistent with previous studies by Singh et al.[7] and Gupta et al.[14] Bhargava and Bhardwaj[15] also reported work on the effect of sodium fluoride on seed germination and seedling growth of *Triticum aestivum* varieties.

The F accumulated in the leaves, as a result of gaseous exchange, negatively affected photosynthesis by reducing the leaf area and the net assimilation rate. In the present study, the leaf area and net assimilation rate were reduced in both genotypes with increasing F concentrations and the maximum effect occurred at 25 ppm of F. F degrades chlorophyllase and iron ions and these effects of F on the leaf area and net assimilation rate might be due to F degrading the chloroplasts, impairing the enzymes required for photosynthesis, and inhibiting the metabolism of reducing sugars. e.g., glucose, fructose, and mannose. At 50 ppm and 100 pm of F, the plants of both genotypes started to recover with the leaves become healthier and the net assimilation rate increasing. Adaptation of plants to this F toxicity, might be the reason why plants exposed to F stress have high concentrations of non-reducing sugars, e.g., sucrose, raffinose, instead of reducing sugars. The degradation of chloroplasts with F stress has been reported by Elloumi et al.,[16] Yamauchi et al.,[17] and Kim et al.[18] Our results showed that the Nemat genotype was more tolerant to the effects on leaf area and net assimilation rate of F stress then the Ghanimat genotype.

The plants affected with F showed decreased growth rate and yield. In the present study, there was decrease in the growth rate as the F concentration increased in both genotypes and the maximum negative effect occurred at 25 ppm of F. This might be due to F causing lipid peroxidation leading to reduced growth and cell death. F stress can reduce glutathione levels resulting in the formation of reactive oxygen species (ROS) at the mitochondrial level leading to damage to the components of cell and also the lipid bilayer. In our experiment, the growth rate showed some recovery in both wheat genotypes at 50 ppm and 100 ppm. This

might be due to the plants have antioxidant defense mechanisms, including superoxide, peroxidases, and catalases. This antioxidant defense system helps keep the plant alive by protecting the cells from ROS and membrane lipid peroxidation.[8] Our findings are consistent with those of Li et al.[19] We found that the Nemat genotype was more tolerant to the effects on growth of F toxicity than the Ghanimat genotype.

The levels of protein, one of the necessary nitrogenous constituents of plants, was also affected by F toxicity. Protein plays a role in the compensatory metabolic changes in plants when they are exposed to F. In present study, the total soluble protein content and the protein profiling of the two wheat genotypes were affected by exposure to F at 10 ppm, 50 ppm, and 100 ppm but the effect was not significant. Compared to the control groups, some protein bands in both genotypes were reduced. This decrease in the protein concentration might be due to either the degradation of protein or the use of protein to provide energy when the plant was experiencing stress. F can also decrease the rate of RNA synthesis by modifying free ribonucleotides and RNA. The decrease also might be due to the transport of F over longer distances by vacuolar sequestration being affected. Plant damage from toxins like F follows their being transported throughout the plant body after first being accumulated in vacuoles. In the present study, an increased content of protein at 25 ppm of F in both genotypes. F might also increase the plant content of the stress protein proline. Plants have a family of fluoride exporter proteins (FEX) which might be expressed when the plants are exposed to F. The function of these proteins is to export extra F out of the cell to prevent cell damage. Some transporter proteins like ATP-binding cassette transporters (ABC transporters) might be over expressed resulting in increased transport of F with a consequent increase in damage to the plant. At 50 ppm and 100 pm there might be a reduced expression of such transport proteins allowing the plants to begin to recover. Similar findings have been reported by Gadi BR et al.[20] and Li et al.[21] The reduced tolerance of the Ghanimat genotype to F stress compared to the Nemat genotype might be related in part to greater changes in the total soluble protein content and the protein profiling in this genotype.

CONCLUSIONS

The present study was carried out to examine the effect of different levels of F on plant growth and development. We found that, in both the Ghanimat and the Nemat genotypes, F had negative effects on the germination rate, the leaf area, the growth rate, the net assimilation rate, the total soluble protein content, and the protein profiling with the maximum adverse effect occurring at 25 ppm of F. The Nemat genotype was more tolerant of F stress than the Ghanimat genotype

REFERENCES

1 Polomski J, Fluhler H, Blaser P. Fluoride induced mobilization and leaching of organic matter, iron, and aluminium. J Environ Qual 1982;11:452-45.
2 Weinstein LH. Fluoride and plant life. J Occup Health 1977;19:49-78.
3 Jacobson J, Weinstein L, McCune D, Hitchcock AT. The accumulation of fluoride by plants. J Air Pollut Control 1966;16:412-7.

4 Kamaluddin M, Januszj, Zwiazek. Effects of root medium pH on water transport in paper birch (*Betula papyrifera*) seedlings in relation to root temperature and abscisic acid treatments. Tree Physiology 2004;24:1173-80.
5 Brahmbhatt NH, Patel RV. Effect of sodium flouride and magnesium chloride and its interaction on seedling growth of *Medicago sativa*, Anand, Gujarat. Asian J Agric Sci 2013; 3(3):80-4.
6 Saini P, Khan S, Baunthiyal M, Sharma V. Effects of fluoride on germination, early growth and antioxidant enzyme activities of legume plant species *Prosopis juliflora*. Journal of Environmental Biology / Academy of Environmental Biology, India 2013;34:205-9.
7 Singh S, Singh J, Singh N. Studies on the impact of fluoride toxicity on growth parameters of *Raphanus sativus* L. Indian Journal of the Science of Food and Agriculture 2013;4(1):61-3.
8 Barbier O, Arreola-Mendoza L, Del Razo LM. Molecular mechanisms of fluoride toxicity. Chem Biol Interact 2010;188:319-33.
9 Adsule RN, Kadam SS. Quality of wheat. In: Salunkhe DK Kadam SS, Austin A, editors. Quality of wheat and wheat products. New Delhi, India: Metropolitan Book Co.; 1986. pp.1-6.
10 Ahmad MN, Ahmad SS, Zia A, Iqbal MS, Shah H, Mian AA, Shah RU. Hydrogen fluoride effects on local mung bean and maize cereal crops from peri-urban brick kilns in South Asia. Fluoride 2014;47(4):315-9.
11 Sabal D, Khan TI, Saxena R. Effect of sodium fluoride on cluster bean (*Cyamopsis tetragonoloba*) seed germination and seedling growth. Fluoride 2006;39(3):228-30.
12 Watson JD. The physiological basis of variation in yield. Advances in Agronomy 1952;4:101-45.
13 Rathore S. Effect of fluoride toxicity on leaf area, net assimilation rate and relative growth rate of *Hordeum vulgare* and *Zea mays*. Fluoride1992;25(4):175-82.
14 Gupta V, Roy A, Tripathy BC. Signaling events leading to red-light-induced suppression of photomorphogenesis in wheat (*Triticum aestivum*). Plant & Cell Physiology 2010;51:1788-99.
15 Bhargava D, Bhardwaj N. Effect of sodium fluoride on seed germination and seedling growth of *Triticum aestivum* var. Rajasthan. J Phytol 2010;2(4):41-3.
16 Elloumi N, Ben Abdallah F, Mezghani I, Rhouma A, Boukhris M. Effect of fluoride on almond seedlings in culture solution. Fluoride 2005;38(3):193-8.
17 Yamauchi M, Chol WK, Yamada Y. Fluoride inhibition of photosynthesis in certain crop plants. Soil Sci Plant Nutr 1983;29(4):549-53.
18 Kim C-G, Power SA, Bel JNB. Effect of cadmium and soil type on mineral and carbon partitioning in seedlings of *Pinus sylvestris*. Water Air Soil Poll 2003;145:253-66.
19 Li C, Y Z, Zhou J, Xu J, D. N. Changes of leaf antioxidant system, photosynthesis and ultrastructure in tea plant under the stress of fluorine. Biol Plantarum 2011;55(3):563-6.
20 Gadi BR, Verma P, Amra R. Influence of NaF on seed germination, membrane stability and some biochemical content in *Vigna* seedlings. J Chem Bio Phy Sci 2012;2(3):1371-8.
21 Li S, Smith KD, Davis JH, Gordon PB, Breaker RR, Strobel SA. Eukaryotic resistance to fluoride toxicity mediated by a widespread family of fluoride export proteins. Proc Natl Acad Sci U S A 2013;110:19018-23.