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# Assessment of effect of fluoride pollution on germination rate, vigor index and fluoride contents in some selected varieties of wheat crop (*Triticum aestivum* L.)

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**Authors' Contribution** Dad, K., F. Zhao & S. Iftikhar designed the experiment, M. N. Khan & F. Ali analyzed the chlorophyll contents, germination percentage and vigor index, M. Nawab & R. M. Khan performed the analysis of protein, proline contents in the plants, K. Majid & K. Bilal determined the F contents in roots & shoots, M. Nawaz analysed the data and conducted statistical analysis.

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## ABSTRACT

Wheat being staple food and a source of economy in Pakistan is cultivated on large scale every year. Due to increasing environmental pollution, this crop is being affected directly or indirectly. Fluoride is one of the most toxic pollutants among rest of the environmental pollutants. A pot experiment was conducted to examine the impacts of fluoride toxicity on the seed germination, seedlings growth and F ion accumulation in locally cultivated varieties of wheat crop. In this regards, 5 wheat varieties (Akbar 2019, Anaj 2017, Ujala 2016, Galaxy 2016 and AARI 2011) were sown in green house at Bahauddin Zakaryia University (BZU), Multan, Pakistan by applying five (3 mM, 5mM, 7mM, 9mM and 11mM) treatments of NaF and control (0mM). Germination percentage, vigor index and F accumulation in roots and shoots were considered as parameters of research. Anaj 2017 and Akbar 2019 were exhibiting the highest germination percentage at 3mM (87% and 75%) while at 11 mM only Akbar 2019 showed 13% of germination rate as compared to that of control and rest of varieties. Vigor index was also found maximum 193.3 at 11mM of NaF in Anaj 2017. The fluoride contents were recorded higher in roots as compared to that of shoots in all varieties of wheat i.e. at 11 mM, 1 ug of F was found in AARI 2011 which was observed higher as compared to that of control as well as rest of varieties.

**Keywords**: Wheat, NaF, germination rate, vigor index, F contents, roots, shoots.

**INTRODUCTION:** Over 22 million acres of Pakistan are used for growing wheat, which contributes 1.8% of GDP and 7.8% of agriculture's value addition. Unfortunately, area sown dropped from 9,168 T/ha in 2021–22 to 8,976 T/ha compared to 27.464 M. tons produced, wheat output decreased to 26.394 M. tons this year (GOP, 2021-22). The decrease in the yield of wheat crop might be due to fluoride pollution among the other reasons. In modest levels, fluoride is required for healthy plant development; nevertheless, at excessive concentrations, it can harm both flora and the ecosystem (Gao *et al.*, 2012). Fluoride occurs naturally in the environment with an estimated total quantity of 0.077% in the earth's crust (Cai *et al.*, 2017). Fluoride is listed sixth among the chemicals that are responsible for contamination in the taxonomy of environmental poisons (Pelc *et al.*, 2020). Industrial activity, the production of artificial fertilizers, and the emission of fluoride compounds into the environment as dust and exhaust gases from aluminum smelters are the primary sources of fluoride pollution in the environment (Gautam and Bhardwaj, 2010). Indeed, F causes a variety of biochemical and physiological shifts in plants that influence plant growth and yield, potentially resulting in a significant drop in economic yield. In some soils, a high F content over >2.5 mM reduces biomass output and crop yield (Ropelewska *et al.*, 2016). The significance of the germination rate in plant development is generally acknowledged. Soil contamination caused by fluoride non-metal is one of the planet's major issues. Fluoride is required for optimal plant growth in modest amounts but, at greater concentrations, it can harm both plants and the ecosystem (Gao *et al.*, 2012). Fluoride-induced germination suppression has been linked to a decrease in enzymatic hydrolysis, which is required for seed germination. Fluoride poisoning has been linked to a reduction in germinating seeds and other metrics in wheat (Iram and Khan, 2016). Singh and his colleagues in 2017 discovered that elevated fluoride levels in the root zone impaired shoot length and root and shoot dry weight per plant. The importance of germinating seeds in plant development is well known, and its research has been utilized as a basis for investigating elements' toxic effects (Dadrwal *et al.*, 2022). As a result, the current study was carried out to visualize the effect of fluoride toxicity on sprouting, germination-related, and morphologic characteristics of wheat during sprouting of seed. Wheat is vital crop for regular consumption, accounting for 44-45.5% of digestible energy and approximately 29-31% of total protein. The increased absorption of F in tissue components causes sickness in sentient creatures as it enters the food webs (Jha *et al.*, 2009).

**OBJECTIVES:** The aim of this study was to observe the growth and physiological responses of five available wheat varieties at Bahauddin Zakariya University Multan under fluoride stress.

**MATERIALS AND METHODS:** Five wheat varieties viz; Akbar 2019, Anaj 2017, Ujala 2016, Galaxy 2013, and AARI 2011, being grown frequently in Pakistan, were selected for the assessment of their response toward fluoride toxicity. Seeds of these varieties were obtained from Konya Industries (Pvt) Ltd. Multan. Healthy seeds were screened by sterilizing with 0.1% of $HgCl_2$ and washing repeatedly with distilled water. About 20 seeds were sown at 3mm depth in the 6 inches plastic pots filled with sandy soil (5kg). The stock solution of fluoride was prepared by adding 42 g of NaF in a liter of distilled water for 1 M solution while 3mM, 5mM, 7mM, 9mM, 11mM concentrations were prepared by addition of distilled water in respective concentration of stock solution and one replicate was kept as control receiving no concentration of NaF. The pots were irrigated with distilled water to keep the moisture contents constant. Each pot was irrigated with respective concentration of NaF twice in a week at fix intervals of time till the observation of different parameters. After 12 days of sowing germination rate was recorded while rests of the plants were sampled after 2 weeks of germination for the observation of impacts fluoride. According Maguire (1962) germination percentage and vigor index were measured by counting the germinated plants in each pot along with the lengths of roots & shoot by following formulas:

$$\text{Germination percentage} = \frac{\text{No.of seeds germinated}}{\text{Total No.of seeds sown}} \times 100$$

$$\text{Vigor index} = \text{Mean root length} + \text{Mean shoot length} \times \text{Germination percentage}$$

The F contents in the roots and shoots of the plants were measured by the method of AOAC (1975) by using potentiometric technique. The dry roots and shoots (2g) were mixed with 8g of sodium carbonate and calcinated at about 450°C for 5 h in a furnace. The calcinated material was mixed with 5 M HCl by adjusting its PH at 5.3 with the addition of glacial acetic acid. The F contents were determined by fluoride electrodes (Model WTW) in a 10mL of extracted solution.

**RESULTS AND DISCUSSION:** Seeds of wheat varieties exposed to different levels of NaF, showed significant effects in term of germination percentage. In control, all varieties showed maximum (100%) while there was subsequent decrease in germination rate by increasing the concentration of NaF. Maximum germination (87%) was found in Anaj 2017 at 3mM while at 5mM (75%) was noted in Akbar 2019 as compared to that of control. While at 7mM of NaF, higher germination percentage (53%) was recorded in Anaj 2017 which was found maximum at that level as compared to that of control as well as rest of varieties. In comparison with the rest of varieties and control, the only lowest germination percentage (13%) at 11 mM was observed in Akbar 2019 and similar pattern of changes were observed in case of vigor index as shown in (table 1). At control maximum vigor index (2510.3) was found in Anaj 2017 as compared to the other treatments and gradual reduction was also noted by increasing NaF. In Anaj 2017 vigor index (2180) and (2058) were found higher at 3mM & 5mM of NaF respectively. Similarly at 7 mM, 9 mM and 11 mM of NaF, Anaj 2017 showed vigor index 776.7, 502.3 and 193.3 which were observed maximum respectively, with respect to control (table 2), F contents in the root of different varieties were found higher with increasing the concentration of NaF.

Table 1: Effects of NaF on germination percentage and vigor index on five selected wheat varities (Mean±SD at P≤ 0.05).

| | Akbar 2019 | Anaj 2017 | Ujala 2016 | Galaxy 2016 | AARI 2011 |
|---|---|---|---|---|---|
| | | | **Germination percentage** | | |
| **0 mM** | 100 ± 0a | 100 ± 0a | 100 ± 0a | 100 ± 0a | 100 ± 0a |
| **3 mM** | 86 ± 5.7b | 87 ± 5.7b | 83± 5.7b | 84 ± 5.7b | 80 ± 5.7b |
| **5 mM** | 75 ± 4.7c | 73 ± 5.7c | 70 ± 10c | 70 ± 10c | 72 ± 11.5c |
| **7 mM** | 50 ± 10d | 53 ± 11.5d | 51 ± 10d | 52 ± 0d | 50 ± 10d |
| **9 mM** | 30 ± 10e | 33 ± 5.7e | 30 ± 10e | 32 ± 5.7e | 31± 10e |
| **11 mM** | 13 ± 5.7f | 12 ± 5.7f | 13 ± 5.7f | 10 ± 0f | 11 ± 5.7f |
| | | | **Vigor index** | | |
| **0mM** | 2316.7 ± 28.9a | 2510 ± 0a | 2433.3 ± 225.4a | 2506.7 ± 76.3a | 2333.3 ± 57.7a |
| **3mM** | 2180 ± 129.3b | 2140 ± 190.5b | 1891.7 ± 202.5b | 1916.7 ± 155b | 1863.3 ± 98.1b |
| **5mM** | 2058.3 ± 334.6c | 1950 ± 216.5c | 1920 ± 280.3c | 1835 ± 418.1c | 1810 ± 423.3c |
| **7mM** | 695 ± 106.4d | 776.7 ± 188.2d | 765 ± 149d | 700 ± 66.1d | 696.7 ± 119.3d |
| **9mM** | 458.3 ± 171.1e | 502.3 ± 66.0e | 465 ± 152.2e | 448.7 ± 99.2e | 501.7 ± 131.6e |
| **11mM** | 191.7± 85.1f | 193.3 ± 75f | 191.7 ± 85.4f | 151.7 ± 2.9f | 213.3 ± 92.3f |

Table 2 : F Contents (µg/g) in roots and shoots of five selected wheat varieties (Mean±SD at P≤ 0.05).

| | Akbar 2019 | Anaj 2017 | Ujala 2016 | Galaxy 2013 | AARI 2011 |
|---|---|---|---|---|---|
| | | | **Roots** | | |
| **0mM** | 0 ± 0a | 0 ± 0a | 0 ± 0a | 0 ± 0a | 0 ± 0a |
| **3mM** | 0.13 ± 0.05b | 0.12 ± 0.05b | 0.15 ± 0.06b | 0.13 ± 0.06b | 0.16 ± 0.06b |
| **5mM** | 0.34 ± 0.05c | 0.33 ± 0.05c | 0.38 ± 0.06c | 0.40 ± 0.05c | 0.32 ± 0.1c |
| **7mM** | 0.46 ± 0.05d | 0.46 ± 0.05d | 0.48 ± 0.1d | 0.50 ± 0.1d | 0.53 ± 0.06d |
| **9mM** | 0.56 ± 0.1e | 0.63 ± 0.05e | 0.60 ± 0.1e | 0.64 ± 0.05e | 0.66 ± 0.06e |
| **11mM** | 0.83 ± 0.1f | 0.91 ± 0.01f | 0.98 ± 0.2f | 0.96 ± 0.15f | 1 ± 0.2f |
| | | | **Shoots** | | |
| **0mM** | 0 ± 0a | 0 ± 0a | 0 ± 0a | 0 ± 0a | 0 ± 0a |
| **3mM** | 0.1 ± 0.01b | 0.07 ± 0.05b | 0.07 ± 0.05b | 0.1 ± 0.01b | 0.08 ± 0.02b |
| **5mM** | 0.17 ± 0.05c | 0.13 ± 0.05c | 0.16 ± 0.05c | 0.16 ± 0.05c | 0.19 ± 0.05c |
| **7mM** | 0.27 ± 0.05d | 0.29 ± 0.1d | 0.24 ± 0.1d | 0.26 ± 0.05d | 0.30 ± 0.1d |
| **9mM** | 0.33 ± 0.1e | 0.36 ± 0.05e | 0.36 ± 0.05e | 0.43 ± 0.3e | 0.33 ± 0.06e |
| **11mM** | 0.47 ± 0.05f | 0.43 ± 0.05f | 0.50 ± 0.1f | 0.53 ± 0.5f | 0.51 ± 0.1f |

At 3mM of NaF, (0.16 µg) was found maximum in AARI 2011 as compared to that of control. Similarly at 5mM of NaF, maximum F ions (0.40 µg) were found in Galaxy 2013 than rest of treatments and varieties while at 7mM, 9mM and 11mM of NaF, the maximum of F contents found in AARI 2011 were (0.53 µg), (0.66 µg) and (1.0 µg) respectively as compared to that of control showing zero contents of fluoride. NaF also exhibited the similar accumulation of F ions in shoots of different varieties like as in roots. The maximum found in AARI2011 at 3mM, 5mM and 7mM of NaF were 0.08, 0.19 and 0.30 µg respectively as compared to that of control as well as rest of the varieties. The 9mM and 11mM of NaF showed higher accumulation of F ions in Galaxy 2013 that were 0.43 and 0.53 ug respectively as compared to that of control which received no F ions. Overall, it was found that F ions accumulation was higher in AARI 2011 and Galaxy 2013 at 11 mM as compared to rest of varieties at that concentration shown in table 2. Over the years, the release of inorganic F has reduced vegetation, forests, and agriculture yield because plants are much sensitive to F, as wider negative impacts have been observed during the growth & development of plants depicting the suppression of growth in this study as well (Yi *et al.*, 2017). The findings of Singh *et al.* (2017) recorded in pea exposed to F stress are also in line with the present research work showing the similarity of building up F contents and metabolic syndrome during early growths in both cases. F levels particularly at 11mM approaching the threshold level, affected the seed germination and primary growth attributes negatively in the current study whereas Gadi *et al.* (2012) also observed the similar effects of fluoride in mung bean when plants were exposed to 7mM of fluoride; due to yellowish appearance of leaves and reduction in the photosynthetic rate. The decrease in seed germination, vigor index and germination failure may be due to decreased water intake, delayed cell division, and a decline in metabolic activity related to different growing phases of plant under fluoride stress (Aske and Iqbal, 2014) analogous to the effects on wheat seedlings in this study. Plant survival and sustainability depend on seed germination and seedling development (Ahmad *et al.*, 2009). While due to toxicity of F contents, growth and yield of crops are hampered by inadequate seed germination & seedling development supporting the findings of current study in which wheat germination has been lowered as a result of soil F toxicity, where high levels of fluoride reduced the growth parameters. The observations of (Kumar *et al.*, 2011; Sodani, 2018) on the seedling growth (shoot length, root length, and vigor index) at 3ml of F toxicity showed the reduction in seed germination rate because 3ml of NaF was found enough to reduce the growth of seedlings, additionally, 15ml of F exposure also showed adverse effects on the different traits of mustard plants (Sodani *et al.*, 2021). It is also evident from the current study that exposure of wheat seeds to high concentration of fluoride for certain period caused injuries to seeds, affecting the growth of root, shoot and germination of seeds. The extent of injuries depends on concentration of fluoride, period of exposure and susceptibility of different species of wheat plant (Joshi and Bhardwaj, 2012). In this study, the young plants of wheat varieties treated with different concentration of NaF showed the varying pattern of distribution of F which was found greater in roots than in shoots. Such variety of distribution pattern of F showing higher in roots than shoots implies the restriction in the translocation of F to the upper parts of plants being employed for photosynthetic activities. Observations on abnormal distribution of F having greater in roots than in shoots & leaves were shown by Elloumi *et al.* (2005) in almond seedlings (*Prunus amygdalus*) and in potato (*Solanum tuberosum*) by Das *et al.* (2015), while Chakrabarti and Patra (2015) showed the higher F in roots of rice (*Oryza sativa*). Through the studies of above authors, the restriction in the distribution of F to the cellular tissues of plants acts as a defensive and protective mechanism of plants against the toxicity of F which is important adaptive strategy being shown in wheat varieties accumulating higher in roots than shoots. Higher ratio of F contents in roots and restriction towards aerial parts of plants.

Baunthiyal *et al.* (2014) referred this distribution of F in olive plants due to efficient activities of roots cells which allowed very little F ions toward upper parts of plants and higher F deposition was seen in cell walls, appoplast & intercellular spaces of plants. This also clues the slow passing of F through symplast which can provide an active barrier for F translocation to the vascular system of plants; hence the photosynthetic activities of plants can be continued without the interruption of F translocation through the soil. The morphological changes in the roots of wheat varieties due to higher accumulation of F contents under different concentration of NaF in this study; is supporting the observations of Jha *et al.* (2009) for F accumulation in onion plant by the order of roots >

shoot > bulb in addition to injures due to excessive accumulations of fluoride through atmospheric deposition.

**CONCLUSION:** The F toxicity reduced the germination percentage of all varieties of wheat with significant reduction in vigor index while this impact gradually increased by increasing the concentration of NaF .The distribution of F was also found higher in roots than in shoot in all varieties and similar increasing trend was observed by increasing the concentration of NaF. Among all varieties of wheat Anaj 2017 and Akbar 2019 showed high percentage of germination than rest of varieties while F accumulation was found higher in AARI 2011 and Galaxy 2013 than rest of varieties. The order of F accumulation was roots > shoot > leaves in all varieties of wheat crop.

**CONFLICT OF INTEREST:** Authors have no conflict of interest.

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