FLUORIDE

Quarterly Journal of The International Society for Fluoride Research Inc.

Antioxidant Defense and Growth Responses of *Vigna radiata L.* Sprouts Under Sodium Fluoride Stress

Unique digital address (Digital object identifier [DOI] equivalent):
https://www.fluorideresearch.online/epub/files/321.pdf

Mona ALONAZI, Sooad Al-DAIHAN[1],Abeer M.ALDBASS[1], Ramesa Shafi BHAT[*1]

[1] Biochemistry Department, Science College of King Saud University, Saudi Arabia

***Corresponding author:**
Dr. Ramesa Shafi Bhat
Biochemistry Department, Science College of King Saud University, PO Box 22452, Zip code 11495, Riyadh, Saudi Arabia
E-mail:rbhat@ksu.edu.sa

Accepted: 2025 Feb 19
Published as e321: 2025 Feb 20

ABSTRACT

Purpose: Fluoride toxicity can disrupt essential physiological and biochemical processes during plants germination and development stages, leading to stunted growth and reduced productivity.

Methods: This study was designed to determine the responses of *Vigna radiata L.* sprouts germinated under sodium fluoride (NaF) stress. Experiments were conducted with 5 different NaF concentrations (20, 40, 60, 80, and 100 ppm). Treated *Vigna radiata* L. sprouts were compared with normal sprouts and their germination was observed over six days. Germination percentage (GP), germination index (GI), vigor index (VI), seedling height reduction (SHR), and relative injury rate were evaluated as growth parameters. Protein content, lipid peroxidation (MDA), glutathione (GSH), vitamin C, and total phenolic content were measured as stress markers.

Results: Results showed that NaF treatment significantly (p-value ≤ 0.001) inhibited sprout germination, with higher concentrations causing marked reductions in GP, GI, and VI, with the maximum inhibition occurring at 100 ppm. Seedling height reduction also increased significantly (p-value ≤ 0.001) with higher NaF concentrations. Biochemical analyses revealed significant changes in protein content, MDA, GSH, vitamin C, and total phenolic content. Protein content declined, while MDA levels increased significantly at higher concentrations of NaF, indicating enhanced oxidative stress, however GSH and phenolic content significantly (p-value ≤ 0.001) increased in response to all concentrations of NaF, suggesting a protective mechanism against fluoride toxicity. Vitamin C levels fluctuated with NaF treatment, showing a non-significant decrease at higher concentrations.

Conclusions: These findings demonstrate that NaF stress adversely affects sprout germination, triggering biochemical changes, particularly in oxidative stress markers in *Vigna radiata L.* seeds. The study also suggests that biochemical markers can be used to evaluate the oxidative stress response of plants subjected to NaF exposure.

Keywords: Fluoride toxicity, Germination, oxidative stress, sprout, *Vigna radiata L.*

INTRODUCTION

Fluorine is Earth's 13th most abundant element, constituting 0.06–0.09% of the surface [1]. It forms soluble compounds like sodium fluoride (NaF) and potassium fluoride (KF), as well as insoluble salts such as calcium fluoride (CaF2) [2]. The toxicity and cumulative effects of fluoride depend on the species' sensitivity and the duration of exposure to fluoride salts' solubility [3]. Industrialization has significantly increased fluoride emissions, mainly due to fertilizer production and aluminum smelting, raising fluoride levels beyond safe limits [4]. Approximately 25% of herbicides currently contain fluoride, either as a fluoride atom or as difluoromethyl and trifluoromethyl groups [5]. This incorporation enhances herbicides' ability to penetrate plant cells and block enzyme activity, boosting their fungicidal properties. However, fluoride is highly toxic to plants, reducing agricultural yields by up to 50% [6]. For every 100 ppm increase in soil fluoride, plant fluoride concentration rises by 3 ppm, negatively affecting plant health [7].

Elevated fluoride levels cause chlorosis, necrosis, and stunted growth, impairing biochemical processes like enzyme activity, pigment synthesis, and protein formation [8]. Fluoride stress also leads to oxidative damage by increasing reactive oxygen species (ROS), causing lipid peroxidation, membrane damage, and electrolyte leakage [9-13]. Fluoride accumulates in edible plant parts, posing human health risks [14]. Fluoride sensitivity is highly species-dependent [15]. Some plants can accumulate fluoride at high concentrations (up to 4000 µg F g^{-1}) without exhibiting toxicity, while others show harmful effects at much lower levels, with certain species being susceptible to concentrations below 20 µg g–1 [16-17]. Fluoride absorbed from the soil is transported to the shoots, causing physiological, biochemical, and structural damage, which varies based on the soil concentration and movement from the roots to the shoots [16-17]. Plants counteract fluoride toxicity through protective mechanisms such as osmolyte production and activating enzymatic and non-enzymatic antioxidant systems [18]. These mechanisms help in managing oxidative stress and damage from fluoride and other abiotic stresses [18].

Germination is a crucial indicator of seed quality, marking the start of a plant's life cycle [19]. While most biochemical analyses focus on later stages, the imbibition phase—when the seed absorbs water and swells—also plays a vital role. Imbibition occurs in three stages: initial swelling, main water absorption, and growth, which includes radicle emergence [20-22]. Mung bean (*Vigna radiata L*) is a vital leguminous crop grown globally for its rich nutritional content, including proteins, vitamins, and minerals, which are crucial for human and animal diets [23]. Mung bean is highly sensitive to stress, and its yield can decline by up to 70% when exposed to various abiotic stresses [24]. Despite its importance, mung beans are vulnerable to multiple environmental stresses, such as fluoride toxicity, which can significantly hinder plant growth and reduce productivity [25]. The germination stage, in particular, is highly susceptible to fluoride exposure, which interferes with vital physiological processes and ultimately affects crop yield [26-28]. Therefore, improving fluoride tolerance in mung beans is critical for sustaining their agricultural output. This study focuses on investigating the effects of sodium fluoride stress on sprout formation in mung bean, using varying concentrations of sodium fluoride, and evaluating its tolerance to fluoride toxicity during the crucial germination phase. This is the first study to report the germination parameters and biochemical profile of *Vigna radiata L*. sprouts influenced by increasing exposure to sodium fluoride salts.

MATERIAL AND METHODS

Seed Material

Mung bean (*Vigna radiata L.)* seeds were procured from a local market in Riyadh, Saudi Arabia. The seeds typically germinate between 18-29°C, with an average sprouting period of 5 days. Seeds were sterilized by soaking in a 1% sodium hypochlorite solution for 10 minutes before conducting germination tests.

Experimental Design

For the experiment, thirty disinfected seeds were placed in Petri dishes lined with double-layered sterile filter paper, which were treated with varying concentrations of NaF (0, 20, 40, 60, 80, and 100 ppm).

The seeds were allowed to sprout over six days under low-light conditions at room temperature. A seed was considered as sprout once the plumule and radicle emerged and exceeded 2 mm in length. After six days, the sprouts were counted and analyzed for various germination parameters and biochemical changes.

Assessment of Growth Parameters of Vigna radiata L. sprouts under NaF Stress

Several growth parameters were evaluated in the treated sprouts and compared to the control group. These included the germination percentage, germination index, vigor index, relative injury rate, and seedling height reduction [29-30].

Biochemical Analysis of Vigna radiata L. Sprouts Under NaF Stress

The fresh sprouts from both control and treated groups were homogenized (using IKA® T 18 digital ULTRA-TURRAX homogenizer Germany) in phosphate buffer (pH 7, 1:10 W/V). The homogenate was centrifuged (using Thermo Fisher MegaFuge 16R Centrifuge Germany) 4°C at 3,000 g for 10 minutes, and the supernatant was used for the following biochemical assays:

The protein content was measured using Bradford's method [31]. Lipid Peroxide Measurement: Lipid peroxidation was quantified using the thiobarbituric acid (TBA) test, as described by Ruiz-Larrea et al [32]. Glutathione levels were determined based on Beutler et al.'s method [33]. The technique described by Jagota and Dani was employed to assess ascorbic acid levels [34] The total phenolic content was determined using the Folin–Ciocalteu method [35]. Biochrom Libra S22 UV/Vis Spectrophotometer was employed to measure the absorbance for all these parameters.

Statistical Analysis

The data were analyzed with GraphPad Prism version 10.4.1 and results are presented as the mean ± standard error of the mean (SEM). Statistical comparisons between the control and NaF-treated sprouts were conducted using one-way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparisons. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Effects of Different Concentrations of Sodium Fluoride Treatment on Vigna radiata L Sprouts formation

Figure 1 illustrates the sprout germination rates of *Vigna radiate* L seeds exposed to different concentrations (0,20,60,80,100 ppm) of NaF. Seeds treated with 20 ppm NaF exhibited almost similar sprout germination rates to the control group. However, the sprout germination potential of *Vigna radiata* L seeds decreased steadily with higher NaF concentrations. Fluoride stress had a considerable impact on germination, with seeds treated with 100 ppm NaF showing significantly lower germination rates.

Effects of different concentrations of sodium Fluoride Treatment on germination parameters Vigna radiata L Sprouts

As shown in Figure 2a, the germination percentage (GP) of *Vigna radiata* L sprouts was significantly (p-value ≤ 0.001) influenced by increasing NaF concentrations. Untreated seeds had a GP of 70%, while seeds treated with 20, 40, 60, 80, and 100 ppm NaF showed GP values of 61%, 49%, 28%, 19%, and 11%, respectively. These results highlight the inhibitory effect of NaF on GP, with the impact becoming more pronounced at higher concentrations. The germination index (GI) values, as depicted in Figure 2b, also declined progressively with increasing NaF concentrations. This decline was particularly evident at higher NaF levels (60–100 ppm) compared to the control. The maximum GI recorded for the control group was 3.4, while the minimum GI of 0.58 was observed at 100 ppm NaF. Figure 2c illustrates the vigor index (VI) of *Vigna radiate* L sprouts under NaF stress. VI decreased significantly (p-value ≤ 0.001) with increasing NaF concentrations, with the control showing a VI of 2.38 and the 100 ppm NaF treatment showing a dramatic reduction to 0.05. Seedling height reduction (SHR), an important indicator of plant response to salt stress, is shown in Figure 2d. SHR increased with higher NaF concentrations, with a reduction of more than 50% at 80 and 100 ppm NaF. The relative injury rate, based on the comparison of germination percentages between the control and NaF-treated seeds, was found to increase significantly

(p-value ≤ 0.001) with higher NaF concentrations, as shown in Figure 2e**.**

Effects of different concentrations of sodium fluoride treatment on biochemical analysis of sprouted Vigna radiata L

The protein content in the sprouts decreased as NaF concentrations increased (Figure 3a). The untreated sprouts (0 ppm) had a protein content of 11 µg, and the level gradually decreased with higher NaF concentrations. At 20 ppm NaF, the protein content remained similar to the control (11 µg), but by 100 ppm NaF, it had dropped significantly (p-value ≤ 0.001) to 7.4 µg. MDA, a marker for lipid peroxidation, showed a slight increase with NaF treatment (Figure 3b). At 0 ppm, MDA was 0.067 µmol, and as NaF concentrations increased, the levels rose slightly, reaching 0.8 µmol/g significantly (p-value ≤ 0.01) at 100 ppm. This rise suggests that higher NaF concentrations led to greater oxidative damage in the sprout tissues. The GSH content in the sprouts significantly increased (p-value ≤ 0.001) with higher NaF concentrations (Figure 3c). At 0 ppm, GSH content was 196 µg, which progressively increased with NaF treatment, reaching 534 µg at 100 ppm. Vitamin C levels displayed variability with NaF treatment (Figure 3d). In untreated sprouts, vitamin C was measured at 28 µg. As NaF concentrations increased, it fluctuated: at 20 ppm, it decreased slightly to 27 µg; at 40 ppm, it increased to 43 µg; and by 100 ppm, it dropped to 25 µg, showing a decrease at higher NaF concentrations. The phenolic content of the sprouts increased significantly (p-value ≤ 0.001) with NaF treatment (Figure 3e). The untreated sprouts showed a phenolic content of 127 µg, which was increased with NaF treatment in a dose-dependent manner. At 100 ppm NaF, the phenolic content had reached 238 µg, indicating that phenolic compounds may contribute to the plant's response to NaF-induced oxidative stress.

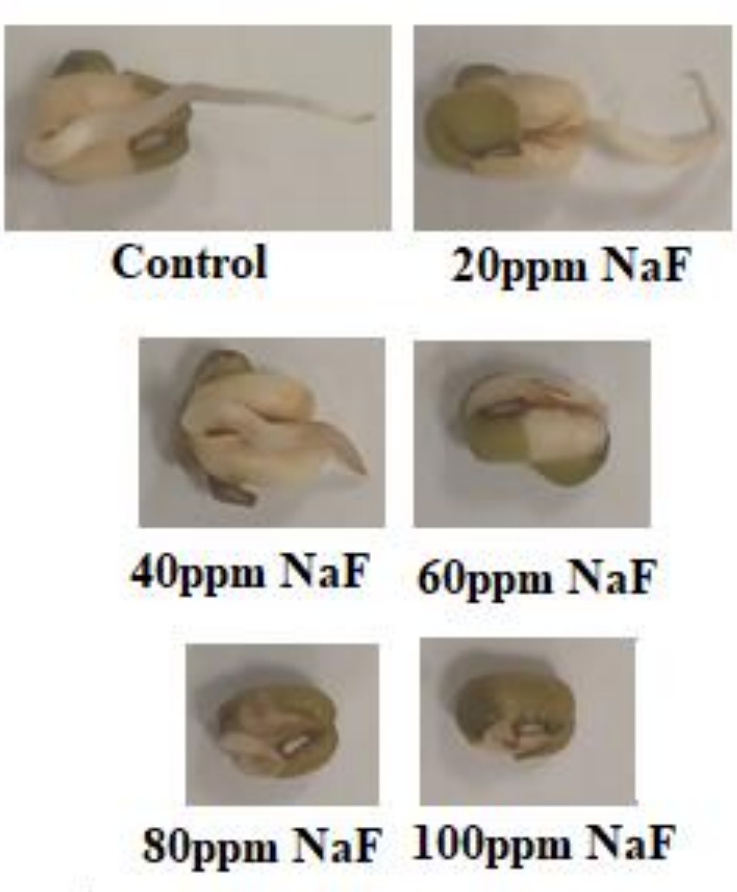
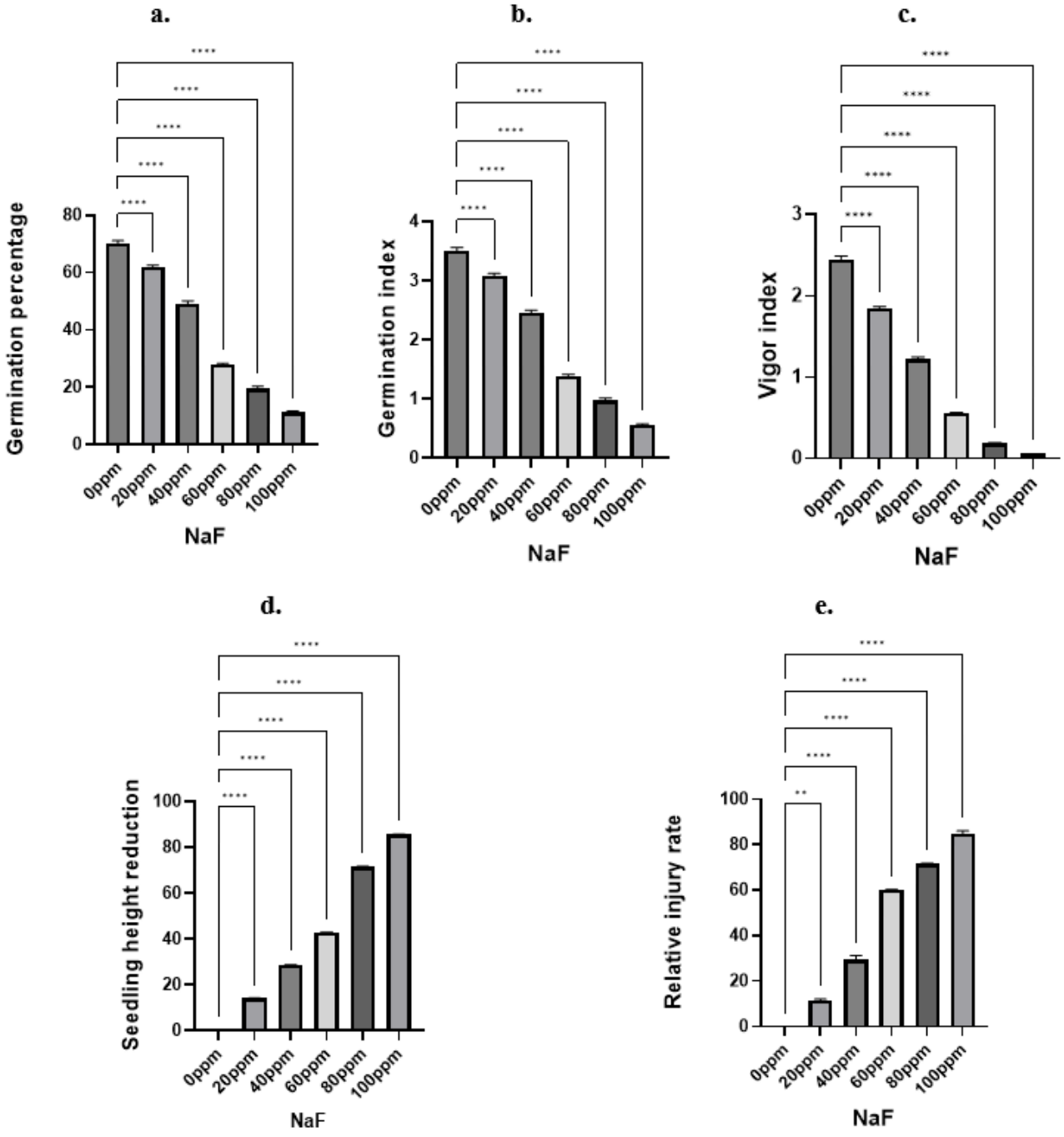
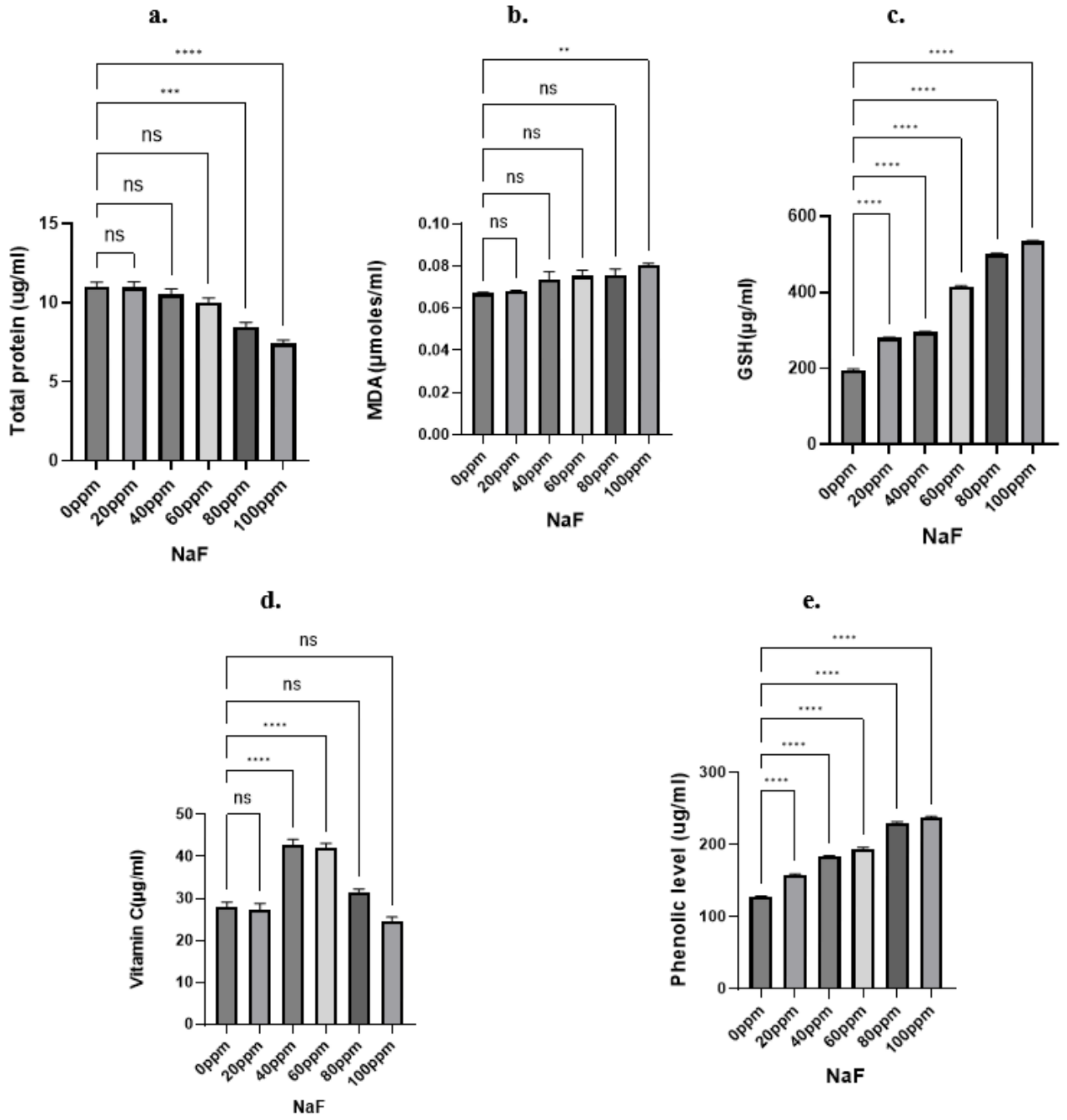


Figure 1. *Vigna radiata* L sprouts under NaF stress at day six.



ns non-significant ; p-value ≤ 0.05; ** p-value ≤ 0.01; *** p-value ≤ 0.001; **** p-value ≤ 0.001*

Figure 2. Growth parameters of *Vigna radiata L* sprouts under NaF. a. Germination percentage; b. Germination index; c. Vigor index; d. Seedling height reduction; e. Relative injury rate.



*ns non-significant; * p-value ≤ 0.05; ** p-value ≤ 0.01; *** p-value ≤ 0.001; **** p-value ≤ 0.001*

Figure 3. Oxidative stress markers of *Vigna radiata L* sprouts under NaF treatment. a. Total protein; b. MDA; c. GSH; d. Vitamin C; e. Total Phenol.

DISCUSSION

Breaking seed dormancy, which triggers sprout formation, is crucial for evaluating crop resistance to stresses [27,36]. Sprouting occurs when the metabolic activity of antioxidant enzymes is restored [37]. However, toxic substances such as NaF can disrupt seed metabolism by altering internal and environmental conditions.

In this study, we examined growth parameters such as germination percentage, germination index, vigor index, relative injury rate, and seedling height reduction of *Vigna radiata L* sprouts exposed to varying concentrations of NaF. The results showed a decline in these indicators as NaF concentration increased, with remarkable differences observed at higher concentrations (Figure 1), indicating a clear inhibitory effect of NaF on the germination process. NaF stress suppressed or delayed sprout formation, likely due to its reduction of metabolic activity, as fluoride acts as a metabolic inhibitor. Previous studies have reported that fluoride inhibits adenosine triphosphatase (ATPase) and 5'-nucleotidase during the germination process of *Vigna radiata L* seeds, which correlates with a reduction in amylase and lipase activity [38]. The reduction in seedling height observed in our study under NaF treatments could be attributed to disrupted nutrient uptake by seedlings [39].

Soluble protein is a key component in various metabolic activities during germination, and changes in its content are important indicators of overall metabolic status [40]. Our findings showed a decline in soluble protein content as NaF concentration increased, further confirming the negative impact of NaF on protein metabolism in plants [41]. These results are also supported by a study on *Spirodela polyrhiza* in which fluoride triggered the oxidative stress [42]. Fluoride stress triggers the production of reactive oxygen species (ROS), leading to oxidative stress and lipid peroxidation, which disrupts biochemical and physiological processes [43-44]. Malondialdehyde (MDA) levels, a marker of lipid peroxidation and oxidative damage, were found to increase in a dose-dependent manner with NaF treatment, indicating membrane rupture, DNA damage, and cell death [45-46]. The cell membrane is typically the first organelle affected by salt stress, as polyunsaturated fatty acids, which are key components of membrane lipids, are particularly vulnerable to peroxidation during stress. This increased membrane permeability compromises membrane integrity [47]. Glutathione (GSH) plays a critical role in plant survival under stress by detoxifying excess ROS, maintaining redox balance, and regulating protein functions [48]. GSH has also been identified as an important signaling molecule involved in abscisic acid signaling, stress response, and related developmental events [49]. The increase in GSH levels in NaF-treated *Vigna radiata L* sprouts observed in this study may represent an adaptive mechanism to combat fluoride stress [50], possibly through the hydrolysis of proteins, which increases the free amino acid pool during the osmotic adjustment process under NaF stress [51]. Our findings align with the observations made in paddy (*Oryza sativa)*) subjected to fluoride stress showing increase in antioxidant enzyme activity [52] .

Vitamin C is an essential metabolite involved in several cellular processes, including cell division [53]. While it is almost absent in dry seeds, its levels increase significantly during germination [54]. NaF treatment resulted in an increase in vitamin C content at lower concentrations in *Vigna radiata L* sprouts, but this was reduced at higher concentrations, confirming fluoride toxicity. Phenolic compounds, which are produced during normal germination, have been shown to increase in response to stress, as ROS generation triggers their overproduction [55]. Our results support this, as high levels of phenolic compounds were observed in NaF-treated sprouts, indicating a protective response against fluoride-induced stress.

CONCLUSIONS

This study emphasizes the negative impact of sodium fluoride (NaF) on the germination and early growth of *Vigna radiata L* sprouts. NaF treatment significantly impairs seedling growth and development, with higher concentrations leading to greater reductions in germination and seedling height. Biochemical analyses indicated an increase in oxidative stress in NaF- treated sprouts. The findings are beneficial for farmers, offering guidance on managing fluoride levels by avoiding the excessive use of fluoride-based fertilizers and irrigation with contaminated water. Furthermore, these insights enhance our understanding of the mechanisms of fluoride toxicity in plants and the potential challenges fluoride contamination presents in agricultural systems.

Limitations of study

Besides the oxidative stress-related events assessed in this study, fluoride has been demonstrated to influence the activity of several antioxidant enzymes. However, enzyme activity was not measured in this study.

Future recommendations

Additional research is required to gain a deeper understanding of the molecular responses involving antioxidant enzymes in sprouts under fluoride stress.

ACKNOWLEDGEMENTS

The authors extend appreciation to the Researchers Supporting Project number (RSP2025R237), King Saud University, Riyadh, Saudi Arabia.

FUNDING

This research project was supported by Researchers Supporting Project number (RSP2025R237), King Saud University, Riyadh, Saudi Arabia.

CONFLICT OF INTERESTS

"None"

REFERENCES

[1] Fluorine is 13th most abundant element on Earth, constituting 0.06–0.09% of the surface.

[2] Martínez-Mier EA, Soto-Rojas AE, Buckley CM, Margineda J, Zero DT. Evaluation of the direct and diffusion methods for the determination of fluoride content in table salt. Community Dent Health. 2009 Dec;26(4):204-10. PMID: 20088217; PMCID: PMC2870713.

[3] Guth S, Hüser S, Roth A, Degen G, Diel P, Edlund K, Eisenbrand G, Engel KH, Epe B, Grune T, Heinz V, Henle T, Humpf HU, Jäger H, Joost HG, Kulling SE, Lampen A, Mally A, Marchan R, Marko D, Mühle E, Nitsche MA, Röhrdanz E, Stadler R, van Thriel C, Vieths S, Vogel RF, Wascher E, Watzl C, Nöthlings U, Hengstler JG. Toxicity of fluoride: critical evaluation of evidence for human developmental neurotoxicity in epidemiological studies, animal experiments and in vitro analyses. Arch Toxicol. 2020 May;94(5):1375-1415. doi: 10.1007/s00204-020-02725-2. Epub 2020 May 8. PMID: 32382957; PMCID: PMC7261729.

[4] Dobaradaran S, Fazelinia F, Mahvi A, Hosseini SS. Particulate airborne fluoride from an aluminium production plant in Arak, Iran Fluoride. 2009;42:228–232

[5] Jeschke, P. (2024), Recent developments in fluorine-containing pesticides. Pest Manag Sci, 80: 3065-3087. https://doi.org/10.1002/ps.7921

[6] Ahmad MN, Zia A, van den Berg L, Ahmad Y, Mahmood R, Dawar KM, Alam SS, Riaz M, Ashmore M. Effects of soil fluoride pollution on wheat growth and biomass production, leaf injury index, powdery mildew infestation and trace metal uptake. Environ Pollut. 2022 Apr 1;298:118820. doi: 10.1016/j.envpol.2022.118820. Epub 2022 Jan 7. PMID: 35007676.

[7] Bharti VK, Giri A, Kumar K (2017) Fluoride sources, toxicity and its amelioration: a review. Ann Environ Sci Toxicol 2:21–32

[8] Ur Rahman S, Qin A, Zain M, Mushtaq Z, Mehmood F, Riaz L, Naveed S, Ansari MJ, Saeed M, Ahmad I, Shehzad M. Pb uptake, accumulation, and translocation in plants: Plant physiological, biochemical, and molecular response: A review. Heliyon. 2024 Mar 7;10(6):e27724. doi: 10.1016/j.heliyon.2024.e27724. PMID: 38500979; PMCID: PMC10945279.

[9] Shuhua X, Ziyou L, Ling Y, Fei W, Sun G. A role of fluoride on free radical generation and oxidative stress in BV-2 microglia cells. Mediators Inflamm. 2012;2012:102954. doi: 10.1155/2012/102954. Epub 2012 Aug 13. PMID: 22933830; PMCID: PMC3425889.

[10] Bhat RS, Soliman DA, Al-Daihan S. Sodium fluoride induces oxidative stress in oral bacteria by altering glutathione (GSH) and glutathione S transferase (GST) activity. Fluoride 2021; 54 (1):90-96.

[11] Al-Daihan S, Ben Bacha A, El-Ansary A, Bhat RS Prenatal bee pollen treatment improves the neurotoxicity in newborn rats during chronic fluoride exposure in relation to propionic acid-induced rodent models of autism. Fluoride 2020;53(1):11-22

[12] Al-Daihan S, Bhat RS Protective Effect of Bee Pollen Against Sodium Fluoride Induced Hepatonephrotoxicity and Serum Electrolyte Changes in Rats. Fluoride 2019; 52(1) 9-17

[13] Bhat RS , Alonazia MA, Al-Daihana S Fluoride triggers oxidative stress and cell damage in antibiotic-resistant Klebsiella pneumonia. Fluoride 57 (11)2024

[14] Essebbahi, I., Ouazzani, C., Moustaghfir, A., Er-Ramly, A., El Baroudi, Y., El Baroudi, H., Dami, A., & Balouch, L. (2024). Assessment of fluoride accumulation in various vegetable and leafy greens consumed in Morocco. Journal of the Saudi Society of Agricultural Sciences.

[15] Tausta SL, Berbasova T, Peverelli M, Strobel SA. The fluoride transporter FLUORIDE EXPORTER (FEX) is the major mechanism of tolerance to fluoride toxicity in plants. Plant Physiol. 2021 Mar 21;186(2):1143–58. doi: 10.1093/plphys/kiab131. Epub ahead of print. PMID: 33744970; PMCID: PMC8195535.

[16] Jha SK, Nayak AK, Sharma YK. Fluoride toxicity effects in onion (Allium cepa L.) grown in contaminated soils. Chemosphere. 2009 Jul;76(3):353-6. doi: 10.1016/j.chemosphere.2009.03.044. Epub 2009 Apr 25. PMID: 19394675.

[17] Mondal NK. Effect of fluoride on photosynthesis, growth and accumulation of four widely cultivated rice (Oryza sativa L.) varieties in India. Ecotoxicol Environ Saf. 2017 Oct;144:36-44. doi: 10.1016/j.ecoenv.2017.06.009. Epub 2017 Jun 7. PMID: 28600945.

[18] Singh A, Roychoudhury A. Deciphering the biochemical regulation of metabolic pathways in abating stress-induced damages during co-exposure of arsenic and fluoride in a non-aromatic and an aromatic rice variety. Physiol Mol Biol Plants. 2023 Oct;29(10):1475-1484. doi: 10.1007/s12298-023-01355-

z. Epub 2023 Sep 23. PMID: 38076766; PMCID: PMC10709284.

[19] Vidak M, Lazarević B, Javornik T, Šatović Z, Carović-Stanko K. Seed Water Absorption, Germination, Emergence and Seedling Phenotypic Characterization of the Common Bean Landraces Differing in Seed Size and Color. Seeds. 2022; 1(4):324-339. https://doi.org/10.3390/seeds104002

[20] Lechowska, K.; Kubala, S.; Wojtyla, Ł.; Nowaczyk, G.; Quinet, M.; Lutts, S.; Garnczarska, M. New insight on water status in germinating Brassica napus seeds in relation to priming-improved germination. Int. J. Mol. Sci. 2019, 20, 540. [Google Scholar] [CrossRef] [Green Version]

[21] Bewley, J.D.; Black, M. Seeds. Physiology of Development and Germination; Springer: New York, NY, USA, 1994. [Google Scholar]

[22] Souza, F.H.D.; Marcos-Filho, J. The seed coat as a modulator of seed-environment relationships in Fabaceae. Rev. Bras. Botânica 2001, 24, 365–375

[23] Hou D, Yousaf L, Xue Y, Hu J, Wu J, Hu X, Feng N, Shen Q. Mung Bean (Vigna radiata L.): Bioactive Polyphenols, Polysaccharides, Peptides, and Health Benefits. Nutrients. 2019 May 31;11(6):1238. doi: 10.3390/nu11061238. PMID: 31159173; PMCID: PMC6627095.

[24] Ghafoor, A.; Zahid, M.A.; Ahmad, Z.; Afzal, M.; Zubair, M. Selecting superior mungbean lines on the basis of genetic diversity and harvest index. Pak. J. Biol. Sci. 2000, 3, 1270–1273.

[25] Aizaz M, Khan I, Lubna, Asaf S, Bilal S, Jan R, Khan AL, Kim KM, Al-Harrasi A. Enhanced Physiological and Biochemical Performance of Mung Bean and Maize under Saline and Heavy Metal Stress through Application of Endophytic Fungal Strain SL3 and Exogenous IAA. Cells. 2023 Jul 28;12(15):1960. doi: 10.3390/cells12151960. PMID: 37566039; PMCID: PMC10417269.

[26] Chakraborty, S.; Singh, A.; Roychoudhury, A. Extensive cross-talk among stress-regulated protective metabolites, biogenic-amines and phytohormone-signalling, co-ordinated by dopamine-mediated seed-priming, governs tolerance against fluoride stress in rice. Plant Cell Rep. 2022, 41, 2261–2278

[27] Fu Y, Ma L, Li J, Hou D, Zeng B, Zhang L, Liu C, Bi Q, Tan J, Yu X, et al. Factors Influencing Seed Dormancy and Germination and Advances in Seed Priming Technology. Plants. 2024; 13(10):1319. https://doi.org/10.3390/plants13101319

[28] Chahine, S., Melito, S., Giannini, V. et al. Fluoride stress affects seed germination and seedling growth by altering the morpho-physiology of an African local bean variety. Plant Growth Regul 102, 339–350 (2024). https://doi.org/10.1007/s10725-023-01064-3

[29] Bhat RS, Alghamdi JM, Aldbass AM, Aljebrin NA, Alangery AB, Soliman DA, Al-Daihan S Biochemical and FT-IR profiling of Tritium aestivum L. seedling in response to sodium fluoride treatment. Fluoride 2022; 55 (1): 81-89.

[30] Bhat RS , Aldbass AM, Alghamdi JM, Alonazia MA, Al-Daihana S Trigonella foenum-graecum L seed germination under sodium halide salts exposure. Fluoride 2023; 56(2):169-179

[31] Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 1976, 72, 248–254

[32] Ruiz-Larrea, M. B., Leal, A. M., Liza, M., Lacort, M., & de Groot, H. (Antioxidant effects of estradiol and 2-hydroxyestradiol on iron-induced lipid peroxidation of rat livermicrosomes. Steroids, 59(6), 383 388. (1994).

[33] Beutler, E., Duran, O., & Kelly, B. M. Improved method for the determination of blood glutathione. Journal of Labor-atory and Clinical Medicine, 61, 882– 888. (1963).

[34] Jagota, S.; Dani, H. A new colorimetric technique for the estimation of vitamin C using Folin phenol reagent. Anal. Biochem. 1982, 127, 178–182

[35] Pérez, M.; Dominguez-López, I.; Lamuela-Raventós, R.M. The Chemistry Behind the Folin-Ciocalteu Method for the Estimation of (Poly)phenol Content in Food: Total Phenolic Intake in a Mediterranean Dietary Pattern. J. Agric. Food Chem. 2023, 71, 17543–17553.

[36] Fu Y, Ma L, Li J, Hou D, Zeng B, Zhang L, Liu C, Bi Q, Tan J, Yu X, et al. Factors Influencing Seed Dormancy and Germination and Advances in Seed Priming Technology. Plants. 2024; 13(10):1319. https://doi.org/10.3390/plants13101319

[37] Benincasa P, Falcinelli B, Lutts S, Stagnari F, Galieni A. Sprouted Grains: A Comprehensive Review. Nutrients. 2019 Feb 17;11(2):421. doi: 10.3390/nu11020421. PMID: 30781547; PMCID: PMC6413227.

[38] Yu MH. Effects of fluoride on growth and soluble sugars in germinating mung bean (Vigna radiata) seeds. Fluoride. 1996;29(1):3–6.

[39] Sabal D, Khan TI, Saxena R. Effect of sodium fluoride on cluster bean (Cyamopsis tetragonoloba) seed germination and seedling growth. Fluoride. 2006;39(3):228–230.

[40] Alharbi, K.; Al-Osaimi, A.A.; Alghamdi, B.A. Sodium chloride (nacl)-induced physiological alteration and oxidative stress generation in Pisum sativum (L.): A toxicity assessment. ACS Omega 2022, 7, 20819–20832. [

[41] López-Cristoffanini, C.; Bundó, M.; Serrat, X.; San Segundo, B.; López-Carbonell, M.; Nogués, S. A comprehensive study of the proteins involved in salinity stress response in roots and shoots of the fl478 genotype of rice (Oryza sativa L. Ssp. Indica). Crop J. 2021, 9, 1154–1168.

[42] Sharma, R., & Kaur, R. (2019). Fluoride toxicity triggered oxidative stress and the activation of antioxidative defence responses in Spirodela polyrhiza L. Schleiden. Journal of Plant Interactions, 14(1), 440–452. https://doi.org/10.1080/17429145.2019.1646826

[43] Ashraf M, Akram NA, Arteca RN, Foolad MR, The physiological, biochemical and molecular roles of brassinosteroids and salicylic acid in plant processes and salt tolerance, CRC Crit. Rev. Plant Sci. 29 (2010) 162–190.

[44] Ali LG, Nulit R, Ibrahim MH, Yien CYS , Efficacy of KNO3, SiO2 and SA priming for improving emergence, seedling growth and antioxidant enzymes of rice (Oryza sativa), under drought, Sci. Rep. 11 (2021) 1–11.

[45] Farooq MA, Li L, Ali B, Gill RA, Wang J, Ali S, Gill MB, ZhouW , Oxidative injury and antioxidant enzymes regulation in arsenic-exposed seedlings of four Brassica napus L. cultivars, Environ. Sci. Pollut. Res. 22 (2015) 10699–10712.

[46] Garcia-Caparros P, de Filippis L, Gul A, Hasanuzzaman M, Ozturk M, AltayV, Lao MT, Oxidative stress and antioxidant metabolism under adverse environmental conditions: a review, Bot. Rev. 87 (2021) 421–466.

[47] Mosahebeh M, Khorshidi M, Faridnoure H, Investigation of physiological responses of wheat under salt stress, Int. J. Farm. Allied Sci. 5 (2016) 199–204.

[48] Hasanuzzaman M, Nahar K, Anee TI, Fujita M. Glutathione in plants: biosynthesis and physiological role in environmental stress tolerance. Physiol Mol Biol Plants. 2017 Apr;23(2):249-268. doi: 10.1007/s12298-017-0422-2. Epub 2017 Mar 10. PMID: 28461715; PMCID: PMC5391355.

[49] Koramutla MK, Negi M, Ayele BT. Roles of Glutathione in Mediating Abscisic Acid Signaling and Its Regulation of Seed Dormancy and Drought Tolerance. Genes (Basel). 2021 Oct 14;12(10):1620. doi: 10.3390/genes12101620. PMID: 34681014; PMCID: PMC8535772.

[50] Nahar, K., Hasanuzzaman, M., Suzuki, T. et al. Polyamines-induced aluminum tolerance in mung bean: A study on antioxidant defense and methylglyoxal detoxification systems. Ecotoxicology 26, 58–73 (2017). https://doi.org/10.1007/s10646-016-1740-9

[51] Pasković I, Popović L, Pongrac P, Polić Pasković M, Kos T, Jovanov P, Franić M. Protein Hydrolysates—Production, Effects on Plant Metabolism, and Use in Agriculture. Horticulturae. 2024; 10(10):1041. https://doi.org/10.3390/horticulturae10101041

[52] Chakrabarti S, Patra PK. 2015. Biochemical and antioxidant responses of paddy (Oryza sativa L.) to fluoride stress. Fluoride. 48(1):56.

[53] Asard, H., Smirnoff, J., & May, N. (2003). Vitamin C: its function and biochemistry in animals and plants. The role of ascorbic acid in defence networks and signalling in plants (Chap. 4, pp. 73-92). Nova York:Garland Science/BIOS Scientific Publishers.

[54] Shah, S. A., Zeb, A., Masood, T., Noreen, N., Abbas, S. J., Samiullah, M., Alim, M. A., & Muhammad, A. (2011). Effects of sprouting time on biochemical and nutritional qualities of Mungbean varieties. African Journal of Agricultural Research, 6(22), 5091-

[55] Tomé-Sánchez I, Martín-Diana AB, Peñas E, Bautista-Expósito S, Frias J, Rico D, González-Maillo L, Martinez-Villaluenga C. Soluble Phenolic Composition Tailored by Germination Conditions Accompany Antioxidant and Anti-inflammatory Properties of Wheat. Antioxidants (Basel). 2020 May 14;9(5):426. doi: 10.3390/antiox9050426. PMID: 32423164; PMCID: PMC7278661.