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Biochemical and Physiological Responses to Sodium Halide Stress in Mung Beans Sprouts

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ABSTRACT

Purpose: Germination is a complex physiological process that triggers biochemical and physiological responses. Abiotic stresses can significantly influence seed germination by inducing various physiological and biochemical alterations, followed by morphological changes that impact seedling survival. Irrigation water plays a crucial role in the early stages of seed germination.

Methods: This study examines the effects of different sodium halides on mung bean germination and early sprout development. Mung bean seeds were exposed to three types of sodium halides: NaCl (50 & 100 ppm), NaI (50 & 100 ppm), and NaF (50 & 100 ppm) for five days. Afterward, sprouts were collected, and growth parameters were measured. Biochemical analyses assessed stress markers in the sprout homogenates, including protein content, lipid peroxidation (MDA), glutathione (GSH), ascorbic acid, and total phenolic content.

Results: Control seeds showed optimal growth, such as germination percentage (GP) 92%, germination index (GI) 4.6, vigor index (VI) 4.8, seedling height reduction (SHR) 0%, Relative injury rate (RIR) 0%. NaF caused the most significant inhibition of sprout growth at both concentrations, followed by NaI and NaCl at 100 ppm, leading to marked reductions in GP of 28%, GI as 1.4, and VI as 0.14, and 90% reduction in seedling height with a sharp rise in RIR of 69.65%. 50 ppm NaI and NaCl-treated sprouts were almost the same as control growth parameters. NaF also induced the most severe oxidative stress, reflected by a significant increase in lipid peroxidation and alterations in secondary metabolites such as ascorbic acid and phenolic compounds as compared to control.

Conclusion: This study highlights the concentration-dependent effects of sodium halides on mung bean germination and early growth. It underscores the importance of further research to understand the molecular mechanisms underlying halide-induced stress, including the roles of enzyme activity, gene expression, and stress signaling pathways.

Key-words: *Germination; Halides; Metabolities, Oxidative stress; Seedling*

INTRODUCTION

Halogens are essential micronutrients found throughout nature, playing a crucial role in the health of all living organisms, including plants.[1,2] Among these, fluoride, iodine, and chlorine are important for human health, but their excessive exposure can lead to toxicity.[3] Fluoride is one of the most affected halogens due to human activity.[4] While it naturally occurs in the Earth's lithosphere, human industries, especially those related to fertilizers, ceramics, and cement, contribute significant amounts of fluoride to the environment. [5] Chlorine, on the other hand, is primarily found in the oceans. Iodine is an essential nutrient and is influenced by various factors in the soil, such as rock types, and the biological interactions between plants and soil.[6] Iodinated irrigation water can also elevate iodine levels in the soil.[7] Among the halogens, iodine is the least abundant in the Earth's crust, while fluorine and chlorine are more commonly found.[3] Sodium readily forms salts with halogens due to its electropositive nature, which allows it to lose an electron and form Na+ ions that combine easily with halide ions to create stable salts.[8] These salts play a significant role in agriculture by supporting plant growth, enhancing soil fertility, and contributing to nutrient availability, although their use must be managed to avoid toxicity.[9-11]

Sodium fluoride (NaF) in soil or water can affect plant physiology, biochemistry, and structural functions.[12] Though Fluoride is not an essential element for plants, its presence in soil, air, and water causes alterations in plants' physiological, biochemical, and structural activities, sometimes even without showing any visible symptoms of injury.[13] NaF affects plant growth and development by interfering with several metabolic pathways associated with photosynthesis, respiration, protein synthesis, carbohydrate metabolism, and nucleotide synthesis.[14-15] Several studies have been reported on fluoride-contaminated soil and its effects on different plant species including cereals and vegetables.[16-17] Sodium chloride (NaCl), present in small quantities, acts as a micronutrient and is involved in several plant physiological processes.[18] However, if it exceeds the limits it can hinder seeds' water absorption capacity resulting in osmotic stress and causing ion imbalance which ultimately inhibits germination.[19] NaCl can disrupt enzyme activity, metabolism, hormonal signaling, and the use of energy reserves.[20] Sodium iodide (NaI) provides iodine to plants, which can help improve growth and protect them from certain diseases.[21] It has gained significant importance in agriculture globally. At low concentrations, it can promote plant growth and development, enhance the production of secondary metabolites, and boost antioxidant enzyme activity. However, at higher concentrations, iodine can become toxic to plants.[22]

Mung beans *(Vigna radiata)* are a key pulse crop grown across more than 6 million hectares in tropical and subtropical regions worldwide. Being a member of the Fabaceae family, it is becoming popular due to its health benefits and high nutritional value, particularly with the growing trend of consuming sprouts.[23] During germination, notable changes in nutrient profile, such as high protein, minerals, dietary fiber, and improved antioxidant activity, occur.[24-25] As the seed undergoes germination, stored reserves are broken down to support respiration and the formation of new cellular structures for embryonic development, and alterations in the seed's biochemical and nutritional characteristics.[26-27] With growing awareness of these health advantages, the consumption of mung beans has risen in recent years. However, plant diseases, particularly those caused by fungi and viruses, and abiotic stresses such as abiotic challenges including waterlogging, salinity, heat, and drought, can adversely affect the nutritional quality and crop yield. There is limited research on how halogen salts influence mung bean sprout development. This study explores the effects of three halogen salts (NaF, NaCl, NaI) at different concentrations on sprout formation, mainly focusing on growth parameters and biochemical changes observed after five days of exposure.

MATERIAL AND METHODS

Seed Material

Mung bean seeds used in this study were purchased from a local Riyadh, Saudi Arabia market.

Sodium Halide solution

Aqueous solutions of NaF, NaCl, and NaI were prepared by using respective anhydrous powder at concentrations of 50 ppm and 100 ppm and used directly.

Experimental design

Before germination, the seeds were sterilized in a 1% sodium hypochlorite solution for 5 minutes. The sterilized mung bean seeds were randomly assigned to three groups, with twenty seeds placed in each Petri dish lined with double layers of sterile filter paper. The seeds were treated as follows:

Group I: Seeds were sprouted in three subgroups, treated with 0, 50 and 100 ppm NaF.

Group II: Seeds were sprouted in three subgroups, treated with 0, 50 and 100 ppm NaCl

Group III: Seeds were sprouted in three subgroups, and treated with 0, 50 and 100 ppm NaI.

The seeds were germinated for 5 days under low-light conditions at room temperature. A seed was considered to have sprouted when the plumule and radicle emerged and grew to a length greater than 1 mm. After five days, the number of sprouts was recorded and analyzed for various germination parameters and biochemical changes.

Germination parameters

On day five sprouted seeds were counted and the shoot and root lengths of sprouts from each replication were measured. The growth parameters of treated Mung bean sprouts were assessed and compared with the control group [29] as mentioned below:

1. *Germination percentage*

Number of sprouted seeds ÷ Total number of seeds× 100

2. *Germination index*

Number of days of sprouting × Number of sprouted seeds ÷ Total number of seeds.

3. *Vigor index*

Germination percentage× Sprout or seedling length ÷ 100

4. *Relative injury rate*

[GP%(control) –GP% (halogen salts treatment) ÷ GP%(control)] × 100

GP% =Germination percentage

5. *Seedling height reduction*

[SH at control) – SH at Specific halide treatment) ÷ SH at control] × 100

Where SH =Sprout height.

Biochemical Analysis

Fresh sprouts from all groups were homogenized in phosphate buffer (pH 7, 1:10 W/V). The homogenate was centrifuged at 4°C at 3500 g for 15 minutes. The supernatant was collected and used for the following biochemical assays:

Total crude protein was measured using Bradford's method with Bovine serum albumin (BSA) as standard.[29]

Lipid peroxidation was assessed using the thiobarbituric acid (TBA) test, as outlined by Ruiz-Larrea et al. [30]

Reduced Glutathione(GSH)- Levels of GSH were determined following Beutler et al.'s method[31] by using GSH as a standard

Vitamin C levels were measured using the technique described by Jagota and Dani with ascorbic acid as standard. [32]

The total phenolic content was quantified using the Folin–Ciocalteu method by using Gallic acid as standard. [33]

Statistical Analysis

GraphPad Prism version 10.4.1 was used to analyses the data and results are mean ± standard deviation (SD). Statistical comparisons between the three different halide treated sprouts were conducted using two-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. A p-value of less than 0.05 was considered statistically significant.

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RESULTS

Figure 1 illustrates the impact of various concentrations of sodium halides on sprout formation. Seeds treated with 50 ppm NaCl and NaI exhibited slightly decreased or similar sprout formation rates. However, seeds treated with 50 ppm NaF showed a significant reduction in sprout length, nearly halving it. At 100 ppm, all sodium halides led to a marked decrease in sprout length, with NaF causing the most significant reduction, followed by NaI, and then NaCl

The growth parameters of seeds treated with different concentrations of sodium halides are summarized in Table 1 and Figure 2. The control group sprouts had a GP of 92%. At 50 ppm, NaCl and NaI treatments showed only a slight reduction in GP to 80 and 90, respectively, whereas NaF decreased to 68%. At 100 ppm, NaF treatment led to a sharp decline in GP to 28%, while NaCl and NaI showed 80%. Control sprouts displayed a GI of 4.6. At 50 ppm, NaF caused a reduction in GI to 3.4, while NaCl and NaI treatments showed minimal changes of 4 and 4.5, respectively. At 100 ppm, NaF treatment resulted in a further decrease in GI to 1.4, while NaCl and NaI maintained it at 4. Control spouts had a vigor index of 4.8. At 50 ppm, NaF-treated seeds showed a substantial decline to 1.7, while NaCl and NaI exhibited moderate reductions to 3.6 for both. At 100 ppm, NaF caused a dramatic drop in vigor to 0.14, while NaCl and NaI treatments maintained values of 3.2 and 2.8, respectively. SHR of control sprouts is taken as 0%. At 50 ppm, NaF caused a significant reduction in seedling height to 50%, whereas NaCl and NaI showed smaller reductions of 10% and 20%, respectively. At 100 ppm, NaF led to the most severe decrease in height at 90%, followed by NaI at 30% and NaCl at 20%. RIR was 0% for the control sprouts. At 50 ppm, NaF resulted in an RIR of 26.0%, while NaCl and NaI showed lower RIR values of 13.04% for both. At 100 ppm, NaF caused a substantial increase in RIR to 69.65%, while NaCl and NaI exhibited much lower rates of 13.04% and 2.17%, respectively. The biochemical changes of seeds treated with different concentrations of sodium halides are shown in Table 2 and Figure 3. The total protein content in the control group was reported as 17.16. At 50 ppm, NaCl and NaI treatments showed negligible changes as compared to NaF where protein level was reduced to 16.71. At 100 ppm, NaCl and NaI still showed minor reductions in protein content to 16.93 and 16.91 respectively while NaF exhibited a slight further decrease to 16.34. Lipid peroxidation (MDA) was 17.9 in the control group. 50 ppm NaF decreased MDA to 18.38, while NaCl (16.99) and NaI (17.30) showed minimal changes. At 100 ppm, NaF resulted in a significant increase in lipid peroxidation to 24.37, whereas NaCl and NaI showed more moderate changes to 17.18 and 13.3 respectively. GSH levels in control seeds were 19.61. At 50 ppm, NaI displayed the highest increase in GSH to 62.25, followed by NaCl to 41.83 and NaF to 35.89. At 100 ppm, NaF caused a marked rise in GSH to 55.82, while NaCl 44.15 and NaI 48.59 maintained elevated levels of 44.15 and 48.59 respectively. Ascorbic acid was 10.63 in control sprouts. At 50 ppm, NaCl showed a significant increase to 15.47, followed by NaI to 12.5 while NaF showed a slight decrease to 10.42. At 100 ppm, NaCl-treated sprouts exhibited the highest ascorbic acid levels at 17.05, while NaI-treated sprouts were 13.68 and NaF showed the lowest level of 4.0. Total phenols in the control sprouts were 23.19. At 50 ppm, NaF treatment showed a marked increase in total phenol content to 43.97, compared to NaI (36.46) and NaCl (32.04). At 100 ppm, NaF (47.59) showed the highest increase again, followed by NaI (38.91) and NaCl (32.38).

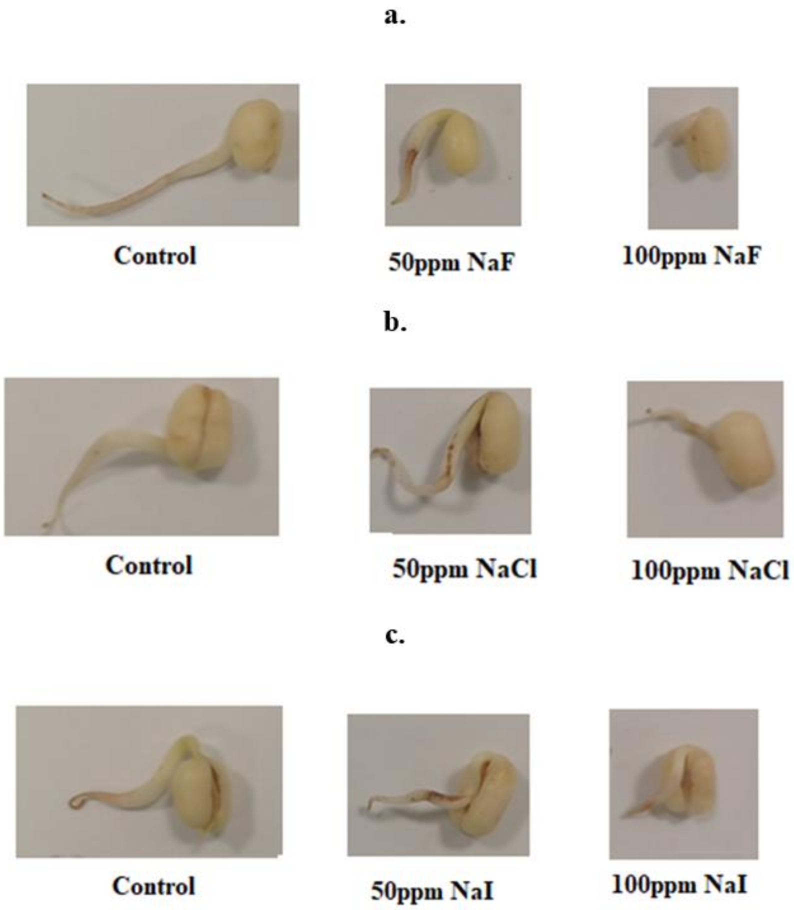


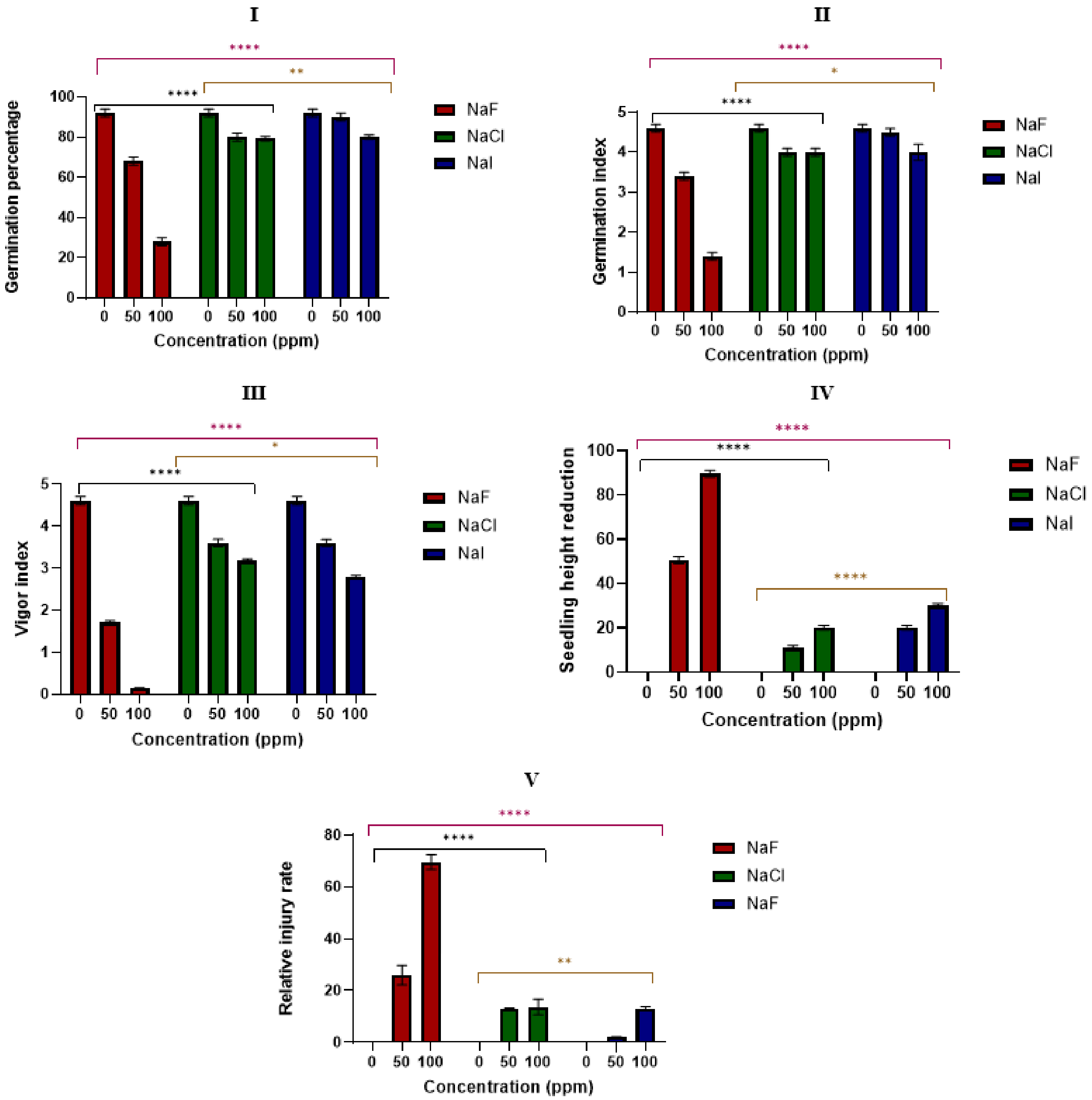
Figure 1. Mung bean sprouts under sodium halide stress on day five. a) NaF; b) NaCl; c) NaF.

Table1- Effect of various concentrations of different sodium halides on growth parameters of Mung bean sprouts

Growth parameters	Sodium halide	Concentration		
		0	50ppm	100ppm
Germination percentage	NaF	92±2.0	68±3.0	28±3.0
	NaCl	92±2.0	80±2.0	80±2.0
	NaI	92±2.0	90±2.0	80±1.0
Germination index	NaF	4.6±0.1	3.4±0.1	1.4±0.1
	NaCl	4.6±0.1	4±0.1	4±0.1
	NaI	4.6±0.1	4.5±0.1	4±0.2
Vigor index	NaF	5.06±0.3	1.7±0.15	0.14±0.11
	NaCl	5.06±0.3	3.6±0.51	3.2±0.13
	NaI	5.06±0.3	3.6±0.15	2.8±0.16
Seedling height reduction	NaF	0	50±00	90±00
	NaCl	0	10±00	20±00
	NaI	0	20±00	30±00
Relative injury rate	NaF	0	26±04	69.65±03
	NaCl	0	13.04±01	13.04±02
	NaI	0	13.04±0.9	2.17±0.18

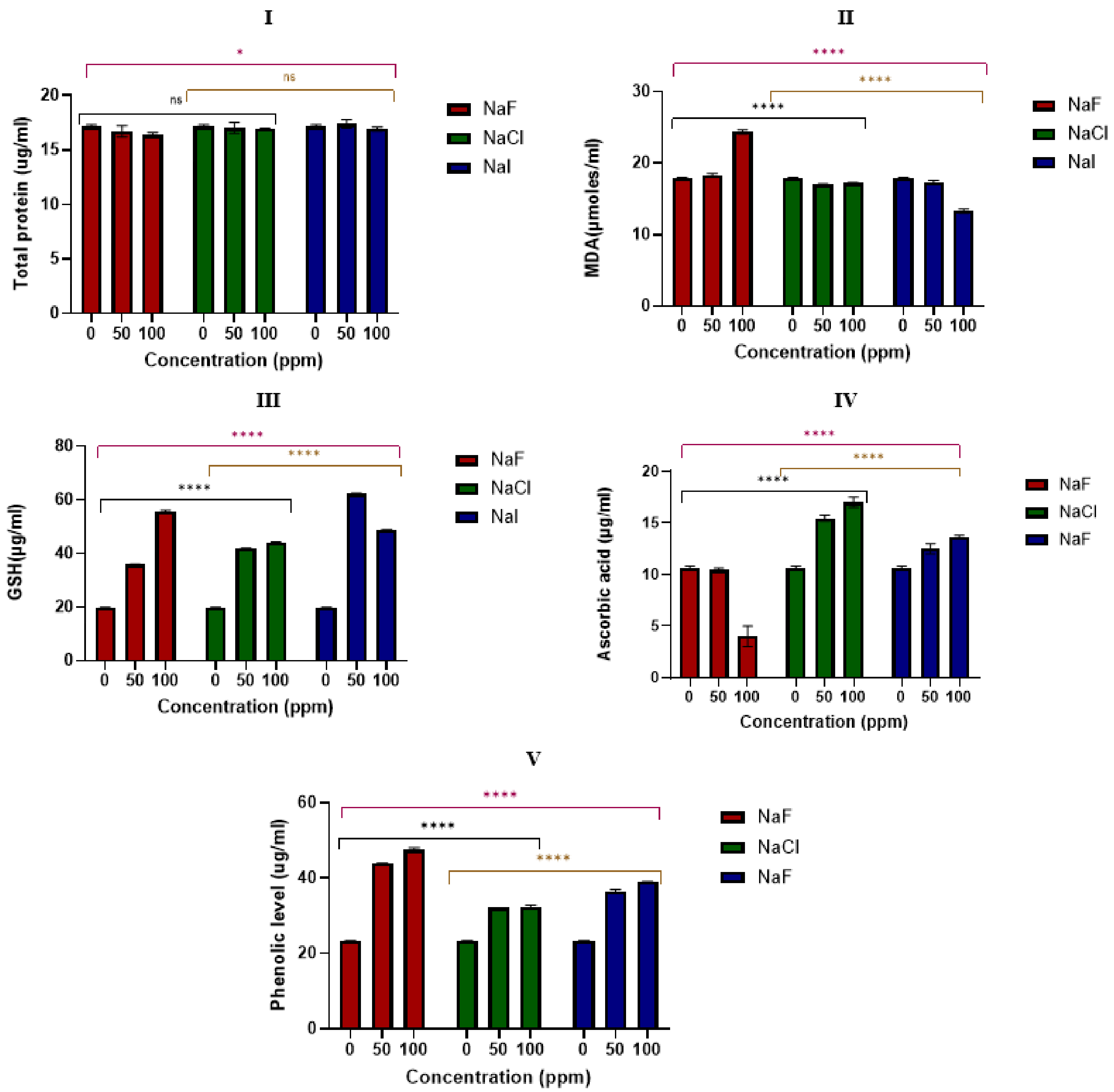
Table 2- Effect of various concentrations of different sodium halides on Oxidative stress markers of Mung bean sprouts

Growth parameters	Sodium halide	Concentration		
		0	50ppm	100ppm
Total protein (μg/ml)	NaF	17.16±01	16.71±02	16.34±02
	NaCl	17.16±01	17±02	16.93±0.9
	NaI	17.16±01	17.37±03	16.91±01
MDA (μmoles /ml)	NaF	17.9±0.9	18.38±0.2	24.37±01
	NaCl	17.9±0.9	16.99±0.9	17.18±0.5
	NaI	17.9±0.9	17.3±0.2	13.3±0.9
GSH (μg/ml)	NaF	19.61±0.4	35.89±0.6	55.82±0.9
	NaCl	19.61±0.4	41.83±0.9	44.15±0.8
	NaI	19.61±0.4	62.25±0.9	48.59±1.0
Vitamin C (μg/ml)	NaF	10.63±1	10.42±1.3	4±1.5
	NaCl	10.63±1	15.47±1.2	17.05±1
	NaI	10.63±1	12.5±1	13.68±1.2
Total Phenol (μg/ml)	NaF	23.19±0.9	43.97±0.2	47.59±0.3
	NaCl	23.19±0.9	32.04±0.1	32.38±0.4
	NaI	23.19±0.9	36.46±0.3	38.91±0.2



*ns non-significant; p-value * ≤ 0.05; ** ≤ 0.01; *** ≤ 0.001; **** ≤ 0.0001*

Figure 2. Growth parameters of Mung bean sprouts under sodium halide stress on day five. I. Germination percentage; II. Germination index; III. Vigor index; IV. Seedling height reduction; V. Relative injury rate.



*ns non-significant; p-value * ≤ 0.05; ** ≤ 0.01; *** ≤ 0.001; **** ≤ 0.0001*

Figure 3. Oxidative stress markers of Mung bean sprouts under sodium halide stress on day five. I Total protein; II. MDA; III. GSH; IV. Vitamin C; V. Total Phenol.

DISCUSSION

Mung beans are highly susceptible to water stress, which can significantly hinder the formation of sprouts.[34-36] Polluted water, particularly during the early germination stages, can disrupt this process, leading to poor sprout development. [37-40] Adequate water availability is crucial for maintaining turgor pressure in plant cells and supporting healthy tissue growth. Halides as water pollutants, pose risks to human health and plant life when their concentrations exceed safe thresholds. [41] These pollutants can enter water bodies through water treatment, disinfection processes, or human activities involving halide-related chemicals.[42] Sprouting is a complex process involving various metabolic and physical changes in mature seeds, beginning with water absorption (imbibition) and then the development of seedlings.[43] Evaluating seed germination is crucial for ensuring successful crop production. Water quality plays a key role in breaking seed dormancy, and initiating various physiological processes that lead to seed germination.[44] In this study, the germination rates of mung beans were influenced by the concentration and specific type of sodium halide, suggesting that these compounds can interfere with the early stages of sprout formation. Among the halides tested, fluoride (NaF) was the most effective inhibitor, followed by iodide (NaI) and chloride (NaCl), as shown in Figure 1. The impact of these compounds was concentration-dependent, with higher levels causing a more significant hindrance to plant development. Gaining a deeper understanding of the mechanisms behind this inhibition could help uncover the specific pathways through which sodium halides affect sprout formation.

NaF had the most detrimental effects on sprout growth, especially at higher concentrations, while NaCl and NaI caused comparatively less damage (Figure 1). This aligns with previous studies on Fenugreek seedlings, where NaF severe impact on seedling length than NaCl and NaI. [45] Many researchers have also reported that elevated fluoride levels hinder germination.[46-48] In this study, the reduced sprout numbers in NaF-treated mung beans may be linked to fluoride's inhibition of phytase enzymes essential during germination for breaking down phytate, a major energy source for germinating seeds.[49] Fluoride also disrupts carbohydrate metabolism by impeding amylase activity, thus slowing seed growth. [50] While NaCl is known to be toxic at higher concentrations, it does not affect plants at lower concentrations and maintains ion balance without interfering with cellular metabolism or nutrient distribution.[18,51] Previous studies have also observed improved growth at lower NaCl concentrations.[45] In contrast, NaI treatment may limit sprout development due to an imbalance in nutrient uptake. [22,52]

Sodium halide-induced oxidative stress negatively affected mung bean sprouts, as shown in Figure 2. NaF led to a decrease in total protein levels and an increase in lipid peroxidation, signaling stress. NaCl and NaI showed moderate effects across these stress markers. Plants have developed various defense mechanisms to minimize damage caused by high halide concentrations. When these substances accumulate within cells, defense mechanisms are activated to protect cells from oxidative stress, which could otherwise lead to cell death or stress-induced adaptation.[53] In the current study, increased oxidative stress was reflected by growth parameters such as sprout length, particularly under NaF exposure, followed by NaI, with NaCl showing the least impact. During germination, seeds employ a range of antioxidant mechanisms to counteract the toxic effects of chemicals. [54] These include regulating antioxidant enzyme activity and metabolite production to maintain reactive oxygen species (ROS) at safe levels.[55] These protective enzymes and metabolites safeguard germinating seeds from cellular damage while metabolic activities are optimized.[56] When stressed, plants produce more secondary metabolites, such as soluble solids, organic acids, proteins, and amino acids.[57] Crude protein levels in NaCl and NaI-treated sprouts indicate a potential protective response. Changes in lipid peroxidation, ascorbic acid, and phenol levels were observed in all treated sprouts and can serve as biomarkers for assessing halide-induced damage. Ascorbic acid is a crucial metabolite involved in several cellular processes, including cell division, and its levels typically rise during germination.[58] NaF exposure led to reduced levels of ascorbic acid, indicating fluoride toxicity. However, NaI and NaCl treatments increased ascorbic acid content, suggesting a potential protective response. Phenolic compounds, produced during normal germination, are also shown to increase under stress conditions, as ROS generation triggers their overproduction.[52,59] Our findings support this, as high levels of phenolic compounds were detected in NaF-treated sprouts compared to NaCl and NaI-treated mung beans. The coordinated action of ROS-scavenging pathways across various cellular compartments likely plays a crucial role in halide tolerance, helping to manage ROS levels, minimize cellular damage, and maintain cellular function.[60]

Limitations and Future Recommendations

The study did not explore the molecular mechanisms underlying the biochemical changes associated with inhibited sprout growth. Further research on enzyme activity, gene expression, and signaling pathways involved in stress responses could provide valuable insights into how mung beans manage halide-induced oxidative stress.

CONCLUSIONS

Exposure to sodium halides triggers oxidative stress in mung bean sprouts, as demonstrated by alterations in protein levels, lipid peroxidation, and secondary metabolites such as ascorbic acid and phenolic compounds. NaF had the most detrimental impact among the three halides, significantly reducing germination, growth, and vigor. It also caused the highest increase in lipid peroxidation and changes in secondary metabolites such as ascorbic acid and phenolic compounds, indicating severe oxidative stress. NaCl and NaI treatments showed relatively milder effects. While seeds have adaptive mechanisms to manage oxidative stress during germination, high halide concentrations can overwhelm these defenses, resulting in reduced growth. Findings of this study suggest that sodium halides, especially NaF, can negatively affect mung bean crop development**.** Understanding the mechanisms behind halide-induced inhibition could help improve agricultural practices and develop strategies to enhance mung bean crop

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CONFLICT OF INTERESTS

"None"

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