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RESEARCH ARTICLE

EFFECTS OF THE TOXICITIES OF ZN AND FE EXPOSURE ON THE GROWTHS OF MUNG BEAN (*VIGNA RADIATA*): AN EXPERIMENTAL LABORATORY STUDY

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ABSTRACT

This study investigates the crucial effects of Zinc (Zn) and Iron (Fe) on the development of mung beans (*Vigna radiata*) at different concentrations. Mung beans were subjected to three treatments: control (distilled water), 25 ppm Zn and Fe, and 50 ppm Zn and Fe. This study measured mung bean growth in length, leaf count, biomass, and stomatal opening. The results indicated that 25 ppm Zn and Fe restricted plant growth compared to the control, with reduced stomatal opening and average length. However, the biomass was slightly higher, suggesting other limiting factors. In contrast, the 50-ppm treatment produced mixed results, with some plants showing stunted growth due to possible nutrient toxicity and the stomatal opening showing tremendous effect on the stem. This study underscores the importance of balanced micronutrient management for optimal plant health and development.

KEYWORDS

Mung bean, metal toxicity, laboratory experiment, growth.

1. INTRODUCTION

Mung beans (*Vigna radiata*), belonging to the family Fabaceae, are often considered the most versatile plant for scientific studies and have unique physiological growth (Huppertz et al., 2023). Mung bean is gaining popularity as a functional food for promoting good health due to its rich content of protein, fibre, minerals, vitamins, and substantial amounts of bioactive compounds such as polysaccharides, polyphenols, and peptides (Hou et al., 2019). It also plays an important role in maintaining soil fertility by enhancing the soil's physical properties and fixing atmospheric nitrogen (Naik et al., 2020). Mung beans are characterised by rot nodules that house nitrogen-fixing bacteria, Rhizobium, which enhances soil fertility with minimal irrigation and is independent of fertilizers (Favero et al., 2021). However, for mung beans to thrive, it is crucial to maintain a balanced supply of both macro and micronutrients.

Micronutrient elements such as Zinc (Zn) and Iron (Fe) needed in minute quantities significantly improve plants' health, developmental growth, and many physiological processes (Ahmed et al., 2024). These two elements are widely used as fertilisers in crop production to increase yields for commercial and sustenance purposes. The presence of Zn in soil could help plants enhance their protein synthesis, cell division, and hormonal controls, such as auxin, which stimulates the nutrition uptake of plants (Umair et al., 2020). Zinc is essential for accelerated growth and reproductive ability of plants. It also enhances the finger proteins that are involved in signal transduction and becomes the regulator and transcription of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) in plants (Bui and Inaba, 2024). Furthermore, iron aids plants in the synthesis of chlorophyll, respiration processes, and nitrogen fixation (Liu et al., 2023). Iron is an important micronutrient in the plant life cycle to ensure its growth is not compromised. Iron also provides a structural element of hemes, hematin, and leghaemoglobin that are engaged in the nitrogen fixation of pulses catalysed metabolically by nitrogenase (Dhayal et al., 2023).

These micronutrients contribute to the plants' productive growth, yield, and nutritional assimilation, particularly the mung beans. However, the optimal concentration of these micronutrients remains largely ambiguous to dictate the deficiency or excessiveness of both Zn and Fe elements present in agricultural practices (Kanwal et al., 2020). This issue encourages the need for scientific intervention to determine the most appropriate levels of Zn and Fe for the development of mung bean growth. Therefore, this study examines the effects of zinc and iron in a range of concentrations of mung bean growth to gain insight into the ideal concentration needed for optimal growth. This study underlines more sustainable guidelines for micronutrient management in the current agriculture sectors.

2. MATERIALS AND METHODS

2.1 Mung Bean Preparation

About 200 pieces of mung beans (*Vigna radiata*) were immersed in 200 mL beakers for 5 hours using tap water from 10 am to 2 pm. These freshly immersed mung beans with prominent hypocotyl (embryonic shoot) and radicle (embryonic root) were then meticulously selected to be cultivated. The seed coat was removed using forceps. Ten selected mung beans were randomly placed onto a moistened cotton wool (about 1.3 g) in each of the 100 mm petri dishes, with three replicates (R1, R2, R3) per treatment group. To study the effects of zinc and iron concentrations on mung bean growth, moistened cotton wool was prepared with three different treatments (control: 50 mL of distilled water, 25 ppm of iron (II) sulphate

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(Fe) + 25 ppm of zinc sulphate (Zn) and 50 ppm of Zn + 50 ppm of Fe. These concentrations were selected as they exhibit an ideal range to examine the effects of micronutrients for enhancing mung bean growth with less toxicity towards the plants (Shepa et al., 2024a). These Petri dishes were positioned adjacent to a window to ensure consistent exposure to natural light conditions, mimicking realistic growth environments for mung beans. The progression of mung bean growth was observed over a week, accompanied by the regular supplementation of water treatments at two-day intervals. After a week, the growth parameters like its length, leaves count, biomass, and stomatal opening per mung bean were noted down. Then, mung bean seedlings were harvested from Petri dishes, dried the excessive moisture with tissues, and weighed using analytical balance to measure the biomass of the respective three concentration treatments.

2.2 Preparation of Fe and Zn Solutions

To prepare a 25 ppm solution of iron (II) sulfate (Fe) and zinc sulfate (Zn), 5 mg of Zn and 5 mg of Fe were dissolved separately in 200 ml of distilled water. For a 50 ppm solution, 10 mg of Zn and 10 mg of Fe were dissolved in 200 ml of distilled water. The control group were treated with 50 mL of distilled water.

2.3 Stomatal Opening and Data Analysis

The stomatal opening of the mung bean was captured under an Olympus CX33 HD Digital Microscope and digital camera Accu-Scope Excelis camera (Olympus Corporation, Japan). One specimen of mung bean from each treatment was selected. The leaf and stem were cut into small segments for microscopic observation. These segments were placed onto the glass slide, and a drop of distilled water was added before carefully covering them with a coverslip to avoid air bubbles. The prepared slides were then observed under the digital microscope. Stomatal opening in both stems and leaves was measured using CaptaVision software, a built-in software in the digital microscope. The observable gap in the stomata represented the variability in stomatal openings. Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) were used to represent all the graphs and calculate the average length, leaves count, stomatal opening (leaf, stem) and biomass of mung beans in Zn and Fe treatments. One-way *ANOVA* and *Tukey t-test* were used to compare the significant difference of Zn and Fe treatment on mung bean growth parameters compared to the control.

2.4 Ethics Statement

The laboratory experiment used commercially available mung beans (*Vigna radiata*) bought from a local supplier without any natural populations sampled. Experiments specialised in the study of mung beans hypocotyl and radicle after the seed coat removal in a controlled laboratory environment inside the Department of Biology, Universiti Putra Malaysia (UPM) on 19 June 2024. Ethical standards were followed strictly without inflicting harm on the plant matters and imposing minimisation of the effects of waste and toxic byproducts from the chemical substances used. All data were specifically documented for research purposes without authoritative ethical approvals due to the nature of the materials being used throughout the experiment.

3. Results

3.1 Stomatal Opening

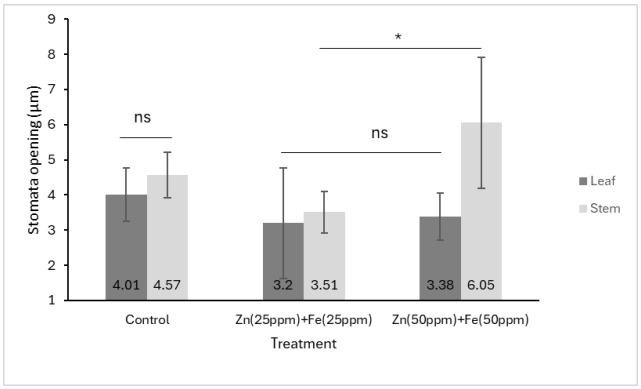

Figure 1: The stomatal opening of mung bean leaves was slightly reduced after 7 days when treated with 25 and 50 ppm of both Zn and Fe, but the reduction was not significant compared to the control. However, on the stem, the stomatal opening significantly increased when treated with 50 ppm of Zn and Fe compared to the control (N =30 for each treatment, *p ≤ 0.05 significantly different from the control)

The stomatal opening in stems significantly increased, particularly with the higher Zn(50ppm)+Fe(50ppm) treatment, nearly doubling compared to the control. However, the stomatal opening in leaves showed a slight decrease with both Zn treatments. The different doses of Zn combined with Fe had a more obvious positive effect on the stomatal opening in stems than in leaves. These findings suggest that higher concentrations of Zn and Fe may enhance stomatal opening in stems, while the effect on leaves is less significant.

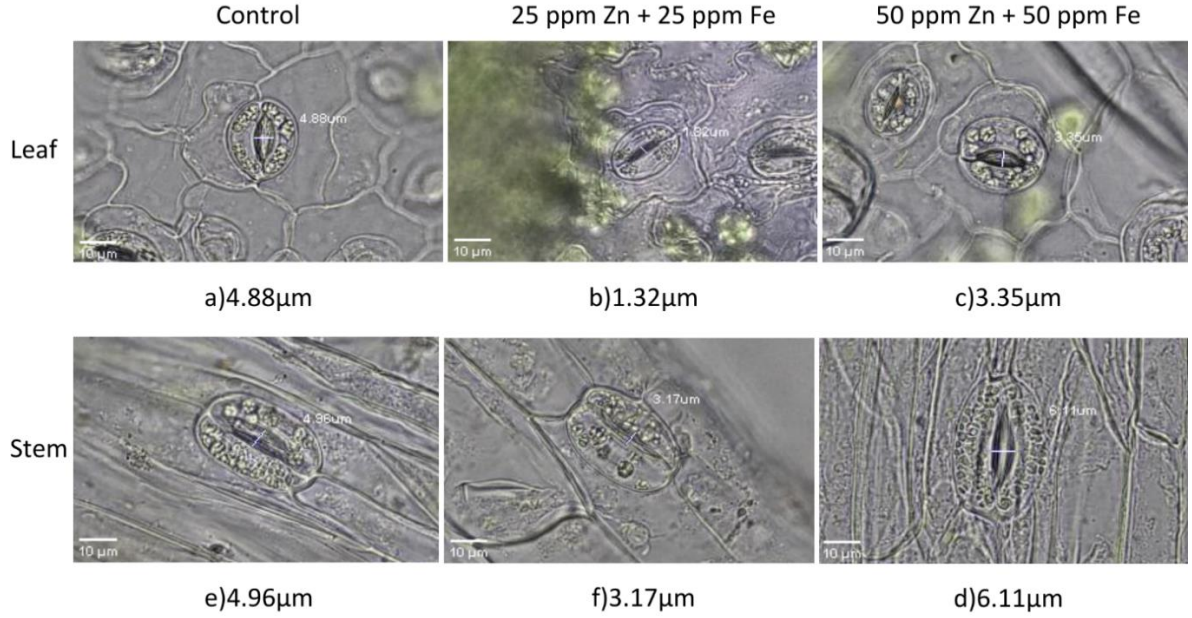

Figure 2: Leaf and stomata opening image under 400x magnification of the compound light microscope.

The representative microscopic images in Figure 2 illustrate leaf and stem stomatal apertures of mung bean (*Vigna radiata*) under different treatments: control (untreated), 25 ppm Zn + 25 ppm Fe, and 50 ppm Zn + 50 ppm Fe treatments. Stomatal apertures are observed in both leaf (a-c) and stem (e-f) tissues. Each image (a-f) represents the best replicate from a set of three replicates for each treatment. Visible changes in stomatal aperture size across treatments are evident, with a decrease in aperture size in the 25 ppm Zn + 25 ppm Fe treatment (b, f) and an increase in the 50 ppm Zn + 50 ppm Fe treatment (c, d). The measurements below each image denote the average stomatal aperture size (µm) for each condition.

3.2 Length

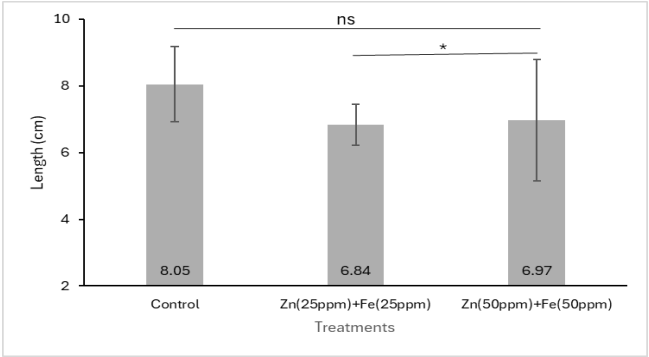

Figure 3: The average length of mung bean after 7 days increased slightly when treated with 25 ppm and 50 ppm of both Zn and Fe, but not significantly different from the control. (N=30 for each treatment, *p ≤ 0.05 significantly different from the control).

The length of the plants decreased when treated with Zn and Fe compared to the control, with both Zn treatments showing a reduction in length by about 1-1.2 cm. There was little difference between the effects of the Zn(25ppm)+Fe(25ppm) and Zn(50ppm)+Fe(50ppm) treatments on plant length, as both doses led to a similar decrease. These results suggest that adding Zn and Fe in the concentrations used negatively impacts plant length, with no significant difference between the two concentrations

3.3 Leaves Count

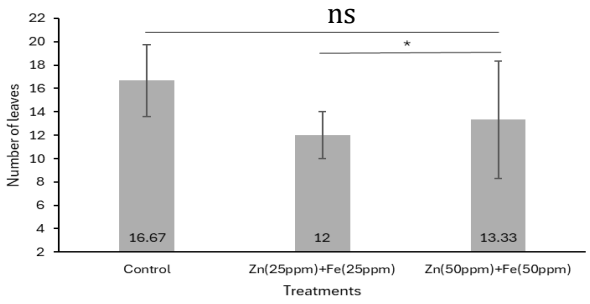

Figure 4: The average leaf count of mung bean after 7 days was reduced when treated with 25 ppm and 50 ppm of both Zn and Fe, but the difference was insignificant compared to the control. (N=30 for each treatment, *p ≤ 0.05 significantly different from the control).

The number of leaves decreased when treated with Zn and Fe compared to the control, with the leaf count dropping from 16.67 in the control to 12 in the Zn(25ppm)+Fe(25ppm) treatment. The Zn(50ppm)+Fe(50ppm) treatment resulted in a slightly higher leaf count of 13.33, but it was still lower than the control. The different doses of Zn combined with Fe negatively impacted the number of leaves, with a more pronounced reduction observed at the lower concentration. These findings suggest that the application of Zn and Fe, particularly at lower concentrations, reduces the number of leaves in plants.

3.1 Biomass

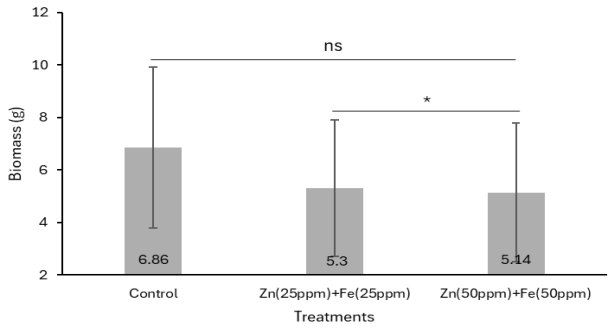

Figure 5: The total biomass of mung bean after 7 days decreased slightly when treated with 25 ppm and 50 ppm of both Zn and Fe solutions, but there was no significant difference compared to the control. (N =30 for each treatments, *p ≤ 0.05 significantly different from the control)

This bar graph shows the effect of different treatments on biomass. The control group has the highest biomass, with a value of 6.86 grams, while the treatments with Zinc (Zn) and Iron (Fe) lead to reduced biomass. The biomass decreases when treated with Zn and Fe compared to the control, showing a reduction of around 1.5 times. Different doses of Zn (25 ppm and 50 ppm) combined with Fe (25 ppm and 50 ppm) showed a negative effect on biomass, resulting in decreased biomass values of 5.3 grams and 5.14 grams, respectively. These findings suggest that treatment with Zn and Fe at the given concentrations reduces biomass compared to the control, indicating a negative impact on plant growth.

4. Discussion

For the stomatal opening at the leaf, the control group demonstrated a moderate average stomatal opening with minimal variability, indicating consistent stomatal function under normal, stress-free conditions. When Zn and Fe were introduced at 25ppm each, the average stomatal opening decreased compared to the control, with an increase in variability. Similarly, research on crop treatment with Iron (Fe) nanoparticles highlighted that the presence of high concentrations of Zn and Fe can influence stomatal functionality by increasing the rate of stomatal conductance and disruption of photosynthetic mechanisms (Moore et al., 2024). This suggests that the added micronutrients may have caused stress or disrupted normal physiological processes at this concentration, leading to reduced and more inconsistent stomatal openings. In more detail, Zinc interferes with photosynthetic processes, stomatal conductance, and various other essential metabolic functions (Chakraborty and Mishra, 2020). The higher variability implies that individual plants reacted differently to this treatment, possibly due to differences in tolerance or nutrient uptake efficiency.

At a higher concentration of 50 ppm for both Zn and Fe, the stomatal opening on the leaf increased slightly compared to the 25ppm treatment but remained below the control level. The reduced variability, relative to the 25ppm treatment, suggests a more uniform response among the plants, although the overall stomatal opening was still inhibited compared to the control. This indicates that while the plants may have partially adapted to the higher concentration, the overall stress or nutrient imbalance was not completely resolved, resulting in a persistent, though less pronounced, reduction in stomatal opening. From the treatment of both 25 ppm and 50 ppm Zn and Fe, we could also see the effect of the influence of water movement through the plant thus also affecting the stomatal opening in the plant. According to a study, under iron (Fe) deficiency of excessive uptake, plants appear to generate a signal that may impact water movement (water potential) within the plant (Barzana et al., 2020).

For the stomatal opening at the stem, in the control group, the stem exhibited a higher average stomatal opening than the leaf, with low variability, reflecting a healthy and stable physiological state. However, when Zn and Fe were applied at 25ppm, there was a noticeable decrease in stomatal opening on the stem, mirroring the response seen in the leaf. This suggests that the lower concentration of micronutrients may have inhibited normal stomatal function, possibly due to a stress response or inadequate nutrient levels. Micronutrient deficiency-induced oxidative stress and associated antioxidant responses of plants (Hou et al., 2019). The relatively low variability indicates a more uniform response among the plants on the stem compared to the leaf.

Interestingly, at the higher concentration of 50 ppm for both Zn and Fe, the stem showed a significant increase in stomatal opening compared to both the control and the 25p pm treatment. This indicates that the 50 ppm concentration had a stimulatory effect on stomatal activity in the stem, in contrast to the inhibitory effect observed on the leaf. The stimulatory effect on stomatal activity could be because of the production of signalling molecules that can promote stomatal opening whilst the inhibitory effect could be of levels of concentration that is considered toxic to the plants. The increased variability in this treatment suggests that while some plants responded positively to the higher concentration, others may have experienced stress or a different response, leading to varied outcomes. This differential response between the leaf and stem could be due to differences in how the micronutrients are distributed, absorbed, or utilized, with the stem potentially being more responsive to higher concentrations of Zn and Fe (Al-Hayami, 2024).

As for the average length of mung bean, the control group, which was treated with distilled water, exhibited the greatest average length of mung beans, indicating that without additional Zn and Fe, the plants achieved their maximum growth potential. When Zn and Fe were applied at 25ppm each, the average mung bean length decreased, likely reflecting a mild stress response where the added micronutrients may have interfered with

normal physiological processes, resulting in stunted growth. For example, plants exposed to either excessive or insufficient levels of zinc may develop abnormalities (Ungadau et al., 2023). At the higher concentration of 50ppm for both Zn and Fe, the average length increased slightly compared to the 25ppm treatment but still fell short of the control group's growth. A recent research on *Linum uitatissimum* (flax plants) exemplified that high Zn concentration can enhance growth metrics without reaching a threshold that could cause stress and stunt the growth process (Sadak and Bakry, 2020). This suggests that although the higher concentration may have mitigated some of the stress caused by the lower concentration, it was still insufficient to reach the growth levels observed in the control group. The plants likely experienced a nutrient imbalance or mild toxicity at these concentrations, which affected their growth.

The control group treated with distilled water had the highest average leaf count, showing moderate variability among the replicates. This indicates that without the addition of Zn and Fe, plants were able to maintain a relatively high and consistent level of leaf production. The application of Zn and Fe at 25 ppm each resulted in a lower average leaf count compared to the control. This decrease, combined with reduced variability among the replicates, suggests that this concentration might slightly inhibit leaf development. The reduced leaf production could be due to a mild stress response or the plants' adjustment to the micronutrient levels. When the Zn and Fe concentrations were increased to 50ppm each, the average leaf count rose slightly compared to the 25ppm treatment but was still lower than the control. This treatment also showed the greatest variability among replicates, indicating an inconsistent response among the plants. This variability might suggest that some plants benefited from the higher micronutrient levels, while others may have experienced stress, leading to fewer leaves. The mixed response could be due to differences in the plant's tolerance to higher micronutrient concentrations or possible micronutrient toxicity affecting some plants more than others (Sherpa et al., 2024b).

Lastly, for the total biomass of mung beans similarly, the control group had the highest total biomass of the mung beans, reflecting ideal growth conditions without additional Zn and Fe. When Zn and Fe were applied at 25ppm each, there was a significant reduction in total biomass. This decline is consistent with the observed decrease in average length, reinforcing the idea that the plants were under stress or faced a less-than-ideal nutrient environment (Kumar et al., 2023). At the higher concentration of 50 ppm for both Zn and Fe, the total biomass decreased slightly compared to the 25 ppm treatment. Comparably, research on *Theobroma cacao* (cocoa plants) indicated that treatment of Zn and Fe did not create noticeable impacts on total biomass but recorded some variations in leaves and stems. This suggested that the effect of Zn and Fe on biomass is inconsistent and dependent on specific environments. But, despite a minor increase in average length, this continued drop in biomass suggests that the higher concentration of micronutrients may have intensified the stress on the plants, likely due to toxicity or nutrient imbalances.

5. Conclusions

This study exhibited variations in stomatal opening, lengths, leaf count, and biomass of mung beans. In the control treatment without zinc (Zn) and iron (Fe), the stomatal opening remains functionally stable at the most optimum level with the highest average growth, leaves count, and total biomass. Exposure to 25 ppm Zn and Fe treatment showed a decrease in leaf and stem stomatal opening, length, and leaf counts but increased slightly in biomass, implying that this concentration inflicts stress or physiological disruption to mung beans. However, the exposure to 50 ppm zinc (Zn) and iron (Fe) enhanced the stomatal opening on the stem. Still, it restricted the length, number of leaves, and biomass compared to the control treatment and 25 ppm treatment, indicating this concentration generated an inconsistent effect towards the mung beans. It is suggested that lower concentrations of Fe and Zn can promote growth while higher concentrations may inhibit their growth. Overall, mung bean represents a promising model system for studying the effect of Zn and Fe concentration on mung bean development leading to the observable effect of those minerals on such plants.

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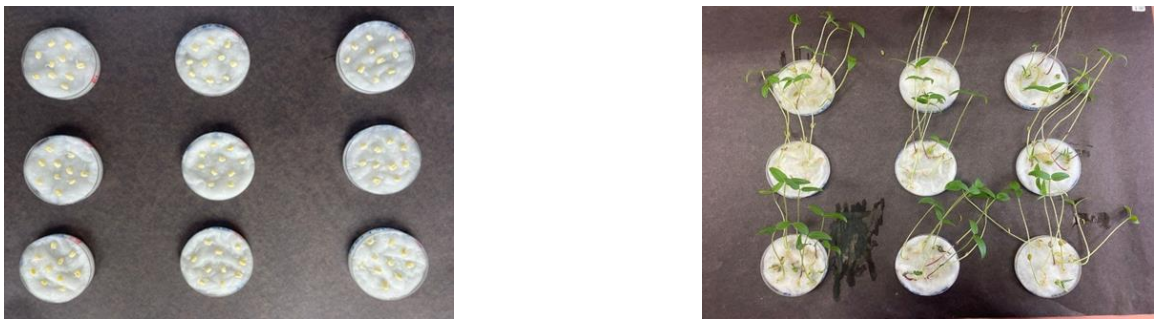
Author Contributions

Conceptualisation, C.K.Y. and N. A. B.; methodology, Z.A.C., M. I. M. H.; M. A. R; M. A. S. B.; and M. M. L; investigation, M. I. M. H.; M. A. R; M. A. S. B.; and M. M. L; writing-original draft preparation, M. I. M. H.; Z. A. C; M. A. R; M. A. S. B.; and M. M. L; data curation, M. I. M. H., Z. A. C. and M. M. L., visualisation, M. M. L, M. I. M. H.; M. A. R; and M. A. S. B.; supervision, N. A. B. All authors have read and agreed to the published version of the manuscript.

Additional Materials



a b

Figure 6: Mung bean condition before and after 7 days of germination. (a) Day 0; (b) Day 7.

a b c

Figure 7: Comparison of mung bean length for each treatment. (a) Control; (b) Zn 25 ppm +Fe 25 ppm; (c) Zn 50 ppm + Fe 50 ppm.

Table 1: Stomatal opening of Mung bean under different heavy metal treatments (N=30 for each treatment)

Treatment	Stomatal opening (µm)									
	Leaf					Stem				
	R1	R2	R3	Avg	SD	R1	R2	R3	Avg	SD
Control	4.88	3.50	3.64	4.01	0.76	3.83	4.91	4.96	4.57	0.64
Zn(25ppm) +Fe(25ppm)	2.87	4.91	1.82	3.20	1.57	3.17	3.17	4.19	3.51	0.59
Zn(50ppm) +Fe(50ppm)	3.35	4.07	2.73	3.38	0.67	4.15	6.11	7.89	6.05	1.87

Table 2: Length of mung bean under different heavy metal treatments (N=30 for each treatment)

Mung bean Length (cm)	Treatment								
	Control			Zn (25 ppm) + Fe (25 ppm)			Zn (50 ppm) +Fe (50 ppm)		
	R1	R2	R3	R1	R2	R3	R1	R2	R3
Individual length	7.00	12.50	15.00	14.00	3.80	13.80	4.80	12.80	9.90
	15.50	15.00	13.00	12.40	10.30	12.40	10.60	14.40	8.10
	15.00	15.50	11.00	12.50	12.10	11.20	9.50	12.70	10.10
	15.00	15.80	7.00	11.40	9.60	14.50	9.70	14.30	12.80
	12.60	10.40	8.70	12.30	13.10	13.60	8.60	0.00	11.00
	10.20	3.00	8.10	0.00	11.60	2.20	12.40	0.00	12.90
	16.60	2.00	3.50	0.00	14.50	0.00	9.60	0.00	11.90
	0.00	2.20	3.00	0.00	0.00	0.00	0.00	0.00	10.30
	0.00	1.80	0.00	0.00	0.00	0.00	0.00	0.00	2.80
	0.00	2.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Average length (replicate)	9.19	8.04	6.93	6.26	7.50	6.77	6.52	5.42	8.98
Average length ± SD (cm)	8.05 ± 1.13			6.84 ± 0.62			6.97 ± 1.82		

Table 3: Leaf count of mung bean under different heavy metal treatments (N=30 for each treatment)

Number of leaves	Treatment								
	Control			Zn (25 ppm) +Fe(25ppm)			Zn (50 ppm) +Fe (50 ppm)		
	R1	R2	R3	R1	R2	R3	R1	R2	R3
Leaves counts	14	20	16	10	14	12	14	8	18
Total	50			36			40		
Average leaves count ± SD	16.67 ± 3.06			12.00 ±2.00			13.33 ± 5.03		

Table 4: Total biomass of mung bean under different heavy metal treatments (N=30 for each treatment)

Treatment	Biomass(g)	Mean	Standard deviation (SD)	Biomass ± SD (g)
Control	8.86	0.27	3.07	6.86 ± 3.07
Zn (25 ppm) + Fe(25ppm)	5.30	0.29	2.60	5.30± 2.60
Zn (50 ppm) + Fe(50ppm)	5.14	0.26	2.64	5.14 ± 2.64

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