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PHYTOMORPHOLOGY

Biochemical response of *Glycine max* (L.) Merr. to zinc stress

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Abstract

A pot experiment was conducted to investigate the impact of varying concentrations of Zinc Sulphate ($ZnSO_4$ $7H_2O$) in soil on selected biochemical parameters in soybean (*Glycine max* (L.). Observations were recorded at pre-flowering (30 days), peak-flowering (45 days), and post-flowering (60 days) stages, with the experiment replicated three times. The highest levels of chlorophyll-a, chlorophyll-b, total chlorophyll, carbohydrate, lipid, and protein content were observed at zinc @500 mg/kg of soil at pre-, peak- and post-flowering stages, respectively. However, increase in the rate of application of zinc resulted in significant reductions in these parameters across all growth stages compared to the control. Proline considered as a strong indicator of environmental stress showed a gradual increase with increase in metal concentration in soil as compared to the control. The findings indicate that lower zinc levels (@250 and @500 mg/kg of soil) led to significant enhancements in plant biochemical parameters, with peak values observed at 500 mg/kg. However, caution is advised to prevent zinc concentrations exceeding 750 mg/kg of soil to mitigate potential phytotoxicity or nutrient imbalances, particularly in soybean cultivation areas. These results underscore the importance of implementing prompt measures to address zinc pollution and ensure optimal productivity of food crops.

Keywords: Heavy metal, Zinc, Chlorophyll, Carbohydrate, Lipid, Protein, Proline

Introduction

Soybean hailed as the "miracle crop", is globally renowned for its significant contributions as the primary source of vegetable oil and protein. It contains approximately 20% oil and 40% protein, far surpassing other staple crops like rice, wheat, maize, and pulses in protein content (Gupta et al., 2017). However, soybean cultivation faces challenges in regions with high temperatures, intense solar radiation, high evaporation rates, and drought conditions, severely impacting productivity. Additionally, agricultural soils are increasingly contaminated by industrial effluents containing toxic heavy metals, posing a significant new challenge. Plants, including soybeans, are susceptible to both

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deficiency and excess heavy metals, which can accumulate in soils and limit crop growth (Abirami & Vikrant, 2023). Zinc, a typical heavy metal found in agricultural soils due to waste contamination, is essential for various plant functions but can become toxic at high concentrations, reducing root and shoot growth and decreasing crop yields (Gupta and Meena, 2024). Given the urgent need to increase crop productivity to meet the growing global population, this study aims to assess how zinc affects the biochemical parameters of *Glycine max* (L.) Merr.

Material and methods

In the present investigation, zinc sulphate ($ZnSO_4$ $7H_2O$) is used for treatment with various concentrations ranging from 0 (control), 250, 500, 750, 1000, and 1250 mg/kg of soil. Certified seeds of the soybean variety JS-95-60 were procured from Agriculture Station, Kota, Rajasthan.

Pot experiment

The experiment was conducted in April at the University of Rajasthan's Botany Department greenhouse. Pots, 30 cm tall and 25 cm in diameter, filled with 4 kg of garden soil, were randomly placed to mitigate environmental variations. Zinc sulfate was applied at concentrations of 250, 500, 750, 1000, and 1250 mg/kg of soil, with untreated pots as controls. Soybean seeds, sterilized with 0.1% $HgCl_2$and rinsed with distilled water, were sown at 2 cm depth in each pot. Consistent plant numbers were maintained with alternate-day watering. Each treatment was replicated thrice across pre-flowering (30 days), peak-flowering (45 days), and post-flowering (60 days) stages to ensure robust data collection on biochemical parameters.

Biochemical analysis

Chlorophyll quantification

Chlorophyll was extracted and quantified according to Arnon's method (1949). Fresh leaves (1 g) from each treatment were macerated in 80% acetone and centrifuged at 2000 rpm for 10 minutes. The supernatant was diluted to 100 ml with 80% acetone in a volumetric flask. The amount of chlorophyll 'a' and 'b' were quantified by measuring the optical density at 663 nm and 645 nm wavelengths using a UV-VIS spectrophotometer. Pigment contents were calculated as mg/g FW (fresh weight).

Carbohydrate estimation

The standard Anthrone method (Yemm & Willis, 1954) was employed for carbohydrate estimation. Samples (0.1 g) were hydrolyzed with 5 ml 2.5N HCl in boiling water for 3 hours, neutralized with solid sodium carbonate, and centrifuged. Supernatants were collected, and aliquots (0.5 and 1.0 ml) were made up to 1 ml with distilled water. Anthrone reagent (4 ml) was added, and tubes were heated for 8 minutes and then cooled. Absorbance at 630 nm was measured to calculate carbohydrate content (mg/g FW) using a glucose standard curve.

Lipid determination

Lipid content was determined using the method described by Jayaram (1981). One gram of dried sample was macerated with 10 ml distilled water and transferred to a conical flask with 30 ml chloroform: methanol (2:1, v/v). After overnight extraction at room temperature in the dark, 20 ml chloroform was added, followed by centrifugation. The transparent lower chloroform layer, containing lipids was collected in pre-

weighed glass vials. After solvent evaporation, samples were weighed. Lipids were expressed as the total lipid/ gm of the dried sample.

Protein estimation

Protein was quantified according to Lowry (1951) method. Samples were homogenized in 0.1M phosphate buffer (pH=6.8). After adding Folin-Ciocalteau reagent, absorbance was recorded at 660 nm, and protein was estimated using a Bovine Serum Albumin (BSA) standard curve.

Proline determination

Proline content was assessed using the acid ninhydrin method (Bates et al., 1973). Fresh plant material (0.5 gm) was homogenized in 10 ml of 3% sulfosalicylic acid, filtered, and mixed with 2 ml each of acid ninhydrin and glacial acetic acid. The mixture was heated, cooled, and extracted with toluene. The absorbance of the toluene phase was measured at 520 nm. Proline concentration was calculated using a standard curve of L-proline and as expressed in mg/gm proline.

Statistical analysis

Statistical analysis employed SPSS ver. 25.0 and Microsoft Office Excel 2016. All parameters studied were expressed as mean ± standard error (S.E.) The data was analyzed by analysis of variance (ANOVA) to determine the statistical significance of the differences between means of treatments.

Results

Chlorophyll-a

At the pre-flowering stage, chlorophyll-a was 0.8290 mg under the control treatment. It increased under zinc 250 mg/ kg (0.8486 mg) and 500 mg/kg (0.9198 mg) treatments in comparison to control but decreased with the increasing concentration of zinc (750-1250 mg/kg of soil). Minimum chlorophyll-a at 1250 mg/ kg zinc treatment was 0.6853 mg, a 17% decrease compared to control.

At the peak-flowering stage, the chlorophyll-a was 0.8445 mg under the control treatment. It increased under zinc 250 mg/kg (0.8625 mg) and 500 mg/kg (0.9009 mg) treatments in comparison to control but decreased with the increasing concentration of zinc (750-1250 mg/kg). Minimum chlorophyll-a at 1250 mg/kg zinc treatment was 0.7306 mg, a 13% decrease compared to the control.

At the post-flowering stage, chlorophyll-a was 0.8027 mg under the control treatment. It increased at zinc 250 mg/kg (0.8182 mg) and 500 mg/kg (0.8522 mg) treatments in comparison to control but decreased with the increasing concentration of zinc (750-1250 mg/kg). The minimum chlorophyll-a at 1250 mg/ kg zinc treatment was 0.5374 mg, a 33% decrease compared to control (Table 1).

Chlorophyll-b

At the pre-flowering stage, chlorophyll-b was 1.0615 mg under control treatment. It increased under zinc 250 mg/kg (0.9470 mg) and 500 mg/kg (1.0544 mg) treatments in comparison to control but decreased with the increasing concentration of zinc (750-1250 mg/kg). The minimum chlorophyll-b at 1250 mg/kg zinc treatment was 0.8472 mg, a 20% decrease compared to control.

The chlorophyll-b was 1.0953 mg under the control treatment at the peak-flowering stage. It increased under 250 mg/kg (1.0974 mg) and 500 mg/kg (1.1840

mg) zinc treatments in comparison to control but decreased with the increasing concentration of zinc (750-1250 mg/kg). The minimum chlorophyll-b at 1250 mg/kg zinc treatment was 0.9379 mg, a 14% decrease compared to control.

At the post-flowering stage, chlorophyll-b was 1.0247 mg under the control treatment. It increased at zinc 250 mg/kg (1.0612 mg) and 500 mg/kg (1.123 mg) treatments in comparison to control but decreased with the increasing concentration of zinc (750-1250 mg/kg). The minimum chlorophyll-b at 1250 mg/kg zinc treatment was 0.6169 mg, a 40% decrease compared to control (Table 1).

Total chlorophyll (a+b)

At the pre-flowering stage, total chlorophyll was 1.8905 mg under control treatment. It increased under 250 mg/kg (1.7956 mg) and 500 mg/kg (1.9742 mg) zinc treatments in comparison to control but decreased with the increasing concentration of zinc (750-1250 mg/kg of soil). The minimum total chlorophyll at 1250 mg/kg zinc treatment was 1.5325mg, a 20% decrease compared to control.

At the peak-flowering stage, total chlorophyll was 1.9398 mg under the control treatment. It increased under zinc 250 mg/kg (1.9599 mg) and 500 mg/kg (2.0849 mg) treatments in comparison to control but decreased with the increasing concentration of zinc (750-1250 mg/kg). The minimum total chlorophyll at 1250 mg/kg zinc treatment was 1.6685 mg, a 14 % decrease compared to control.

At the post-flowering stage, total chlorophyll was 1.8301 mg under the control treatment. It increased at 250 mg/kg (1.8794 mg) and 500 mg/kg (1.9752 mg) zinc treatments in comparison to control but decreased with the increasing concentration of zinc (750-1250 mg/kg).

Table 1. Impact of zinc on chlorophyll (mg/gm) in *Glycine max* at different stages of plant growth

Treatment	Pre-flowering stage			Peak-flowering stage			Post-flowering stage		
	Chl-a	Chl-b	Total Chl	Chl-a	Chl-b	Total Chl	Chl-a	Chl-b	Total Chl
Control	0.8290±0.012	1.0615±0.026	1.8905±0.043	0.8445±0.034	1.0953±0.036	1.9398±0.023	0.8027±0.023	1.0274±0.032	1.8301±0.023
250 mg/kg	0.8486±0.023^{a}	0.9470±0.032^{b}	1.7956±0.023^{b}	0.8625±0.032^{a}	1.0974±0.033^{a}	1.9599±0.026^{b}	0.8182±0.013^{a}	1.0612±0.032^{c}	1.8794±0.034^{b}
500 mg/kg	0.9198±0.037^{b}	1.0544±0.018^{c}	1.9742±0.012^{b}	0.9009±0.024^{b}	1.1840±0.021^{b}	2.0849±0.030^{a}	0.8522±0.018^{b}	1.123±0.023^{c}	1.9752±0.023^{b}
750 mg/kg	0.8410±0.026^{c}	.9082±0.042^{c}	1.7492±0.020^{c}	0.8417±0.045^{c}	.9846±0.024^{c}	1.8263±0.029^{a}	0.7715±0.054^{c}	.8373±0.017^{b}	1.6088±0.026^{c}
1000 mg/kg	0.8064±0.045^{c}	.8924±0.023^{c}	1.6988±0.026^{c}	0.8284±0.023^{b}	.9741±0.041^{c}	1.8025±0.023^{b}	0.6685±0.045^{b}	.7220±0.056^{c}	1.3905±0.037^{c}
1250 mg/kg	0.6853±0.023^{c}	.8472±0.012^{c}	1.5325±0.037^{c}	0.7306±0.032^{c}	.9379±0.016^{c}	1.6685±0.026^{c}	0.5374±0.038^{c}	.6169±0.027^{c}	1.1543±0.059^{c}

Values were expressed as mean± SEM, Significance level: $^{a}p \leq 0.1$, $^{b}p \leq 0.05$, $^{c}p \leq 0.01$

The minimum total chlorophyll at 1250 mg/kg zinc treatment was 1.1543 mg, a 40% decrease compared to control (Table 1).

Carbohydrate

At the pre-flowering stage, carbohydrate was 41.56 mg under the control treatment. It increased under 250 mg/kg (41.89 mg) and 500 mg/kg (43.23 mg) zinc treatments in comparison to control but decreased with the increasing concentration of zinc (750-1250 mg/kg of soil). The minimum carbohydrate at 1250 mg/kg zinc treatment was 27.36 mg, a 34% decrease compared to the control.

At the peak-flowering stage, carbohydrate was 60.22 mg under the control treatment. It increased under 250 mg/kg (60.75 mg) and 500 mg/kg (63.03 mg) zinc treatments in comparison to control but decreased with the increasing concentration of zinc (750-1250 mg/kg). The minimum carbohydrate at 1250 mg/kg zinc treatment was 40.00 mg, a 34% decrease compared to the control.

At the post-flowering stage, carbohydrate was 57.04 mg under the control treatment. It increased at 250 mg/kg (57.89 mg) and 500 mg/kg (60.59 mg) zinc treatments in comparison to control but decreased with the increasing concentration of zinc (750-1250 mg/kg). The minimum carbohydrate at 1250 mg/kg zinc treatment was 33.10 mg, a 42% decrease compared to control (Table 2).

Table 2. Impact of zinc on Carbohydrate (mg/gm) in *Glycine max* at different stages of plant growth

Treatment	Pre-flowering stage	Peak-flowering stage	Post-flowering stage
Control	41.56±0.46	60.22±0.38	57.04±0.51
250 mg/kg	41.89±0.64 [a]	60.75±0.53 [a]	57.89±0.46 [a]
500 mg/kg	43.23±0.82 [a]	63.03±0.24 [a]	60.59±0.61 [b]
750 mg/kg	33.68±0.47 [c]	54.62±0.40 [c]	49.26±0.39 [c]
1000 mg/kg	29.57±0.64 [c]	46.04±0.58 [c]	42.12±0.40 [c]
1250 mg/kg	27.36±0.31 [c]	40.04±0.56 [c]	41.1±0.61 [c]

Values were expressed as mean± SEM, Significance level: [a]$p \leq 0.1$, [b]$p \leq 0.05$, [c]$p \leq 0.01$

Table 3. Impact of zinc on lipid (mg/gm) in *Glycine max* at different stages of plant growth

Treatment	Pre-flowering stage	Peak-flowering stage	Post-flowering stage
Control	45.30±0.57	46.48±0.48	44.67±0.41
250 mg/kg	45.77±0.49 [a]	46.81±0.49 [a]	46.09±0.39 [a]
500 mg/kg	46.81±0.58 [a]	47.05±0.39 [a]	47.62±0.50 [c]
750 mg/kg	42.94±0.48 [b]	43.76±0.48 [b]	42.54±0.38 [b]
1000 mg/kg	39.50±0.28 [b]	40.71±0.49 [c]	38.31±0.36 [c]
1250 mg/kg	35.28±0.37 [c]	36.98±0.98 [c]	38.47±0.53 [c]

Values were expressed as mean± SEM, Significance level: [a]$p \leq 0.1$, [b]$p \leq 0.05$, [c]$p \leq 0.01$

Lipid

At the pre-flowering stage, lipid was 45.30 mg under the control treatment. It increased under 250 mg/kg (45.77 mg) and 500 mg/kg (46.81 mg) zinc treatments in comparison to control but decreased with the increasing concentration of zinc (750-1250 mg/kg). The minimum lipid at 1250 mg/kg zinc treatment was 35.28 mg, a 22% decrease compared to control.

At the peak-flowering stage, lipid was 46.48 mg under the control treatment. It increased under 250 mg/kg (46.81 mg) and 500 mg/kg (47.05 mg) zinc treatments in comparison to control but decreased with the increasing concentration of zinc (750-1250 mg/kg). The minimum lipid at 1250 mg/kg zinc treatment was 36.98 mg, a 20% decrease compared to control.

At the post-flowering stage, lipid was 44.67 mg under the control treatment. It increased at 250 mg/kg (46.09 mg) and 500 mg/kg (47.62 mg) zinc treatments in comparison to control but decreased with the increasing concentration of zinc (750-1250 mg/kg). The minimum lipid at 1250 mg/kg zinc treatment was 34.47 mg, a 23% decrease compared to control (Table 3).

Protein

At the pre-flowering stage, the protein was 9.6512 mg under the control treatment. It increased under 250 mg/kg (10.223 mg) and 500 mg/kg (12.336 mg) zinc treatments in comparison to control but decreased with the increasing concentration of zinc (750-1250 mg/kg of soil). The minimum protein at 1250 mg/kg zinc treatment was 3.220 mg, a 66% decrease compared to control.

The protein was 12.332 mg under the control treatment at the peak-flowering stage. It increased under 250 mg/kg (12.853 mg) and 500 mg/kg (13.894 mg) zinc treatments in comparison to control but decreased with the increasing concentration of zinc (750-1250 mg/kg). The minimum protein at 1250 mg/kg zinc treatment was 5.979 mg, a 51% decrease compared to control.

The protein was 10.509 mg under the control treatment at the post-flowering stage. It increased at 250 mg/kg (11.550 mg) and 500 mg/kg (12.488 mg) zinc treatments in comparison to control but decreased with the increasing concentration of zinc (750-1250 mg/kg). The minimum protein at 1250 mg/kg zinc treatment was 5.199 mg, a 50% decrease compared to control (Table 4).

Proline

At the pre-flowering stage, proline was 5.144 µmoles under the control treatment. It slightly increased under 250 mg/kg (6.602 µmoles) and 500 mg/kg (9.065 µmoles) zinc treatments in comparison to control but highly increased with the increasing concentration of zinc (750-1250 mg/kg of soil). The maximum proline was observed at 1250 mg/kg zinc treatment (28.490 µmoles).

At the peak-flowering stage, proline was 7.879 µmoles under the control treatment. It slightly increased under 250 mg/kg (8.791 µmoles) and 500 mg/kg (14.263 µmoles) zinc treatments in comparison to control but highly increased with the increasing concentration of zinc (750-1250 mg/kg). The maximum proline was observed at 1250 mg/kg zinc treatment (30.862 µmoles).

At the post-flowering stage, proline was 10.1596 µmoles under the control treatment. It slightly increased at 250 mg/kg (11.071 µmoles) and 500 mg/kg (17.911 µmoles) zinc treatments in comparison to control but highly increased with the increasing concentration of zinc (750-1250 mg/kg). The maximum proline was

Table 4. Impact of zinc on protein (mg/gm) in *Glycine max* at different stages of plant growth

Treatment	Pre-flowering stage	Peak-flowering stage	Post-flowering stage
Control	9.651±0.37	12.332±0.42	10.509±0.44
250 mg/kg	10.223±035 [a]	12.853±0.47 [a]	11.550±0.38 [a]
500 mg/kg	12.336±0.37 [c]	13.894±0.48 [a]	12.448±0.39 [b]
750 mg/kg	6.083±0.44 [c]	9.442±0.59 [c]	8.192±0.41 [b]
1000 mg/kg	4.677±0.39 [c]	7.697±0.33 [c]	6.761±0.43 [c]
12500 mg/kg	3.220±0.35 [c]	5.979±0.44 [c]	5.199±0.38 [c]

Values were expressed as mean± SEM, Significance level: [a]$p \leq 0.1$, [b]$p \leq 0.05$, [c]$p \leq 0.01$

Table 5. Impact of zinc on proline (µmoles/gm) in *Glycine max* at different stages of plant growth

Treatment	Pre-flowering stage	Peak-flowering stage	Post-flowering stage
Control	5.144±0.42	7.879±0.47	10.159±0.69
250 mg/kg	6.602±0.48 [a]	8.791±0.45 [a]	11.071±0.41 [a]
500 mg/kg	9.065±0.44 [c]	14.263±0.33 [c]	17.911±0.42 [c]
750 mg/kg	18.641±0.42 [c]	21.833±0.48 [c]	23.383±0.38 [c]
1000 mg/kg	22.471±0.35 [c]	27.578±0.54 [c]	28.217±0.41 [c]
1250 mg/kg	28.490±0.58 [c]	30.862±0.36 [c]	33.506±0.42 [c]

Values were expressed as mean± SEM, Significance level: [a]$p \leq 0.1$, [b]$p \leq 0.05$, [c]$p \leq 0.01$

observed at 1250 mg/kg zinc treatment (33.506 µmoles) (Table 5).

Discussion

The current study demonstrates that lower concentrations of zinc (250 and 500 mg/kg) enhance pigment composition, carbohydrate, lipid, and protein content in soybean (*Glycine max*). This positive effect diminishes as zinc levels increase (750-1250 mg/kg), leading to a gradual decline in these biochemical parameters during the pre-, peak-, and post-flowering stages. The most significant reduction occurs at the highest concentration (1250 mg/kg), indicating a dose-dependent adverse impact on soybean growth. Gupta & Meena (2024) highlight zinc's role as a micronutrient beneficial for soybean growth up to 500 mg/kg of soil, emphasizing its moderating effect on pigment composition even under zinc stress conditions within this range. Beyond this threshold, increased zinc levels notably decrease chl-a, chl-b, and total chlorophyll, consistent with findings in other plant species like Cluster Bean (Manivasagaperumal et al., 2011) and *Triticum aestivum* (Kumar et al., 2012). Studies suggest that plant zinc exposure may induce iron deficiency, inhibit chlorophyll synthesis, or accelerate chlorophyll degradation through heightened chlorophyllase activity (Kazemi et al., 2010). This adversely affects photosynthesis, a critical process for plant biomass production, by

disrupting chlorophyll synthesis, photochemical enzyme activity, and plant water balance (Maxwell & Johnson, 2000). The results indicate a consistent decrease in carbohydrate content with increasing zinc levels. However, there was a notable positive effect on carbohydrate levels at 250 and 500 mg/kg of zinc concentrations. This finding aligns with previous studies on various plants, including Cluster Bean *(Cyamopsis tetragonoloba* (L.) Taub., (Manivasagaperumal et al., 2011), *Triticum aestivum* L. (Kumar et al., 2012), in tomato seedling (Saeed et al., 2013), Wheat leaves (Lanaras et al., 1993), lettuce plants (Farshian et al., 2007), *Phaseolus vulgaris* (Hamid et al., 2010) *Sesuvium portulacastrum* (Kalaikandhan et al., 2018) under zinc treatment. The decline in carbohydrate content at higher zinc levels may be attributed to zinc's involvement in enzymatic reactions associated with carbohydrate catabolism cycles (Rabie et al., 1992; Manivasagaperumal et al., 2011). This decrease in total carbohydrates corresponds to either inhibition of photosynthesis or increased respiration rates (Zengin & Kirbag, 2007). In the present study, low zinc levels showed a slight increase in lipid content over the control. This value indicates that zinc at a lower level had a significant stimulatory, beneficiary, and nutritional effect. The growth process beyond these levels has an adverse effect. It was also reported by Manivasagaperumal et al. (2011) in zinc treated Cluster Bean (*Cyamopsis tetragonoloba* (L.) Taub).

In the current study, protein content exhibited higher levels at lower zinc concentrations (500 mg/kg), with a subsequent decrease as zinc levels increased (750-1250 mg/kg). This trend is consistent with findings reported by previous studies (Zengin et al., 2007; Jayakumar et al., 2010; Manivasagaperumal et al., 2011), which observed similar patterns in different plant species such as *Triticum aestivum* (Kumar et al., 2012) and *Sesuvium portulacastrum* (Kalaikandhan et al., 2018). It might be due to binding of metals to the sulfhydryl protein group and causing deleterious effects in the standard protein form. It could be due to decreased protein synthesis or increased protein degradation (Balestrasse et al., 2003). Heavy metals are known to promote protein denaturation (Gadd & Griffth, 1978) and increase the activities of proteases, RNAase and DNAase enzymes (Lee et al., 1976). These findings underscore the sensitivity of plant protein metabolism to varying zinc levels, highlighting the need for careful management of zinc concentrations to support optimal protein synthesis and overall plant health. The results of our study indicate a progressive increase in proline content with higher concentrations of metals in the soil compared to the control group. This accumulation of proline may have mitigated the negative impacts of metal stress on soybean growth, thereby helping to maintain normal plant functioning.

These findings align with previous research that has shown increased proline concentrations under metal stress in various plant species, including *Sesuvium portulacastrum* (Kalaikandhan et al., 2018), *Vigna mungo* (Singh et al., 2012), tomato seedlings (Saeed et al., 2013), *Triticum aestivum* (Kumar et al., 2012), Cluster bean (Manivasagaperumal et al., 2011), *Brassica juncea* (John et al., 2009), *Brassica oleracea* var. botrytis (Theriappan et al., 2011), *Vigna radiata*, *Phaseolus vulgaris* cv Strike (Fikriye,

2006), and *Lemna minor* (Radic et al., 2010). Under stress conditions, proline serves multiple roles, such as nitrogen and energy source in plant metabolism. It also acts as a compatible solute, aiding in maintaining osmotic balance between the cytoplasm and vacuoles, thus supporting cellular function under stress (Asgharipour et al., 2011). The accumulation of proline in response to heavy metal stress underscores its importance as a protective mechanism in plants exposed to environmental challenges (Fatima et al., 2007). These consistent findings underscore the reliability of the observed trends and enhance our understanding of how zinc levels impact biochemical aspects in soybean plants. The accumulated evidence suggests that zinc concentrations beyond 500 mg/kg of soil may result in diminishing returns or negative impacts on plant growth. To avoid potential phytotoxicity or nutrient imbalances in soybean, it is recommended to refrain from exceeding 750 mg/kg of soil. This highlights the importance of implementing careful management practices to mitigate the adverse effects of heavy metal exposure in agricultural environments.

Conclusion

The study findings indicate that zinc imposition significantly influenced various biochemical parameters throughout different growth stages of *Glycine max* (L.) Merr. (soybean). Lower zinc concentrations (250 and 500 mg/kg) increased pigment composition, carbohydrate, lipid, and protein content. However, as zinc concentration increased (750-1250 mg/kg), these parameters gradually declined, with the most pronounced reductions observed at 1250 mg/kg. Lipid content was relatively less affected compared to other parameters. Chlorophyll, carbohydrate, and protein levels decreased under higher zinc exposure, while proline content in plant tissues increased, suggesting a stress response. The study recommends avoiding zinc concentrations exceeding 750 mg/kg in soil to mitigate potential phytotoxicity and nutrient imbalances in soybean. The highest concentration tested (1250 mg/kg) had the most adverse effects across all parameters studied. It underscores the importance of informing farmers about soil heavy metal levels. For future research, strategies focusing on enhancing enzymes that remove reactive oxygen species (ROS) and increasing antioxidant compounds are proposed to improve oxidative stress tolerance in plants exposed to heavy metal pollution on agricultural lands. These approaches could mitigate the detrimental effects observed in this study.

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