International Journal of Ecology and Environmental Sciences 50: 617-622, 2024
ISSN: 2320-5199 (Online); https://doi.org/10.55863/ijees.2024.0185

Impact of Zinc on Growth of Soybean (*Glycine max* L.)

SIDDHI GUPTA[1]* AND MANOJ KUMAR MEENA[2]
[1]Department of Botany, S.B.D. Government College, Sardarshahar, 331403, Rajasthan, India
[2]Department of Botany, University of Rajasthan, Jaipur, 302033, Rajasthan, India
E-mail: siddhigupta24@gmail.com, smartmann1989@gmail.com
***Corresponding author**

ABSTRACT

This study investigated the impact of five different concentrations of Zinc Sulphate ($ZnSO_4.7H_2O$), ranging from 0 (control), 250, 500, 750, 1000, 1250 mg/kg., on soybean (*Glycine max* (L.) Merr.) plant growth attributes like root and shoot length, as well as dry weight at pre, peak, and post-flowering stages. The experiments were set up in pots with three replications in green house of Department of Botany of University of Rajasthan, Jaipur during the month of April in natural outdoor conditions, where the photoperiod was 12 h and the average temperature was 30°C. The effect of Zinc on almost all the growth parameters under study was found to be statistically significant. The study revealed that at 500 mg/kg had maximum root length (56.04 cm), shoot length (74.06 cm), root dry weight (1.321 g) and shoot dry weight (5.106 g) at post flowering stage. In contrast, at 1250 mg/kg application resulted substantial reduction in root length (31.10 cm), shoot length (46.22 cm), root dry weight (0.519 g) and shoot dry weight (2.923 g) at post flowering stage as compared to control. The experiment finding showed that at low levels of zinc (250 and 500 mg/kg) showed a significant increase in growth parameter, with maximal growth observed at 500 mg/kg soil concentration. Considering this, a recommended zinc application rate of 0.75 kg/ha is suggested to optimize plant growth and nutrient uptake in agricultural fields. Furthermore, caution should be exercised to avoid soil concentrations surpassing 750 mg/kg to mitigate potential phytotoxicity or nutrient imbalances, particularly in soybean cultivation areas. These findings suggest that urgent measures are warranted to mitigate zinc pollution and ensure the productivity of food crops.

Key words: Heavy metal, Zinc, Root length, Shoot length, Root dry weight (RDW), Shoot dry weight (SDW), Soybean

INTRODUCTION

The escalating pace of anthropogenic activities has resulted in pervasive environmental contamination, exerting influence across diverse ecological dimensions. Among these contaminants, heavy metals (HMs) have become a focal point due to their persistent, bio-accumulative, and deleterious characteristics, significantly impinging upon plant yields (Repkina et al. 2023). Heavy metals can be classified in two groups which are essential and nonessential. Essential heavy metals such as copper (Cu), zinc (Zn), iron (Fe), molybdenum (Mo), nickel (Ni) and cobalt (Co) are required for normal physiological function of plants (redox reactions, electron transfer and structural functions in nucleic acid metabolism) at very low concentration. Zinc (essential heavy metal), despite its micronutrient status, manifests heightened effects at elevated concentrations and prolonged exposures. Zinc exposure triggers oxidative damage to cellular constituents, including lipids, proteins, and nucleic acids, leading to compromised host metabolism via the generation of reactive oxygen species (ROS) (Qadir et al. 2023). Manifestations of this exposure include chlorotic spots, necrotic lesions on leaf surfaces, leaf senescence, and inhibited growth (Gupta et al. 2016). Soybean, lauded as the Miracle crop for its pivotal role as a primary global source of vegetable oil and protein, encounters detrimental consequences on yield and nutritional quality due to heavy metal exposure. Recognizing the pressing imperative to augment crop productivity in response to the burgeoning global population, this study endeavors to appraise the effects of zinc on the growth parameters of *Glycine max* (L.) Merr. (soybean).

MATERIALS AND METHODS

Study area

The study area of Kota City, situated within the geographical coordinates of 24°32’02.17" to 25°51’19.33" North latitude and 75°36’55.19” to

76°34’57.10” East longitude in Rajasthan, India, boasts a semiarid climate (Köppen climate classification BSh) with an average annual rainfall of 660.6 mm. The region’s soil composition is primarily alluvial, characterized by deep to very deep soils ranging from clayey loam to clay, typically non-calcareous, and varying in color from brown to dark brown. Relative humidity is at its minimum during the summer months, notably in April and May, with morning levels ranging from 35 to 60% and afternoon levels from 10 to 30%. Soil samples were collected from the different area of Kota such as industrial area, agriculture field and human urban area for estimation of pH, organic carbon, Phosphate, Potassium, metals (Zn and Pb) concentration which ranged from 6.3 to 6.7, 5.46 to 9.82%, 119.2 to 152.8 kg/ha, 288 to 486 kg/ha, 188 to 354 ppm and 21 to 61 ppm, respectively. It provides essential information for understanding the soil fertility status, nutrient availability, and heavy metal concentrations in the region.

Plant material and chemicals

Certified seeds of soybean (*Glycine max* (L.) Merr.) produced by Vigor Biotech Pvt. Ltd., variety JS-95-60 were procured from Agriculture Station, Kota, Rajasthan. The seeds with uniform size, color and weight were chosen for the experimental purpose. Zinc sulphate ($ZnSO_4.7H_2O$) is used for the treatment purpose with various concentrations ranging from 0 (control), 250, 500, 750, 1000, 1250 mg/kg of soil.

Physico-chemical properties of water samples

Physico-chemical analysis of water samples collected from Department of Botany, University of Rajasthan, Jaipur was done for estimation of pH, electrical conductivity, chlorides, total dissolved solids, calcium hardness, magnesium hardness, total hardness and metal ion concentration.

Experimental setup

The experimental setup was established within the confines of the greenhouse facilities at the Department of Botany, University of Rajasthan, Jaipur, during the month of April, under ambient outdoor conditions. The natural photoperiod prevailing at the time was 12 hours, with an average temperature of 30°C. Each experimental unit consisted of pots measuring 30 cm in height and 25 cm in diameter, filled with four kgs of garden soil. To minimize bias and account for environmental variations inherent to the garden setting, pot placement was randomized. This strategic randomization aimed to attenuate potential impacts of factors such as differential sunlight exposure, temperature gradients, and soil composition heterogeneity. Five distinct concentrations of zinc, namely 250, 500, 750, 1000, and 1250 mg/kg of soil, were applied in the form of Zinc sulphate ($ZnSO_4$ $.7H_2O$). No additional nutrient supplements were introduced. Control pots devoid of zinc supplementation served as the baseline comparison. Prior to sowing, soybean seeds underwent surface sterilization using a 0.1% mercuric chloride ($HgCl_2$) solution for two minutes, followed by thorough rinsing with distilled water (DW). Subsequently, ten sterilized soybean seeds were evenly sown at a depth of 2 cm within each pot. Throughout the experimental period, efforts were made to maintain a consistent number of growing plants within each pot. Any observed deviations in plant count were meticulously documented and addressed to ensure uniformity across treatment groups. Watering was administered on alternate days to sustain optimal moisture levels for plant growth. Each experimental treatment comprised three replicates, thereby ensuring robustness and reliability of the data. This entailed the allocation of three sets of pots for each stage of the experimental timeline - pre-flowering (30 days), peak-flowering (45 days), and post-flowering (60 days) - within each treatment group. Consequently, every developmental stage was represented by its own set of replicates across all treatment conditions. For the assessment of growth parameters, plant harvesting was conducted at designated intervals corresponding to the pre, peak, and post-flowering stages, ensuring comprehensive data collection across the entire growth cycle.

Plant height

For plant height determination, plants of each concentration were carefully removed from pots keeping the root and shoot system intact. Shoot length was measured as length from the surface of soil to the tip of the highest leaf or flowering axis and in root length from the surface of the root tip in centimeter’s and mean values were calculated.

Root and shoot dry weight

For root and shoot dry weight determination, plants were carefully removed from pots keeping the root and shoot system intact. Plant roots were thoroughly washed with water to remove soil particles. Roots and shoots were separated and dried in the oven at 80°C for 48 hours to get constant dry weight. Six plants of each concentration were sampled and mean values were calculated. These weights were expressed in g/plant.

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.). In order to detect differences in the determined growth parameters of plants treated with different concentrations of heavy metals, analysis of variance (ANOVA) was performed. Significant differences between the mean values of variables of treatments were determined at 0.05 level.

RESULTS

The physico-chemical properties of water and soil samples used in the present study are presented in Tables 1, 2.

Root length

At pre-flowering stage, root length was 28.58 cm under control treatment. Root length increased under zinc 250 mg/kg (30.76 cm) and 500 mg/kg (35.02 cm) treatments in comparison to control but decreased with the increasing concentration of zinc (750-1250 mg/kg of soil). Minimum root length was observed at 1250 mg/kg zinc treatment 11.72 cm, a 59% decrease compared to control.

At the peak-flowering stage, the root length was 36.02 cm under the control treatment. It increased under zinc 250 mg/kg (41.12 cm) and 500 mg/kg (46.04 cm) treatments in comparison to control but decreased with the increasing concentration of zinc (750-1250 mg/kg). Minimum root length was observed at 1250 mg/kg zinc treatment 21.78 cm, a 40% decrease compared to control.

At the post-flowering stage, root length was 48.18 cm under the control treatment. It increased at zinc 250 mg/kg (54.12 cm) and 500 mg/kg (56.04 cm) treatments in comparison to control but decreased with the increasing concentration of zinc (750-1250 mg/kg). Minimum root length was observed at 1250 mg/kg zinc treatment 31.10 cm, a 35% decrease compared to control (Table 3).

Table 1. Physico-chemical properties of water samples

Soil properties	Values
pH	7.3±0.370
Electrical Conductivity (mmhos/cm)	1.15±0.477
Total solids (mg/l)	1082.2±120.3
Chloride (mg/l)	128.36±36.33
Total hardness (mg/l)	444±127.39
Calcium hardness (mg/l)	186±71.274
Zinc (Zn) (ppm)	0.006±0.002
Lead (Pb) (ppm)	0.00±0.001

Values were expressed as mean± SEM

Table 2. Physiochemical properties of garden soil

Soil properties	Values
pH	7.9±0.4
Electrical Conductivity (DS/m)	0.11±0.01
Organic carbon (%)	0.16±.02
Phosphate (Kg/ha)	24±1.1
Potash (Kg/ha)	290±5.5
Zinc (Zn) (ppm)	0.60±0.06
Iron (Fe) (ppm)	7.52±0.44
Copper (Cu) (ppm)	0.68±0.04
Maganese (Mn) (ppm)	6.56±0.70
Lead (Pb) (ppm)	0.0 +0.01

Values were expressed as mean± SEM

Shoot length

At pre-flowering stage, shoot length was 47.46 cm under control treatment. Shoot length increased under zinc 250 mg/kg (50.30 cm) and 500 mg/kg (58.62 cm) treatments in comparison to control but decreased with the increasing concentration of zinc (750-1250 mg/kg of soil). Minimum shoot length was observed at 1250 mg/kg zinc treatment 30.17 cm, a 36% decrease compared to control.

At peak-flowering stage, the shoot length was 55.94 cm under the control treatments. It increased under zinc 250 mg/kg (59.02 cm) and 500 mg/kg

Table 3. Impact of zinc on root and shoot length (cm) in *Glycine max*

Treatment	Pre-flowering		Peak-flowering		Post flowering	
	Root length	Shoot length	Root length	Shoot length	Root length	Shoot length
Control	28.58±0.43	47.46±0.54	36.02±0.44	55.94±0.82	48.18±0.78	67.02±0.62
250 (mg/kg)	30.76±0.54 ^{a}	50.3±0.59 ^{b}	41.12±0.42 ^{c}	59.02±0.49 ^{b}	54.12±0.56 ^{c}	70.76±0.69 ^{a}
500 (mg/kg)	35.02±0.46 ^{c}	58.62±0.71 ^{c}	46.04±0.52 ^{c}	62.56±0.70 ^{c}	56.04±0.59 ^{c}	74.06±0.50 ^{c}
750 (mg/kg)	22.19±0.51 ^{c}	42.48±0.60 ^{c}	35.36±0.61 ^{a}	56.78±0.78 ^{a}	43.30±0.68 ^{c}	62.54±0.67 ^{c}
1000 (mg/kg)	16.23±0.35 ^{c}	37.30±0.58 ^{c}	29.05±0.43 ^{c}	48.12±0.61 ^{c}	36.42±0.70 ^{c}	54.80±0.75 ^{c}
1250 (mg/kg)	11.72±0.35 ^{c}	30.17±0.72 ^{c}	21.78±0.68 ^{c}	35.56±0.70 ^{c}	31.10±0.66 ^{c}	46.22±0.76 ^{c}

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

(62.56 cm) treatments in comparison to control but decreased with the increasing concentration of zinc (750-1250 mg/kg). Minimum shoot length was observed at 1250 mg/kg zinc treatment 35.56 cm, a 36% decrease compared to control.

At the post-flowering stage, shoot length was 67.02 cm under control treatment. It increased at zinc 250 mg/kg (70.76 cm) and 500 mg/kg (74.06 cm) treatments in comparison to control but decreased with the increasing concentration of zinc (750-1250 mg/kg). Minimum shoot length was observed at 1250 mg/kg zinc treatment 46.22cm, a 31% decrease compared to control (Table 3).

Root dry weight (RDW)

At pre-flowering stage, root dry weight was 0.462 g under control condition. Root dry weight increased under zinc 250 mg/kg (0.495 g) and 500 mg/kg (0.527 g) treatments in comparison to control but decreased with the increasing concentration of zinc (750-1250 mg/kg of soil). Minimum root dry weight was observed at 1250 mg/kg zinc treatment 0.296 g, a 36% decrease compared to control.

At peak-flowering stage, root dry weight was 0.724 g at the control condition. Root dry weight increased under zinc 250 mg/kg (0.806 g) and 500 mg/kg (0.876 g) treatments in comparison to control but was decreased with the increasing concentration of zinc (750-1250 mg/kg). Minimum root weight was observed at 1250 mg/kg zinc treatment 0.448 g, a 38% decrease compared to control.

At post-flowering stage, root dry weight was 0.902 g at the control condition. Root dry weight increased under zinc 250 mg/kg (1.110 g) and 500 mg/kg (1.321 g) treatments in comparison to control but decreased with the increasing concentration of zinc (750-1250 mg/kg). Minimum root weight was observed at 1250 mg/kg zinc treatment 0.519 g, a 43% decrease compared to control (Table 4).

Shoot dry weight (SDW)

At the pre-flowering stage, shoot dry weight was 2.695 g under control treatment. It increased under zinc 250 mg/kg 2.961 g and 500 mg/kg 3.223 g treatments in comparison to control but decreased with the increasing concentration of zinc (750-1250 mg/kg of soil). Minimum shoot weight was observed at 1250 mg/kg zinc treatment 1.906 g, a 43% decrease compared to control.

At the peak-flowering stage, shoot dry weight was 4.476g under control treatment. It increased under zinc 250 mg/kg (4.691 g) and 500 mg/kg (5.221 g) treatments in comparison to control but decreased with the increasing concentration of zinc (750-1250 mg/kg). Minimum shoot weight was observed at 1250 mg/kg zinc treatment 2.419 g, a 46% decrease compared to control.

At the post-flowering stage, shoot dry weight was 4.741 g under control treatment. It increased under zinc 250 mg/kg (4.916 g) and 500 mg/kg (5.106 g) treatments in comparison to control but decreased with the increasing concentration of zinc (750-1250 mg/kg). Minimum shoot weight was observed at 1250 mg/kg zinc treatment 2.923 g, a 38% decrease compared to control (Table 4).

DISCUSSION

The findings of this study underscore the significance of growth parameters in assessing growth

Table 4. Impact of zinc on root and shoot weight (g) in *Glycine max*

Treatment	**Pre-flowering**		**Peak-flowering**		**Post flowering**	
	Root weight	**Shoot weight**	**Root weight**	**Shoot weight**	**Root weight**	**Shoot weight**
Control	0.462±0.023	2.695±0.086	0.724±0.032	4.476±0.085	0.902±0.025	4.741±0.046
250 (mg/kg)	0.495±0.016^{a}	2.961±0.092^{b}	0.806±0.028^{a}	4.691±0.010^{c}	1.110±0.011^{a}	4.916±0.055^{a}
500 (mg/kg)	0.527±0.021^{b}	3.223±0.063^{c}	0.874±0.016^{b}	5.221±0.065^{c}	1.321±0.014^{c}	5.106±0.066^{b}
750 (mg/kg)	0.410±0.023^{a}	2.816±0.081^{a}	0.671±0.026^{a}	3.912±0.0.1^{b}	0.758±0.021^{a}	4.124±0.066^{c}
1000 (mg/kg)	0.323±0.027^{a}	2.251±0.098^{b}	0.562±0.023^{c}	3.161±0.065^{c}	0.631±0.033^{a}	3.611±0.052^{c}
1250 (mg/kg)	0.296±0.029^{c}	1.906±0.054^{c}	0.448±0.18^{c}	2.419±0.01^{c}	0.519±0.030^{b}	2.923±0.058^{c}

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

performance and crop productivity. Notably, at lower concentrations of zinc (250 and 500 mg/kg), a substantial increase in growth parameters was observed. Marschner (2012) notes that zinc, as a micronutrient, is required in moderate amounts, for soybean generally having low to moderate needs. This suggests that lower concentration of zinc content may support normal growth. However, this positive trend was reversed with an increase in zinc concentration (750-1250 mg/kg), leading to a gradual decline in growth parameters across the pre, peak, and post-flowering stages of soybean (*Glycine max* (L.) Merr.) plants. The most pronounced reduction in these growth parameters was evident at the highest concentration of 1250 mg/kg, indicating a dose-dependent adverse effect on soybean growth. Heavy metal toxicity primarily affects root physiology, inhibiting root elongation (Woolhouse 1983). Zinc toxicity notably impacted root development, resulting in blunt and thickened roots, and restrained cell division and elongation (Barcelo and Poschenrieder, 1990). Sresty and Madhava Rao (1999) observed that radicle elongation suffered more than plumule extension, with extensive root cortical cell damage and significant nuclear changes in root tip cells treated with high zinc levels. Zinc toxicity can inhibit plant growth by causing chlorosis, reducing root development, and interfering with the uptake of other essential nutrients such as iron and manganese (Broadley et al. 2007). Elevated zinc levels can impose toxicity on plants, primarily by disrupting enzymatic activities and metabolic pathways crucial for growth and development. This observation aligns with previous studies conducted by various scientists. Atici et al. (2005) and Aydinalp and Marinova (2009) reported that in *Cicer arietinum* cv. Aziziye-94 and *Medicago sativa* Zn promoted the root and shoot growth. Similarly Manivasagaperumal et al. (2011) in Cluster Bean (*Cyamopsistetra gonoloba* (L.) Taub), Mahmoudi et al. (2021) in *Ocimum basilicum* L., Smaoui et al. (2023) in *Carthamus tinctorius* L. also reported sensitivity of growth attributes to varying concentrations of zinc. These consistent patterns across studies highlight the robustness of the observed phenomenon and strengthen the understanding of how zinc concentrations influence growth parameters in soybean plants. The collective body of evidence supports the conclusion that Zinc concentrations above 500 mg/kg of soil may lead to diminishing returns or adverse effects on plant growth. Therefore, it is advisable to avoid concentrations exceeding 750 mg/kg of soil to prevent potential phytotoxicity or nutrient imbalances of soybean, emphasizing the need for careful management strategies to mitigate the adverse effects of heavy metal exposure in agricultural settings. Based on a soil zinc concentration of 500 mg/kg, a recommended zinc application rate of 0.75 kg/ha is suggested to maintain or supplement soil zinc levels in agricultural fields, ensuring optimal plant growth and nutrient uptake.

CONCLUSIONS

Zinc is an essential element for plant growth because it is also necessary for chlorophyll synthesis, carbohydrate formation, membrane integrity and enzyme activation and biosynthesis of indole acetic acid. The current study's data indicate a concentration-dependent effect of zinc on critical growth parameters in soybean seedlings, including root length, shoot length, root weight, and shoot weight. The data reported in this study that zinc at a 500 mg/kg of soil had a significant stimulatory, beneficiary and nutritional effect. A recommended zinc application rate of 0.75 kg/ha is suggested to maintain or supplement soil zinc levels in agricultural fields, ensuring optimal plant growth and nutrient uptake. The growth process beyond these levels has an adverse effect. Therefore, it is advisable to avoid concentrations exceeding 750 mg/kg of soil to prevent potential phytotoxicity or nutrient imbalances of soybean. Notably, the highest zinc concentration (1250 mg/kg) manifests the most adverse impact on all examined parameters. It is imperative to disseminate information to farmers regarding their soil's heavy metal status. For future endeavors, two overarching strategies for engineering oxidative stress tolerance emerge. Firstly, elevating the levels of enzymes responsible for reactive oxygen species (ROS) removal is proposed. Secondly, augmenting the levels of antioxidant compounds capable of reacting with ROS presents a viable avenue. Implementing such strategies holds promise for alleviating the pollution induced by heavy metals on agricultural lands.

Authors' contributions: All authors contributed equally.

Conflict of interest: Authors declare no conflict of interest.

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Received: 7th February 2024
Accepted: 18th May 2024