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Foliar application of Zn-lysine enhances growth, biochemical traits, and shoot mineral ion composition in rice compared to zinc sulphate and L-lysine

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Abstract

Zinc sulphate, when used as a fertilizer, often causes low bioavailability, soil fixation, leaching, and environmental risks. These limitations can be mitigated by using zinc amino acid chelates, which offer superior plant absorption and environmental sustainability. This study aimed to evaluate the effects of foliar-applied zinc sulphate ($ZnSO_4$), zinc-lysine chelate (Zn-Lys), and lysine on the biochemical attributes and growth of rice (*Oryza sativa* L.). A pot trial was conducted using a completely randomized design with eight treatments (No Spray, Water Spray, 0.50% and 1% $ZnSO_4$, 0.50% and 1% Zn-Lys, and 0.50% and 1% L-Lysine). Compared to the control (no spray), 1% Zn-Lys significantly enhanced biochemical, growth, and mineral parameters, with the highest increases in zinc content (28.9%), flavonoids (23.8%), total chlorophyll (39.4%), and superoxide dismutase activity (23.9%). Additionally, it improved shoot calcium, potassium, and magnesium contents while effectively reducing oxidative stress markers, including malondialdehyde (MDA) and hydrogen peroxide (H_2O_2). Growth indicators such as root dry weight (5.6%) and shoot fresh weight (15.5%) were also maximized with Zn-Lys. In contrast, 1% $ZnSO_4$ moderately improved nutrient content and enzyme activity, while 1% Lysine had minimal or inconsistent effects. These findings highlight Zn-Lys as the most effective treatment for enhancing rice growth, biochemical attributes, and stress resilience.

Keywords: Zinc biofortification, Oryza sativa L., foliar nutrition, antioxidant defence, chelated minerals

1. Introduction

Zinc (Zn) deficiency is a major global concern, particularly in developing countries where it affects millions of people. As an essential micronutrient for human health, zinc plays a crucial role in immune function, growth, and development. A deficiency in zinc can lead to stunted growth, weakened immunity, and increased susceptibility to diseases [1]. In agriculture, zinc deficiency is a common issue that hampers crop productivity, particularly in rice, a staple food for billions of people worldwide. Rice cultivation is crucial to food security, yet zinc deficiency in soils is widespread, leading to reduced yields and poor nutritional quality of rice grains. The practice of biofortification, which involves increasing the zinc content in crops, has become an important strategy to address both human and agricultural zinc deficiency [1-2].

The current methods of addressing zinc deficiency in crops primarily involve the application of zinc fertilizers, such as zinc sulphate ($ZnSO_4$). However, the efficiency of these fertilizers is often limited by factors like soil pH, soil organic matter, and the availability of zinc for plant uptake. Moreover, the application of $ZnSO_4$ can sometimes result in environmental issues, such as the accumulation of excess zinc in the soil, which may lead to toxicity [1]. Therefore, alternative approaches, such as the use of zinc chelates, have been explored to improve zinc bioavailability to plants. Zinc amino acid chelates, like Zinc Lysine (Zn-Lys), are believed to enhance zinc uptake and transport within plants more efficiently than traditional $ZnSO_4$ [3].

Research on zinc chelates has demonstrated their potential in improving plant growth, nutrient uptake, and crop yield. For instance, studies have shown that the application of Zn-Lys chelates results in better growth

parameters, including plant height, biomass, and chlorophyll content, compared to $ZnSO_4$ [3]. Additionally, Zn-Lys has been found to improve the bioavailability and uptake of zinc, leading to enhanced photosynthesis and better overall plant health. Several studies on crops such as maize [3], wheat [4], and radish [5] have suggested that zinc chelates outperform $ZnSO_4$ in improving plant productivity by enhancing nutrient uptake, promoting efficient photosynthesis, and boosting grain yield. These findings highlight the importance of amino acid chelates as a promising alternative to conventional zinc fertilizers [3-5].

Despite these advances, the effectiveness of Zn-Lys as a foliar application in rice, specifically regarding growth enhancement, yield improvement, and nutrient uptake, remains underexplored. Most studies have focused on the seed priming application of zinc chelates [5], leaving a significant research gap in understanding the potential benefits of foliar Zn-Lys application in rice. Moreover, there is a need for a deeper mechanistic understanding of how zinc chelates interact with plant metabolic pathways to optimize growth and nutrient uptake.

In this context, the objective of the present study is to compare the effects of $ZnSO_4$, L-Lysine, and Zinc Lysine (Zn-Lys) on the growth, yield, and nutrient uptake of rice plants. We hypothesize that foliar application of Zn-Lys will result in better growth, higher yields, and improved nutrient uptake compared to $ZnSO_4$ and L-Lys alone. This hypothesis is based on previous research suggesting that amino acid chelates enhance the bioavailability of zinc, leading to improved plant health and productivity [6]. Furthermore, this study aims to investigate the potential benefits of Zn-Lys in rice biofortification, considering both economic and environmental aspects. By exploring the efficacy of Zn-Lys as a foliar treatment, we hope to contribute to the development of more sustainable and efficient approaches for improving rice productivity and addressing zinc deficiency in agricultural systems.

2. Materials and methods

2.1. Experimental conditions and treatments application

The pot trial was conducted in the research area of Government College University Faisalabad (31.4187° N, 73.0791° E). Forty pots were arranged in eight rows, with each row comprising five pots. Each pot was initially seeded with five rice plants, which were later thinned to three plants per pot, resulting in a total of 120 plants. The soil used for the experiment was analysed prior to planting. The soil had a pH of 7.6 (measured in a 1:2.5 soil-to-water suspension) and an electrical conductivity (EC) of 0.35 dS/m. The organic matter content was 0.72%, while macronutrient concentrations were as follows: nitrogen at 0.045%, phosphorus at 8.5 mg/kg (Olsen P), and potassium at 175 mg/kg [7].

The completely randomized design (CRD) was deemed suitable for this pot trial replicating previous trials of Shehzad et al. [4]. Each treatment was randomly assigned within rows to minimize bias. 1. No Spray (NS), 2. Water Spray (WS), 3. 0.50% $ZnSO_4$, 4. 1% $ZnSO_4$, 5. 0.50% Zn-Lys Chelate, 6. 1% Zn-Lys Chelate, 7. 0.50% L-Lysine, 8. 1% L-Lysine. Treatments were randomly assigned to pots within rows to avoid systematic errors or environmental gradients in the experimental area. The foliar treatments were prepared by diluting 3 mL/L of each material in distilled water and were applied using a hand sprayer. Applications were conducted between 8:00 and 10:00 AM at 10-day intervals throughout the experiment [6].

The Zn-Lys chelate used in the treatments was synthesized following the method described by Leu [8]. A total of 260 g of $ZnSO_4 \cdot 7H_2O$ was dissolved in 150 mL of distilled water. Subsequently, 146.12 g of L-lysine monohydrochloride were added to the solution, which was heated at 95°C for 3 hours. The resulting chelate solutions were diluted to concentrations of 0.50% and 1% for the respective treatments.

The climatic conditions in Faisalabad during the experimental period were monitored [4]. Daytime temperatures ranged between 30°C and 40°C, while night-time temperatures varied from 18°C to 26°C. Relative humidity fluctuated between 50% and 75%, with minimal rainfall and approximately 10–12 hours of sunlight daily. Sampling for various parameters was carried out at defined intervals. Growth measurements were recorded at crop maturity (120 days' post-germination), while photosynthetic parameters were measured at 60 days' post germination. Antioxidant enzyme activity and biochemical parameters were assessed 90 days' post germination. Shoot mineral content was determined at the harvest stage. The experiment lasted for a total of 120 days, from the time of germination to the final harvest. No additional fertilizers were administered during the experiment, ensuring that the effects observed were attributable solely to the applied treatments [9].

2.2 Biomass production

The biomass production, specifically the fresh and dry weights of roots and shoots, was recorded after 15 days from the final germination count of the seeds. The plants' root and shoot weights were measured individually. To determine the fresh weight, the plants were subjected to oven-drying at 70°C for 48 hours, and the resulting dry biomass was measured and recorded following Mazhar et al. [10].

2.3 Chlorophyll contents and carotenoid contents

The concentrations of total chlorophyll, chlorophyll a, chlorophyll b, and carotenoids were determined following the method of Lichten haler and Wellburn [11]. Fresh leaf samples (0.25 g) were collected from each treatment. The samples were ground in 80% acetone, and the homogenate was incubated in darkness at 4°C overnight to ensure complete pigment extraction. The extract was centrifuged at 10,000 × g for 10 minutes at 4°C. The clear supernatant was collected, and its absorbance was measured at 663 nm, 645 nm, and 480 nm using a UV-Vis spectrophotometer (Hitachi-U2001, Tokyo, Japan). The concentrations of chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll, and carotenoids were calculated using the following equations:

$$Chl\ a\ (mg/g\ FW) = 12.25 \times A\_663 - 2.79 \times A\_645$$

$$Chl\ b\ (mg/g\ \ FW) = 21.50 \times A\_645 - 5.10 \times A\_663$$

$$Total\ chlorophyll\ (mg/g\ \ FW) = 7.15 \times A\_663 + 18.71 \times A\_645$$

$$Carotenoids\ (mg/g\ \ FW) = ((1000 \times A\_480 - 1.82 \times Chl\ a - 85.02 \times Chl\ b))/198$$

The calculations were adjusted for the volume of acetone used and the fresh weight of the samples.

2.4. Determination of flavonoids and Tocopherol contents

The tocopherol content in various plant parts was determined following the method described by Baker and Davies [12] with some modifications. For each plant sample, 0.5 g of fresh ingredient was homogenized in a mixture of 10 mL petroleum ether and ethanol (2:1.6, v/v). The homogenate was then centrifuged at 10,000 × g for 20 minutes. Next, 1 mL of the supernatant was mixed well with 200 µL of 2% 2,2-dipyridyl in ethanol and kept in the dark for 5 minutes. Subsequently, 4 mL of d.d. H_2O was added to the mixture and thoroughly mixed. The resulting chromophore in the aqueous layer was measured at 520 nm. The tocopherol content was calculated using a standard curve generated with known concentrations of α-tocopherol. Leaf flavonoid ingredients were determined as described by Mazhar et al. [13].

2.5 Anthocyanin content

Fresh plant material weighing 50 mg was taken and mixed with 250 µL of acidic methanol (1% HCl, w/v). The ice plant material was homogenized and then incubated at 4°C for one hour with moderate shaking. After incubation, the suspended material was centrifuged at 14,000 rpm for 5 minutes at room temperature. The extracted material was measured for absorption at 530 nm and 657 nm using a photometer. The measurement of anthocyanin was determined using the following formula:

$$Q_{Anthocyanin} = \frac{(A_{530} - 0.25 \times A_{657})}{m}$$

Q is the correction factor used to determine the accurate absorption values related to the amount of anthocyanin at wavelengths A530 and A657. M represents the weight of the plant material used for extraction (g) [14].

2.6. Seedling antioxidant enzyme activities

2.6.1 Extraction of enzymes

For each treatment, 0.15 g of fresh plant material was homogenized in 10 mL of chilled 50 mM phosphate buffer (pH 7.8). The homogenate was then centrifuged for 20 minutes at 10,000 × g at 4°C. The resulting supernatant contained the and antioxidant enzyme activities [15].

2.6.2 Estimation of superoxide dismutase (SOD), and peroxidase (POD) activity

The functioning of superoxide dismutase (SOD) was determined using the method developed by Giannopolitis and Ries [16]. The process is based on the photochemical reduction of Nitro Blue Tetrazolium (NBT) at a wavelength of 560 nm. To assess SOD activity, an enzyme extract of 50 µL was mixed with a solution containing 50 µM NBT, 1.3 µM Vitamin B2 (riboflavin), 13 mM methionine, 75 mM EDTA, and 50 mM phosphate buffer (pH 7.8). The reaction was initiated by exposing the solution to a 30 W fluorescent light source within a chamber

coated with aluminum on its inner side. The lamp was turned on, and the reaction proceeded for 15 minutes before being stopped by turning off the light. The blue formazone resulting from the photo reduction of NBT was measured at 560 nm using a UV-visible spectrophotometer. A control sample without added enzymes was also included for comparison and to account for any non-enzymatic reactions. In the reaction mixture, the functioning of peroxidase (POD) was determined based on the complete oxidation of guaiacol. The measurements were conducted at a pH of 7.8. The POD solution contained 50 mM phosphate buffer, 20 mM guaiacol, 40 mM H_2O_2, and 100 units of enzyme extract. The reaction was initiated by adding guaiacol to the solution, and the changes in absorbance at 470 nm were monitored after 20 seconds. Total soluble sugar contents were measured using the Anthrone reagent method, as described by Ebell [17].

2.7. Malondialdehyde (MDA) and Hydrogen peroxide (H_2O_2) contents

The oxidative loss in membrane lipids (lipid peroxidation) was estimated by measuring the quantity of malondialdehyde (MDA) in the tissue, following the methodology as described by Cakmak and Horst [18] with slight modifications. Leaf samples weighing 1.0 g were homogenized in 3 mL of 0.1% (w/v) trichloroacetic acid (TCA) solution. The homogenate was then centrifuged at 20,000 × g for 15 minutes, and 0.5 mL of the supernatant was mixed with 3 mL of a 0.5% thiobarbituric acid (TBA) solution in 20% TCA. The mixture was heated in a water bath for 50 minutes at 95°C. To prevent the reaction from cooling, the tubes were kept in a cold water bath with water. Subsequently, the samples were centrifuged for 10 minutes at 10,000 × g and the absorbance was measured at 532 nm and 600 nm using a spectrophotometer. The concentration of MDA was calculated as the difference in absorption between 600 nm and 532 nm using the following formula:

MDA level (nmol) = Δ (A 532 nm-A 600 nm)/1.56×105

The absorption coefficient for calculating MDA is 156 mol/cm^3.

Fresh leaves weighing 0.15g were homogenized using a mortar and pestle, and then 5 mL of 0.1% (w/v) trichloroacetic acid (TCA) was added. The mixture underwent a centrifugation process for 10 minutes at 12,000 × g After centrifugation, 0.5 mL of the supernatant was mixed with 0.5 mL of potassium phosphate buffer and 1 mL of 1 M potassium iodide. The resulting mixture was vortexed, and the absorbance was measured at 390 nm using a spectrophotometer, following the methodology as described by Velikova et al. [19].

2.8. Determination of shoot minerals

Shoot minerals were evaluated using Mazhar et al. [10] protocols. The concentrations of Ca, K, Mg, and Zn were expressed as mg/g of dry weight, while nitrogen content was reported as a percentage of dry weight.

2.9. statistical analysis

The mean, standard error (SE), and least significant difference (LSD) at the 5% significance level were calculated and plotted using R-Studio (version R 4.4.1). The LSD test grouped treatments with statistically similar means by assigning them group letters. All statistical analyses and visualizations were executed in R-Studio (version R 4.4.1) using the following R packages: ggplot2 for bar chart plotting, “agricolae” for the LSD test, and “dplyr” for data manipulation [20].

3. Results

In terms of growth parameters, the treatments involving zinc-based compounds showed positive effects on several key indicators compared to lysine alone. Root dry weight (Figure 1(A)) and fresh weight (Figure 1(B)) with 1% Zn-Lys showed the highest increases in both, reflecting improved root growth. Additionally, shoot dry weight (Figure 1(C)), shoot fresh weight (Figure 1(D), shoot length (Figure 1(E)), and root length (Figure 1(F)), showed slight to moderate increases under the zinc-based treatments, with 1% Zn-Lys resulting in the highest improvements in root and shoot growth attributes (Table 1; Figure 1).

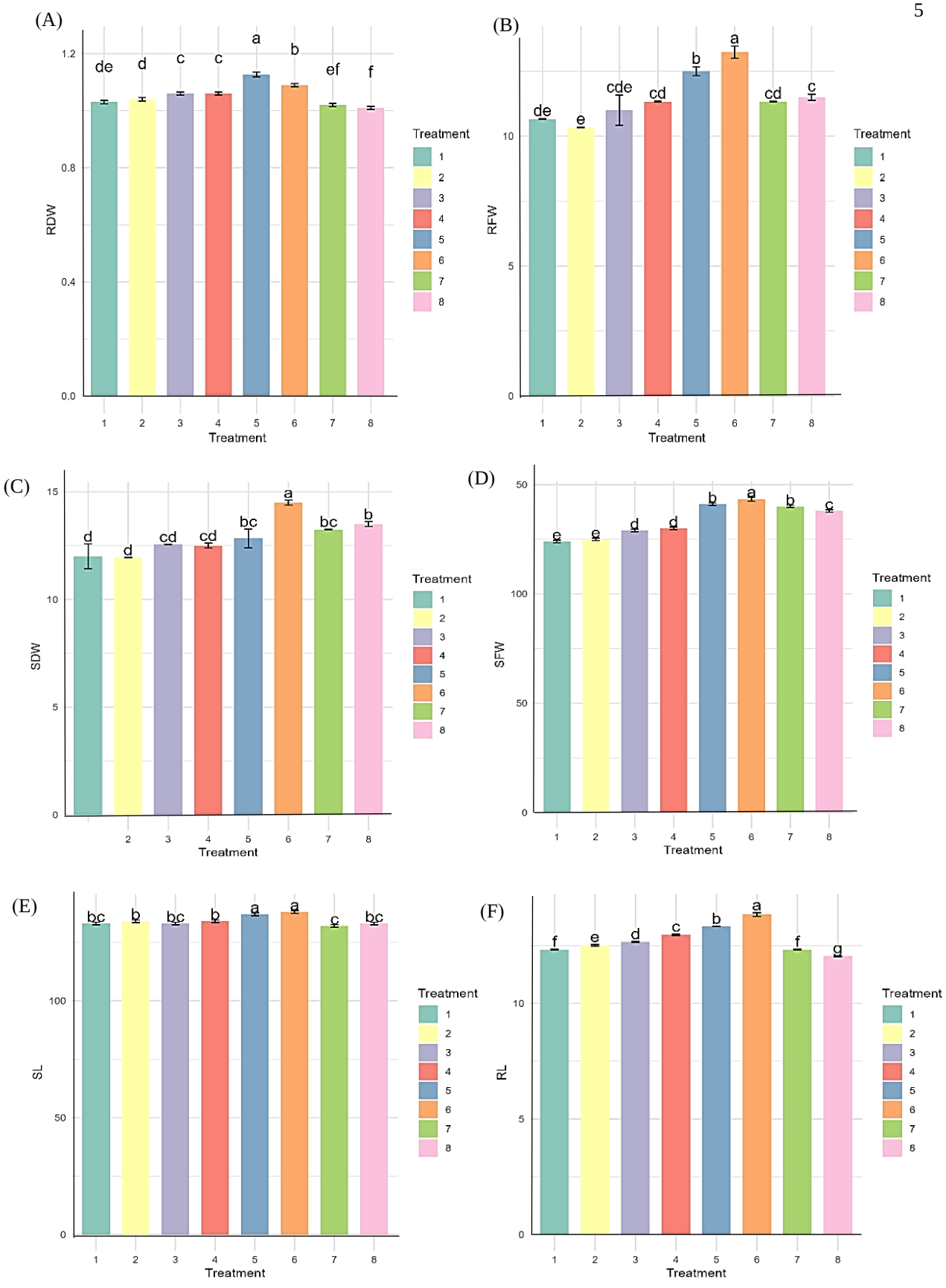

Figure 1 Bar charts (mean ± S.E.) of growth variables in rice plants as affected by various foliar treatments: (A) root dry weight, (B) root fresh weight, (C) shoot dry weight, (D) shoot fresh weight, (E) shoot length, and (F) root length. Treatments: 1 - No spray, 2 - Water spray, 3 - 0.5% $ZnSO_4$, 4 - 1% $ZnSO_4$, 5 - 0.5% Zn-Lys, 6 - 1% Zn-Lys, 7 - 0.5% Lys, 8 - 1% Lys. Bars with different letters indicate means that differ significantly at LSD 5%.

Regarding photosynthetic attributes total chlorophyll contents (Figure 2 (A)) exhibited significant increases with the 1% Zn-Lys treatment, showing the highest improvement of 39.42% compared to the control (NS). 1% $ZnSO_4$ and 1% Lys also showed notable increases in TC, with 15.51% and 21.23% improvements, respectively. The increase in chlorophyll content suggests a potential enhancement in photosynthetic capacity under these treatments. 1% $ZnSO_4$ and 1% Zn-Lys showed the highest increases in Chlorophyll A (Figure 2B) and Chlorophyll B (Figure 2C), with 1% Zn-Lys exhibiting the most significant increase in both chlorophyll types. This suggests that the 1% Zn-Lys treatment has a strong positive impact on the photosynthetic machinery of the plants. Carotenoids (Figure 2D) also saw improvements across treatments, with 1% Zn-Lys showing the highest increase (Figure 2; Table 1).

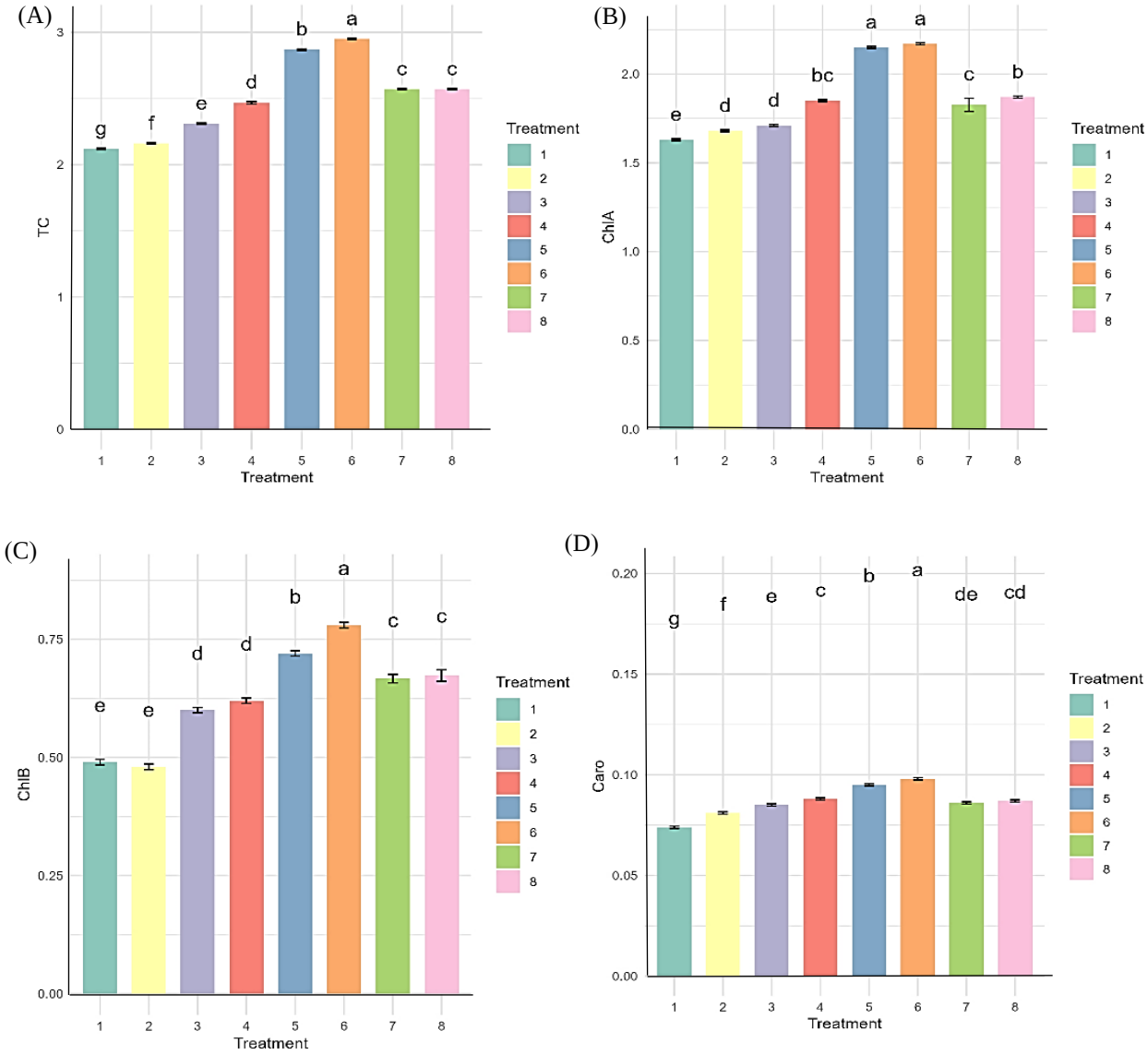

Figure 2 Bar charts (mean ± S.E.) of photosynthetic pigments and osmotic stress variables in rice plants as affected by various foliar treatments: (A) total chlorophyll, (B) chlorophyll A contents, (C) chlorophyll B contents, (D) carotenoids, (E) hydrogen peroxide, and (F) malondialdehyde levels. Treatments: 1 - No spray, 2 - Water spray, 3 - 0.5% $ZnSO_4$, 4 - 1% $ZnSO_4$, 5 - 0.5% Zn-Lys, 6 - 1% Zn-Lys, 7 - 0.5% Lys, 8 - 1% Lys. Bars with different letters indicate means that differ significantly at LSD 5%.

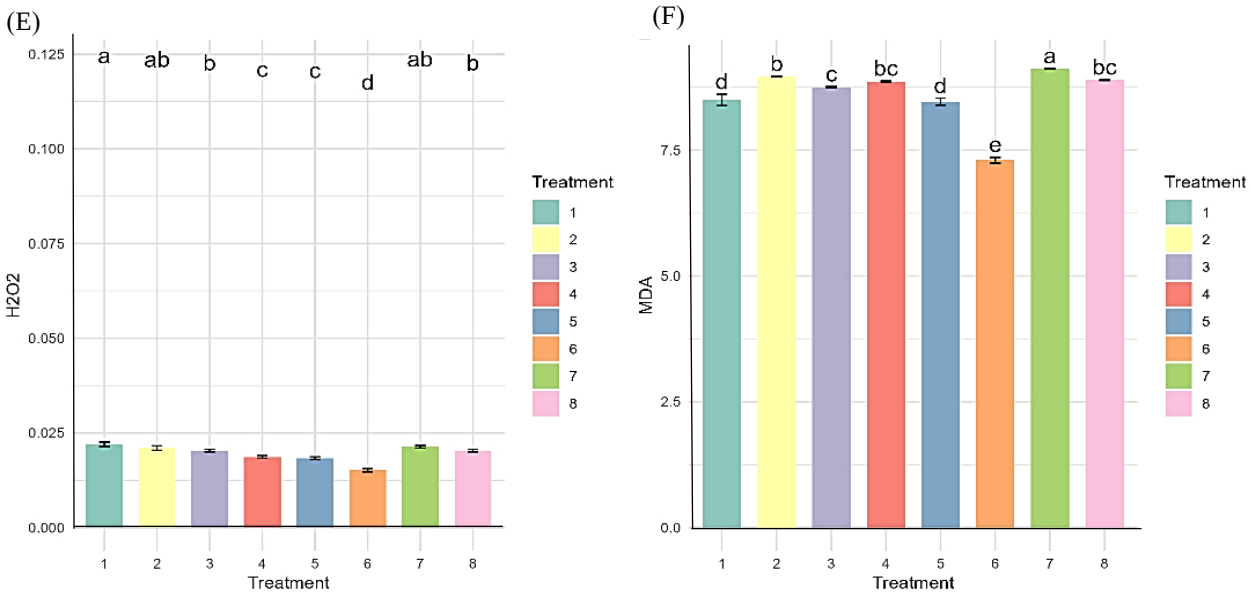

Figure 2 (Cont.) Bar charts (mean ± S.E.) of photosynthetic pigments and osmotic stress variables in rice plants as affected by various foliar treatments: (A) total chlorophyll, (B) chlorophyll A contents, (C) chlorophyll B contents, (D) carotenoids, (E) hydrogen peroxide, and (F) malondialdehyde levels. Treatments: 1 - No spray, 2 - Water spray, 3 - 0.5% $ZnSO_4$, 4 - 1% $ZnSO_4$, 5 - 0.5% Zn-Lys, 6 - 1% Zn-Lys, 7 - 0.5% Lys, 8 - 1% Lys. Bars with different letters indicate means that differ significantly at LSD 5%.

The 1% Zn-Lys treatment led to the most significant decrease in H_2O_2 (Figure 2E) and MDA levels (Figure 2F), with a reduction of 37.62% and 14.76% compared to the no spray. This is closely followed by 0.5% Zn-Lys, which showed a decrease of 30.57% and 10.65% reduction in both variables respectively. 1% $ZnSO_4$ also showed a considerable reduction in H_2O_2 (15.47%), followed by 0.5% Lys (16.67%). 1% Lys and 0.5% Lys also showed considerable reductions in MDA by 9.11% and 14.24%, respectively. The 1% $ZnSO_4$ and Water Spray (WS) treatments showed minimal decreases in MDA (Figure 2).

Table 1 Percentage increase for each variable by treatment (1% $ZnSO_4$, 1% Zn-Lys, and 1% Lys) compared to the control (NS).

Treatment	RDW	RFW	SDW	SFW	SL	RL	TC	Chl A	Chl B	Caro
1% $ZnSO_4$	3.8	6.2	4.1	4.8	0.7	5.1	15.5	13.4	26.7	18.9
1% Zn-Lys	5.6	23.6	20.8	15.5	3.7	8.4	39.4	33.1	44.4	28.3
1% Lys	-1.1	7.9	12.5	11.2	0	-0.8	21.2	14.5	37.4	17.3

RDW: root dry weight; RFW: root fresh weigh; SDW: shoot dry weight; SFW: shoot fresh weight; SL: shoot length; RL: root length; TC: total chlorophyll; Chl A: Chlorophyll A; Chl B: Chlorophyll B; Caro: Carotenoids

In terms of shoot biochemical attributes, 1% Zn-Lys consistently outperformed other treatments. It showed the highest increase in flavonoids by 23.8% (Figure 3A), total soluble sugars (Figure 3B) (2.43%), reducing sugars (Figure 3C) (35.84%), and non-reducing sugars (Figure 3D) (16.66%).

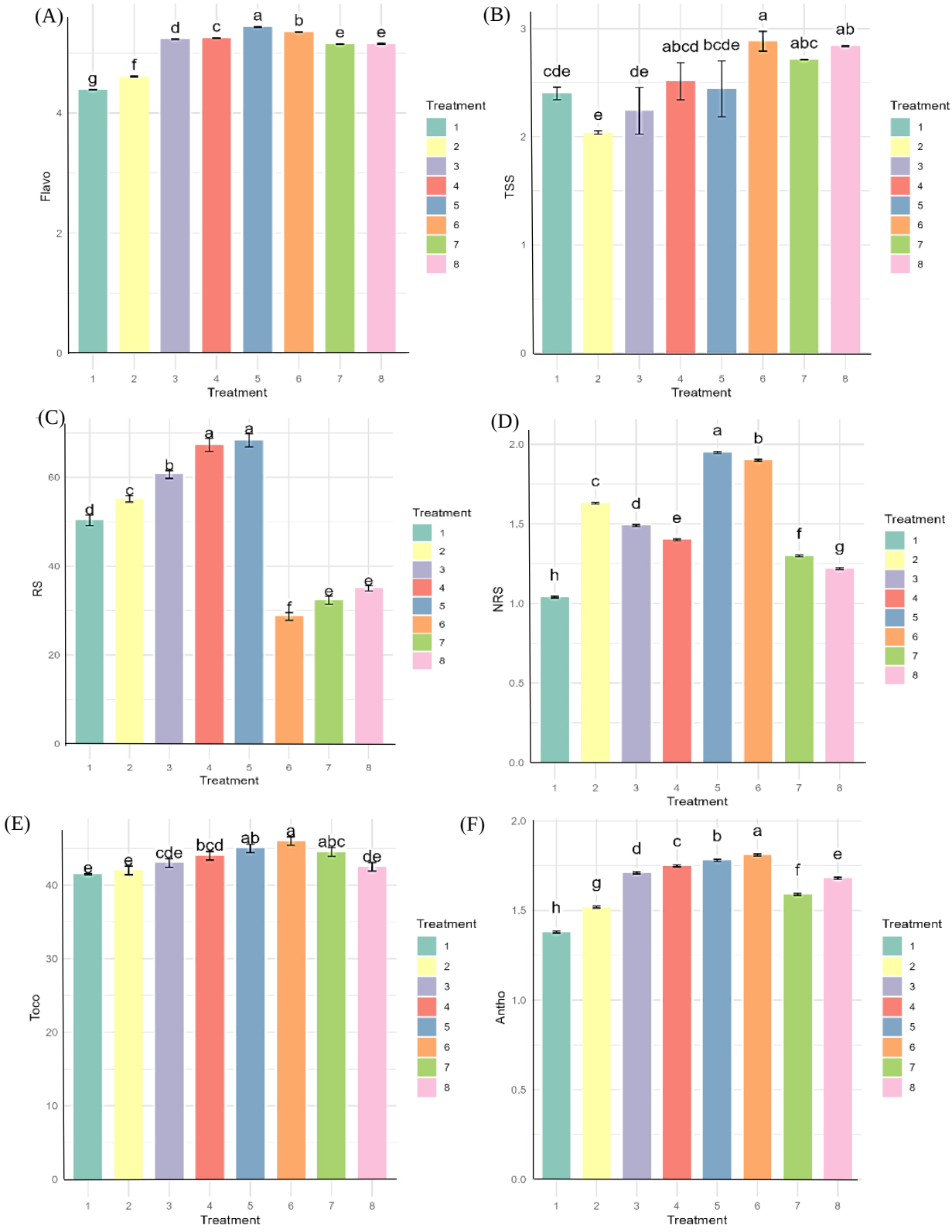

Figure 3 Bar charts (mean ± S.E.) of biochemical variables in rice plants as affected by various foliar treatments: (A) flavonoids contents, (B) total soluble sugars, (C) reducing sugars, (D) non reducing sugars, (E) tocopherol contents, and (F) anthocyanin contents. Treatments: 1 - No spray, 2 - Water spray, 3 - 0.5% $ZnSO_4$, 4 - 1% $ZnSO_4$, 5 - 0.5% Zn-Lys, 6 - 1% Zn-Lys, 7 - 0.5% Lys, 8 - 1% Lys. Bars with different letters indicate means that differ significantly at LSD 5%.

The increased sugar content under 1% Zn-Lys indicates a potential enhancement in the plant's ability to store energy and manage osmotic pressure, especially under stress conditions. Moreover, all the treatments showed significant increases in tocopherols (Figure 3(E)) and anthocyanin accumulation (Figure3F(F)), but the increase in these parameters was not as prominent with Lys treatments (Table 2).

Table 2 Percentage increase for each variable by treatment (1% $ZnSO_4$, 1% Zn-Lys, and 1% Lys) compared to the control (NS).

Treatments	TSS	RS	Toco	Antho	Flavo.	Ca	K	Mg	Zn	N	POD	SOD
1% $ZnSO_4$	4.8	33.8	6.8	0.9	19.5	7.9	3.6	19.1	0.9	10.7	5.7	16.4
1% Zn-Lys	2.4	35.8	14.4	6.1	23.8	14.5	5.7	28.9	7.1	16.6	9.2	23.9
1% Lys	5.5	23.5	9.5	1.6	17.1	2.9	3.8	16.6	4.3	16.6	4.7	29.6

TSS: total soluble sugars; RS: Reducing sugars; Toco: Tocopherol; Antho: Anthocyanin; Flavo: Flavonoids; Ca: Calcium; K: Potassium; Mg: Magnesium; Zn: Zinc; N: Nitrogen; POD: peroxidase; SOD: superoxide dismutase

Both 1% Zn-Lys and 1% $ZnSO_4$ treatments demonstrated higher levels of calcium (Figure 4A), potassium (Figure 4B), and magnesium (Figure 4C), with 1% Zn-Lys showing the highest increases in Ca (14.51%) and Mg (28.95%), indicating better nutrient uptake and mineral balance under zinc-enriched treatments. These improvements suggest that zinc-based treatments positively influence the mineral nutrition of the plants, supporting overall growth and development. For shoot mineral contents, the 1% Zn-Lys treatment showed remarkable improvements, particularly in Zinc (Figure 4D), where it had a 7.06% increase compared to NS. The 1% $ZnSO_4$ treatment also resulted in a moderate increase in Zn (0.95%). For shoot nitrogen contents (Figure 4E), significant improvements were observed with all treatments with 1% Zn-Lys proving the best treatment (Table 2). Lastly, regarding antioxidant status, the 1% Zn-Lys treatment showed the most significant enhancement in SOD (Figure 5A) by 23.94% and POD (Figure 5B) by 9.22%. These enzymes play crucial roles in mitigating oxidative stress and protecting plant cells from damage caused by reactive oxygen species (ROS). 1% ZnSO4 also showed substantial increases in antioxidant enzymes, with SOD increasing by 16.40%. Overall, the 1% Zn-Lys treatment showed the most consistent and significant improvements across various tested parameters (Table 2).

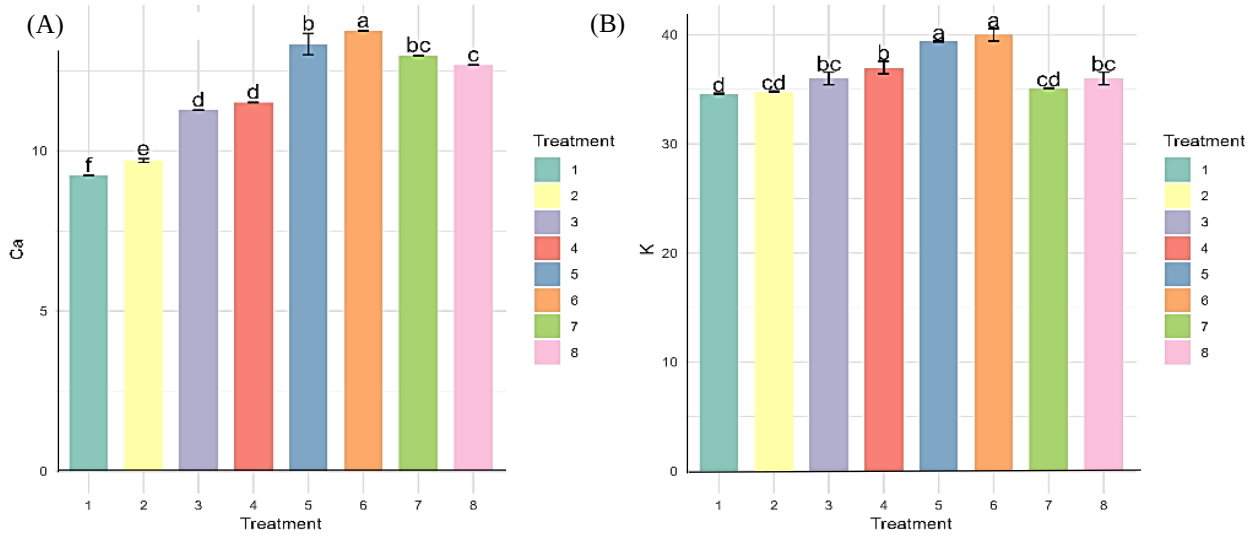

Figure 4 Bar charts (mean ± S.E.) of shoot mineral ions in rice plants as affected by various foliar treatments: (A) calcium, (B) potassium, (C) magnesium, (D) zinc, and (E) nitrogen. Treatments: 1 - No spray, 2 - Water spray, 3 - 0.5% $ZnSO_4$, 4 - 1% $ZnSO_4$, 5 - 0.5% Zn-Lys, 6 - 1% Zn-Lys, 7 - 0.5% Lys, 8 - 1% Lys. Bars with different letters indicate means that differ significantly at LSD 5%.

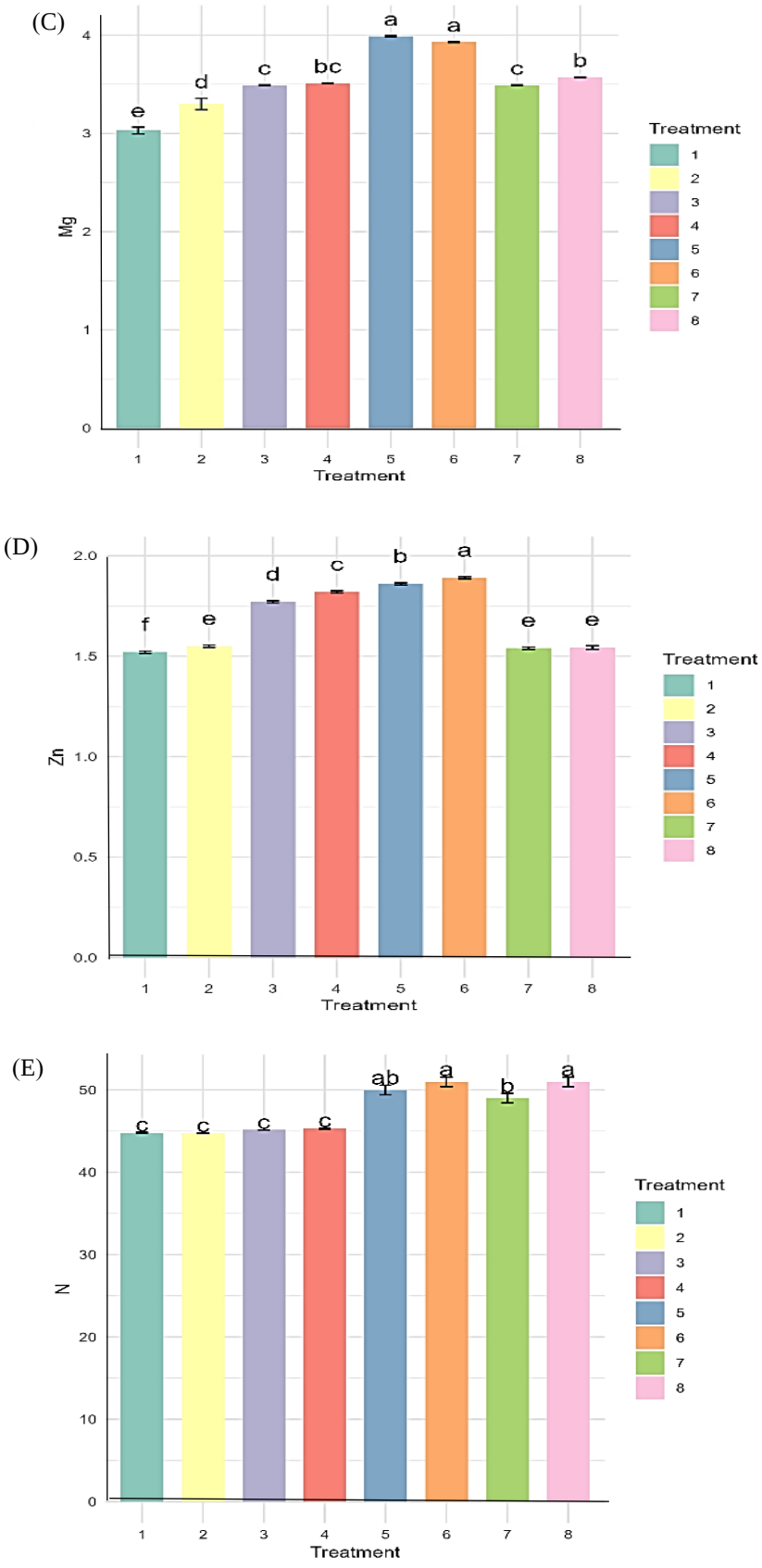

Figure 4 (Cont.) Bar charts (mean ± S.E.) of shoot mineral ions in rice plants as affected by various foliar treatments: (A) calcium, (B) potassium, (C) magnesium, (D) zinc, and (E) nitrogen. Treatments: 1 - No spray, 2 - Water spray, 3 - 0.5% $ZnSO_4$, 4 - 1% $ZnSO_4$, 5 - 0.5% Zn-Lys, 6 - 1% Zn-Lys, 7 - 0.5% Lys, 8 - 1% Lys. Bars with different letters indicate means that differ significantly at LSD 5%.

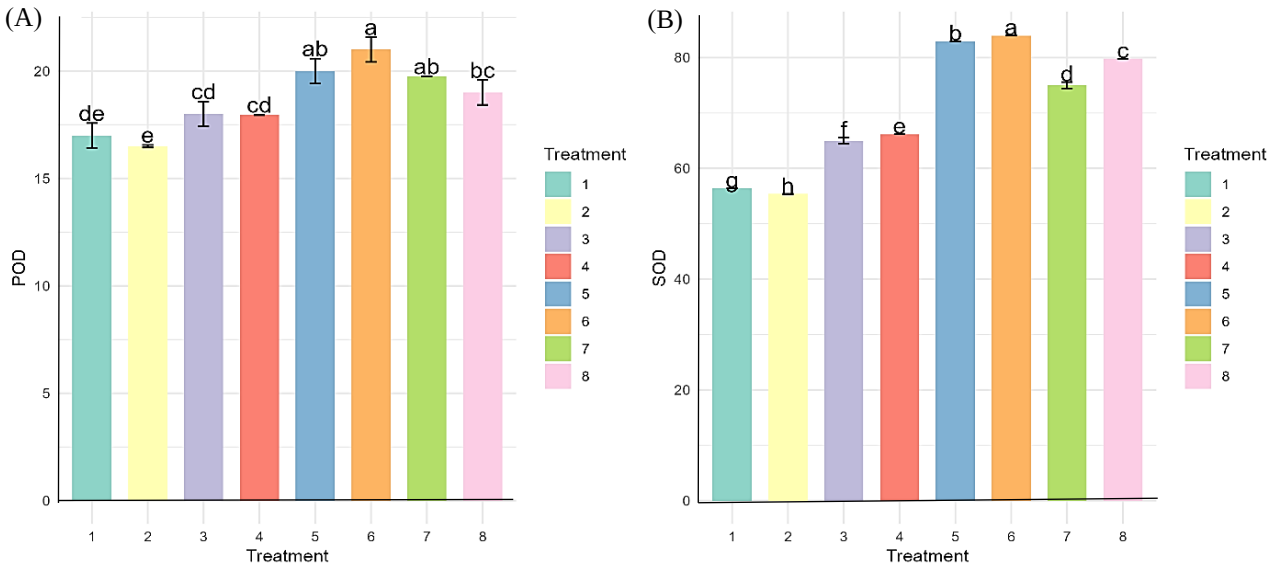

Figure 5 Bar charts (mean ± S.E.) of the studied antioxidant enzymes in rice plants as affected by various foliar treatments: 1 - No spray, 2 - Water spray, 3 - 0.5% $ZnSO_4$, 4 - 1% $ZnSO_4$, 5 - 0.5% Zn-Lys, 6 - 1% Zn-Lys, 7 - 0.5% Lys, 8 - 1% Lys. (A) peroxidase, (B) superoxide dismutase. Bars with different letters indicate means that differ significantly at LSD 5%.

4. Discussions

The results of this study indicate that Zn-Lys foliar application significantly enhances the osmolytes contents, growth of rice plants, as evidenced by increased plant height, biomass, and chlorophyll content. The superior growth response, accumulation of sugars, flavonoids, anthocyanin, and tocopherols to Zn-Lys over $ZnSO_4$ and L-Lys alone can be attributed to the chelation effect of the amino acid [4], which facilitates a more efficient transport of zinc into the plant maintaining the micronutrient status within the plant required to regulate several biosynthetic pathways involved in production of these phytochemicals. Zinc plays a crucial role in chlorophyll biosynthesis and enzyme activation, both of which are vital for photosynthesis and overall plant growth [9-10]. Zinc chelation via amino acids such as L-lysine enhances the bioavailability of zinc, making it more readily available to plants, thus promoting better growth parameters [5]. Recent studies support this finding [4-5]. For example, zinc amino acid chelates have been shown to improve growth traits in various crops by enhancing nutrient absorption and the efficiency of biochemical pathways, such as protein synthesis, which is directly linked to cell division and elongation [1, 4-5]. These studies emphasize that the chelation of zinc with amino acids results in a more stable, plant-available form of zinc, promoting better growth and productivity.

Zinc is involved in numerous biochemical pathways, including enzyme activation, protein synthesis, and nucleic acid metabolism [10]. In plants, zinc plays a pivotal role in the activity of enzymes such as carbonic anhydrase [21], ribulose-1,5-bisphosphate carboxylase, and other enzymes involved in photosynthesis and respiration [10]. The enhanced chlorophyll content observed in the Zn-Lys-treated plants can be attributed to increased zinc availability [5], which in turn supports the chlorophyll biosynthesis pathway. Studies have shown that the availability of zinc directly influences chlorophyll content, as zinc acts as a cofactor for chlorophyll synthase [9-10]. Furthermore, Zn-Lys's ability to improve chlorophyll content supports the higher photosynthetic activity observed in this treatment group, leading to improved growth and biomass accumulation. The mechanism behind Zn-Lys efficacy lies in its superior transport and uptake compared to other forms of zinc, such as $ZnSO_4$. Zinc amino acid chelates, including Zn-Lys, bypass the need for the plant to expend significant energy in ion uptake, facilitating more efficient nutrient use [3-5]. This efficiency in nutrient uptake directly translates to improved metabolic functions, enhancing overall growth and development. Zn-Lys's role in improving nutrient uptake is central to the observed growth benefits. By enhancing zinc bioavailability, Zn-Lys facilitates more efficient uptake of not only zinc but also other essential nutrients, such as nitrogen and phosphorus [10]. This is consistent with previous studies that have shown that zinc chelates enhance the uptake of other nutrients by improving root system development and ion transport efficiency [22-23]. Zinc is involved in many metabolic processes that regulate nutrient uptake, including root growth, ion transport, and the activation of nutrient transporters [23].

In this study, Zn-Lys application resulted in higher concentrations of zinc and other key minerals in the shoots of rice plants, supporting the hypothesis that Zn-Lys enhances the uptake of essential nutrients. This improved

uptake can be attributed to the enhanced transport properties of amino acid chelates, which increase zinc availability in the root zone, improving nutrient transport to the shoots [10]. This result aligns with findings from other studies [3-4, 22-23], which report that chelated forms of micronutrients lead to more efficient nutrient uptake and better growth performance. The antioxidant response of rice plants was significantly improved with Zn-Lys treatment, as evidenced by enhanced activity of key antioxidant enzymes such as superoxide dismutase (SOD), and POD. The increased antioxidant activity suggests a robust response to oxidative stress, which is crucial for improving stress tolerance in plants. Under environmental stress conditions, such as drought or salinity, plants produce reactive oxygen species (ROS) that can damage cellular components. Zinc plays a key role in maintaining cellular integrity under stress by supporting the antioxidant defence system [1]. The foliar application of Zn-Lys not only boosts zinc availability but also activates antioxidant enzymes, thereby improving the plant's capacity to counteract oxidative stress [4]. This is consistent with studies that have linked zinc with enhanced antioxidant defence mechanisms. For example, zinc has been shown to stabilize cell membranes and increase the activity of antioxidant enzymes in response to stress [1, 24]. Zn-Lys's ability to improve antioxidant enzyme activity and mitigate oxidative stress is particularly important for improving the resilience of rice plants to biotic and abiotic stresses. This enhancement of antioxidant defence is a critical factor contributing to the overall stress tolerance of rice plants under suboptimal growing conditions.

The results of this study have significant practical implications for improving rice productivity and sustainability. Foliar application of Zn-Lys provides an efficient means of increasing zinc bioavailability in rice plants, leading to improved growth, yield, and nutrient uptake [3]. This approach could be particularly beneficial in regions with zinc-deficient soils, where conventional soil applications of zinc fertilizers may not be sufficient. The use of Zn-Lys can be an effective strategy for biofortification, potentially increasing the nutritional content of rice grains, particularly zinc, and addressing zinc deficiency in populations dependent on rice as a staple food [25]. From an economic perspective, Zn-Lys treatment is a cost-effective solution compared to other methods, as it enhances nutrient uptake and growth while requiring lower zinc input than traditional fertilizers. Given the higher efficiency of Zn-Lys improving plant growth and yield, it offers a cost-benefit advantage in rice farming by potentially reducing the need for excessive fertilizer applications, while still achieving higher yields and improved grain quality. In terms of environmental impact, the use of Zn-Lys in rice cultivation offers several advantages. Unlike $ZnSO_4$, which can lead to soil zinc accumulation and environmental toxicity when applied in excess [3-4], Zn-Lys enhances zinc uptake without resulting in detrimental build-up in the soil. This reduces the risk of zinc toxicity and ensures that zinc is utilized efficiently by plants. Furthermore, foliar application of Zn-Lys minimizes nutrient losses due to leaching, making it a more sustainable alternative to soil-based applications of zinc.

5. Conclusions

In conclusion, foliar application of Zn-Lys significantly enhances the growth, and fortification of rice plants. The superior performance of Zn-Lys over $ZnSO_4$ and L-Lysine can be attributed to its improved bioavailability and efficiency in nutrient transport. This study not only supports the efficacy of Zn-Lys as a foliar treatment but also provides new insights into its potential for biofortification and stress tolerance in rice. By improving growth, yield, and antioxidant defence, Zn-Lys offers a promising solution for enhancing rice productivity in zinc-deficient soils. The findings also highlight the need for further research to explore the long-term effects of Zn-Lys application for sustainable agricultural practices.

6. References

[1] Mazhar MW, Ali Q, Ishtiaq M, Ghani A, Maqbool M, Hussain T, Mushtaq W. Zinc-aspartate-mediated drought amelioration in maize promises better growth and agronomic parameters than zinc sulfate and L-aspartate. 2021; 53(2):290–310.

[2] Mazhar MW, Ishtiaq M, Maqbool M, Hussain SA. Foliar application of zinc oxide nanoparticles improves rice yield under biotic stress posed by *Magnaporthe oryzae*. Arch Phytopathol Plant Prot. 2023;56(14):1093–1111.

[3] Shehzad F, Ali Q, Ali S, Al-Misned FA, Maqbool S. Fertigation with Zn-Lysine confers better photosynthetic efficiency and yield in water-stressed maize: water relations, antioxidative defense mechanism and nutrient acquisition. Plants. 2022;11(3):404.

[4] Rizwan M, Ali S, Hussain A, Ali Q, Shakoor MB, Zia-ur-Rehman M, Farid M, Asma M. Effect of zinc-lysine on growth, yield and cadmium uptake in wheat (*Triticum aestivum* L.) and health risk assessment. Chemosphere. 2017; 187:35–42.

[5] Noman A, Ali Q, Maqsood J, Iqbal N, Javed MT, Rasool N, Naseem J. Deciphering physio-biochemical, yield, and nutritional quality attributes of water-stressed radish (*Raphanus sativus* L.) plants grown from Zn-Lys primed seeds. Chemosphere. 2018; 195:175–189.

[6] Mazhar MW, Akram R, Shahid A. Foliar application of iron glutamate improves yield and growth of tomatoes compared to iron sulphate and L-glutamate. Int J Veg Sci. 2022;28(6):511–520.

[7] Sparks DL, Page AL, Helmke PA, Loeppert RH, eds. Methods of Soil Analysis, Part 3: Chemical Methods. Vol. 14. John Wiley & Sons; 2020.

[8] Leu M. Metal lysine complexes and method for producing metal lysine complexes. US Patent 5,061,815. 1991.

[9] Mazhar MW, Ishtiaq M, Maqbool M, Mahmoud EA, Ullah F, Elansary HO. Optimizing bitter gourd (*Momordica charantia* L.) performance: exploring the impact of varied seed priming durations and zinc oxide nanoparticle concentrations on germination, growth, phytochemical attributes, and agronomic outcomes. Cogent Food Agric. 2024;10(1):2313052.

[10] Mazhar MW, Ishtiaq M, Hussain I, Parveen A, Bhatti KH, Azeem M, Thind S, Ajaib M, Maqbool M, Sardar T, Muzammil K. Seed nano-priming with zinc oxide nanoparticles in rice mitigates drought and enhances agronomic profile. PLoS One. 2022;17(3): e0264967.

[11] Wellburn AR, Lichtenthaler H. Formulae and program to determine total carotenoids and chlorophylls a and b of leaf extracts in different solvents. In: Advances in photosynthesis research: Proceedings of the VIth International Congress on Photosynthesis, Brussels, Belgium, August 1–6, 1983. Volume 2. Springer Netherlands; 1984:9–12.

[12] Baker RT, Davies SJ. Changes in tissue α-tocopherol status and degree of lipid peroxidation with varying α-tocopheryl acetate inclusion in diets for the African catfish. Aquac Nutr. 1996;2(2):71–79.

[13] Mazhar MW, Ishtiaq M, Maqbool M, Jafri FI, Siddiqui MH, Alamri S, Akhtar MS. Synergistic effects of selenium nanoparticles and LED light on enhancement of secondary metabolites in sandalwood (*Santalum album*) plants through in-vitro callus culturing technique. Peer J. 2024;12: e18106.

[14] Sardar T, Maqbool M, Ishtiaq M, Mazhar MW, El-Sheikh MA, Casini R, Mahmoud EA, Elansary HO. Synergistic influence of yeast extract and calcium oxide nanoparticles on the synthesis of bioactive antioxidants and metabolites in *Swertia chirata* in vitro callus cultures. Molecules. 2023;28(12):4607.

[15] Maqbool M, Ishtiaq M, Mazhar MW, Casini R, Mahmoud EA, Elansary HO. Enhancing bioactive metabolite production in *Aerva sanguinolenta* callus cultures through silver nanoparticle and salicylic acid elicitation. Sustainability. 2023;15(13):10395.

[16] Giannopolitis CN, Ries SK. Superoxide dismutases: I. Occurrence in higher plants. Plant Physiol. 1977;59(2):309–314.

[17] Ebell LF. Variation in total soluble sugars of conifer tissues with method of analysis. Phytochemistry. 1969;8(1):227–233.

[18] Cakmak I, Horst WJ. Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). Physiol Plant. 1991;83(3):463–468.

[19] Velikova V, Yordanov I, Edreva AJ. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Sci. 2000;151(1):59–66.

[20] Ishtiaq M, Sardar T, Hussain I, Maqbool M, Mazhar MW, Parveen A, Ajaib M, Bhatti KH, Hussain T, Gul A, Azeem M. Traditional ethnobotanical knowledge of important local plants in Sudhnoti, Azad Kashmir, Pakistan. Sci Rep. 2024;14(1):22165.

[21] Singh P, Shukla AK, Behera SK, Tiwari PK. Zinc application enhances superoxide dismutase and carbonic anhydrase activities in zinc-efficient and zinc-inefficient wheat genotypes. J Soil Sci Plant Nutr. 2019;19(3):477–87.

[22] Hussain A, Ali S, Rizwan M, Zia-ur-Rehman M, Hameed A, Hafeez F, Alamri SA, Alyemeni MN, Wijaya L. Role of zinc–lysine on growth and chromium uptake in rice plants under Cr stress. J Plant Growth Regul. 2018; 37:1413–1422.

[23] Bashir S, Basit A, Abbas RN, Naeem S, Bashir S, Ahmed N, Ahmed MS, Ilyas MZ, Aslam Z, Alotaibi SS, El-Shehawi AM. Combined application of zinc-lysine chelates and zinc-solubilizing bacteria improves yield and grain biofortification of maize (*Zea mays* L.). PLoS One. 2021;16(7): e0254647.

[24] Mazhar MW, Ishtiaq M, Maqbool M, Akram R. Efficacy of zinc oxide nanoparticles on bitter gourd growth, yield, and some phytochemicals through seed priming. Int J Veg Sci. 2023;29(2):145–155.

[25] Mazhar MW, Ishtiaq M, Maqbool M, Akram R. Seed priming with zinc oxide nanoparticles improves growth, osmolyte accumulation, antioxidant defense, and yield quality of water-stressed mung bean plants. Arid Land Res Manag. 2023;37(2):222–246.