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Dose-Dependent Effects of Zinc Oxide Nanoparticles on Growth, Antioxidative Enzymes, and Yield of Pearl Millet

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Abstract

Zinc is an essential micronutrient required for optimal plant growth, metabolism, and yield; however, zinc deficiency remains a major constraint to cereal production under intensive agricultural systems. Recent advances in nanotechnology have highlighted zinc oxide nanoparticles (ZnO-NPs) as a promising alternative to conventional Zn fertilizers due to their enhanced solubility, bioavailability, and targeted delivery. The present study evaluated the dose-dependent effects of ZnO-NPs on growth performance, antioxidative enzymes activity, and yield attributes of pearl millet [*Pennisetum glaucum* (L.) R. Br.]. Plants were treated with graded concentrations (0.001, 0.01, 0.1, 1, 2 and 10 ppm) of ZnO-NPs through foliar application, and their effects were compared. Plants exhibited a concentration-dependent response to ZnO nanoparticle application, with significant enhancement in vegetative growth, chlorophyll biosynthesis, and enzymatic antioxidant defense up to 2 ppm. However, at higher concentrations (10 ppm), vegetative growth and chlorophyll biosynthesis declined, indicating the onset of phytotoxic effects. The application of ZnO nanoparticles at 2 ppm was the most effective treatment, significantly enhancing shoot and root biomass, chlorophyll content, tiller production, panicle weight, and seed weight compared to other concentrations. Growth parameters, including plant height, biomass accumulation, and leaf area, were significantly enhanced at optimal concentrations of ZnO nanoparticles (ZnO-NPs). Furthermore, the activities of key antioxidant enzymes—superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD)-were markedly increased, indicating improved regulation of oxidative stress and enhanced cellular protection. However, higher doses of ZnO nanoparticles (ZnO-NPs) led to a decline in physiological performance, indicating potential phytotoxic effects at excessive concentrations. Yield attributes, such as grain weight and panicle length, were optimized at moderate ZnO-NP application rates. Overall, the findings demonstrate that judicious application of ZnO-NPs can improve growth, antioxidant defense, and yield of pearl millet, highlighting their potential role in sustainable micronutrient management.

Keywords: Antioxidative enzymes, Pearl millet, Foliar application, Sustainable agriculture, Zinc fertilizer, Zinc oxide nanoparticles.

1. Introduction

Zinc deficiency in agricultural soils remains a major constraint to crop productivity and nutritional quality of millets cultivated in semi-arid environments. Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a crucial food for millions of populations in Asia and Africa. This cereal is typically cultivated in areas characterized by low rainfall and is important in economically sensitive areas where food security is a problem due to recurrent droughts and infertile soils[1]. Widespread zinc deficiency in Indian agricultural soils is primarily attributed to intensive cultivation of cereals and legumes without adequate crop rotation or fallow periods, resulting in reduced yield and mineral content[2]. So, pearl millets might help with managing long-term illnesses like diabetes, obesity, heart disease, cancer, and some types of migraines and asthma[3, 4]. According to Kankarwal it can help with different kinds of hunger and make sure that people eat a variety of foods[5].

Zinc has an important role in mitigating nutritional deficiencies and malnutrition prevalent in many underdeveloped countries, including India, which particularly impacts children's health[6]. Pearl millet is grown with a zinc deficient area which is common micronutrient deficiency, despite its hardiness. In addition to lowering the nutritional value of grains and crop productivity, diets low in Zinc can have negative health effects. Many physiological and biochemical functions in plants, including protein synthesis, Photosynthesis, cell transparency, enzyme activation, and membrane system stabilization, synthesis of lipid, protein and carbohydrate metabolism[7, 8]. Zinc also increases water uptake from root and transport to other parts which reduce the stress effects[9, 10, 11, 12]. Often, traditional fertilization approaches such as the application of zinc sulphate are insufficient in Zn deficient soils due to leaching, fixation, and poor root uptake. There is, however, the pioneering field of nanotechnology which looks like it will be helpful in enhancing the nutrient-use efficiency and productivity of crops. Nanoparticles effectively deliver essential nutrients to plants, enhancing absorption and utilization, minimizing losses, and increasing agricultural productivity. Additionally, nanoparticles can stimulate plant growth[13]. They have the potential to enhance crop resilience within sustainable agriculture by reducing traditional chemical uses and adapting to variable climate conditions and environmental challenges[14].

Zinc oxide nanoparticles (ZnO-NPs) are trending because of their advantages over traditional zinc fertilizers, such as greater bioavailability, controlled release of minerals, and high surface area. Zinc oxide nanoparticles interact with the cell wall to create apertures, facilitating their entry and enabling more rapid movement through the apoplastic and symplastic pathways [15]. ZnO-NPs, among the other metal oxides, are most significant nanoparticle which is widely utilized across various industries due to their unique physiochemical characteristics[16, 17]. ZnO-NPs provide plants with a form of zinc that is more soluble and accessible, hence alleviating zinc shortage difficulties, which are mostly caused by the restricted solubility of zinc resources in the soil[18].

Foliar application of ZnO-NPs serves as a more practical method over others because of fast nutrient delivery through the leaf's pores or stomata, rather than through the roots and promotes photosynthesis, enzyme activity, and grain filling[19, 20]. Few studies have demonstrated that foliar application of zinc oxide nanoparticles (ZnO-NPs) not only significantly enhances grain yield and zinc accumulation but also improves growth parameters in millets [21]. The study intends to determine the effect as a foliar supplement in pearl millet cultivation. It should focus on the effect of ZnO nanoparticles on yield and nutritional value, and the plants performance in zinc deficient environments under controlled environment.

2. Material and methods

2.1 Experimental Setup:

Plastic pots (10-inch diameter) were used for the experiment. Each pot was filled with silica sand sourced from Shankargarh, Prayagraj, Uttar Pradesh, India. The sand was sieved to obtain uniform particle sizes ranging from 0.20 to 0.84 mm. Prior to use, the sand was thoroughly washed with water and initially treated with hydrochloric acid. It was then repeatedly rinsed with distilled water and subjected to further chemical treatment using a mixture of 17% hydrochloric acid and 1% oxalic acid to eliminate residual impurities. After treatment, the sand was again extensively rinsed with distilled water and subsequently used for filling the pots[22]. After each acid treatment, the sand was meticulously cleaned with water. Prior to commencing the experiment, the treated sand was leached with a 4 mM calcium nitrate solution, purified using phosphate adsorption and dithizone extraction[22], to reduce its pH to about neutral (around 6.5).

The pots used for plant cultivation were made of high-quality white plastic and were provided with drainage holes at the base. An inverted watch glass was placed over each hole to retain the sand within the container while allowing free drainage of excess nutrient solution and adequate aeration of the root zone.

2.2 Seed Sowing:

Seed which are healthy and uniform looking were surface sterilized with mercuric chloride to eliminate pathogen infections like bacteria, fungal, and viral infections. Seed were soaked with water for 24 h after that seed were grown under glasshouse conditions. The seeds were shown in 10-inch sand pot in depth of 0.5cm. Seedlings started appearing on the 4th day after sowing. Thereafter, they were supplied with distilled water for 48 days without any nutrient supplementation.

2.3 Nutrient Supply:

After the plants reached a certain height, they were supplied with the nutrient medium. For the control (normal) plants, the medium was applied without zinc are 4 mM KNO_3, 4 mM Ca $(NO_3)_2$, 2 mM $MgSO_4$, 1.33 mM NaH_2PO_4, 0.33 µM HBO_3, 0.1 mM Fe EDTA, 10 µM $MnSO_4$, 1 µM $CuSO_4$, 0.1 µM Na MoO_4, 0.1 M NaCl, 0.1 µM $CoSO_4$, and 0.1 µM $NiSO_4$, Fe-EDTA[23]. The amount of DNS applied to the pots varied depending on the growth stage of the plants and prevailing weather conditions. After 48 days, zinc micronutrient (DNS) was not applied, followed by a 30-day treatment period. For this, pre-prepared zinc oxide nanoparticles (ZnO NPs), purchased from CDH with a size range of 90-200 nm, and were used. These nanoparticles were applied at different concentrations: 0.001, 0.01, 0.1, 1, 2, and 10 ppm. These zinc oxide nanoparticles are used on plant for foliar treatment in different pot setup. Foliar treatment was performed twice per week.

2.4 Morphological parameters measurements:

Plants were harvested 45 days after sowing under controlled conditions. Phenotypic parameters, including leaf, root, and shoot lengths, were measured using a scale. Fresh weights of these plant parts were recorded using an electronic balance. For dry weight determination, the samples were dehydrated in an oven at 105°C for 48 hours, after which the dry weights of the leaves, roots, and shoots were measured.

2.5 Chlorophyll Pigment estimation:

Chlorophyll content was determined using the standard method described by Lichtenthaler[24]. Fresh leaf samples of pearl millet (0.1 g) were washed thoroughly with distilled water to remove dust and impurities. The samples were then ground using a mortar and pestle in 10 mL of 80% chilled acetone. The homogenate was centrifuged at 5000 rpm for 10 minutes, and the supernatant was collected. The absorbance of the supernatant was measured using a dual-beam spectrophotometer at wavelengths of 663 nm, 645 nm, 510 nm, and 480 nm, with 80% acetone serving as a blank. The concentrations of chlorophyll **a**, chlorophyll **b**, and total carotenoids were calculated using the standard Lichtenthaler and Wellburn equations.

2.6 Catalase:

Catalase activity was determined using the classical titrimetric method of Euler and Josephson[25]. Briefly, the enzyme extract was incubated with 0.005 N hydrogen peroxide (H_2O_2) in 0.025 M phosphate buffer for a fixed reaction period. The reaction was terminated by adding 2 ml of 2 N H_2SO_4, and the residual H_2O_2 was titrated against 0.1 N $KMnO_4$ until a persistent pink color appeared. The activity of the enzyme was calculated based on the amount of H_2O_2 decomposed during the incubation period.

2.7 Peroxidase:

For peroxidase test, we used Luck's methodology[26]. An experiment was performed to ascertain the reaction temperature at 25°C. The reaction mixture comprises 0.1M $KMnO_4$ at 6.0 buffer pH, 1 ml of 0.5% p-phenylene diamine, and0.01% $KMnO_4$. After reaction mixture is prepared,1 ml of enzyme extract added and allow it to incubate for few minutes to initiate the reaction. Two ml of 4N H_2SO_4 are used to terminate the process. Introduce 2 mL of H_2SO_4 into the blanks before addition of the fresh leaf

enzyme extract and execute the procedure concurrently. Subject the reaction mixture to centrifugation at 4000rpm after a 20-minute cooling period. We measured the color intensity using a spectrophotometer calibrated and using blank reference to 485 nm wavelength.

2.8 Superoxide Dismutase (SOD):

Enzyme was estimated through standard method of Beauchamp and Fridovich[27]. First, we begin by preparing a mixture consisting of a phosphate buffer with a concentration of 0.05 M, 0.013 M methionine, 75 µM NBT, 0.1 mM EDTA, and 2 µM riboflavin. To start the reaction, 100 µL of enzyme were added and exposed to fluorescent light (approximately 4000–5000 lux) for 10 minutes. For control we used mixture without enzyme extract, representing maximum NBT reduction. The reaction was stopped by placing in dark condition, and at 560 nm absorbance taken by using spectrophotometer.

2.9 Ascorbate Peroxidase (APX): Ascorbate peroxidase enzyme was determined by Nakano and Asada upgrade method[28]. The mixture contains 50 mM phosphate buffer, 0.5 mM ascorbate, and 0.1 mM freshly prepared H_2O_2. To start the reaction enzyme added, and absorbance was recorded at 290 nm for few minutes. Enzyme activities were expressed in absorbance per minute.

2.10 Hydrogen Peroxide (H_2O_2): Concentration of hydrogen peroxide analyzed by Brennan and Frenkel[29]. In this fresh leaf of millet was homogenized with acetone and filter with Whatman paper 1, add 2.5 ml H_2O_2, 0.5 ml Titanium tetrachloride and 1 ml ammonium then centrifuge it 10,000 rpm for 5 min. Precipitate solubilized into 5 N H_2SO_4 so that yellow color is found. Read absorbance at 415 nm by spectrophotometer.

2.11 Lipid Peroxidation Assay: We used Heath and Packer modified method to analyze Lipid Peroxidation in Peral millets[30]. First fresh leaf 0.5g was homogenized in 5ml of TCA. Then centrifuge at 10,000rpm for 5 minutes. Now, use 2ml supernatant and 2ml of TBA. Boil for 30 minutes at 95°C and quickly cool on ice. After centrifuge at 10,000rpm for 15 minutes, the absorbance at 532 and 600nm with spectrophotometer is taken.

2.12 Statistical Analysis:

Data was analyzed using SPSS version 27 One-way analysis of variance was analyzed. Mean of Triplicate data were ± standard error (SE) and analyzed using Duncan's new multiple range test at a significance level of ($P \leq 0.05$). Principal component analysis (PCA) and Pearson's correlation analysis apply with version 2025b of Origin Pro analysis software.

3. Result and Discussion

3.1 Effect of ZnO nanoparticle on Morpho - Physiological traits

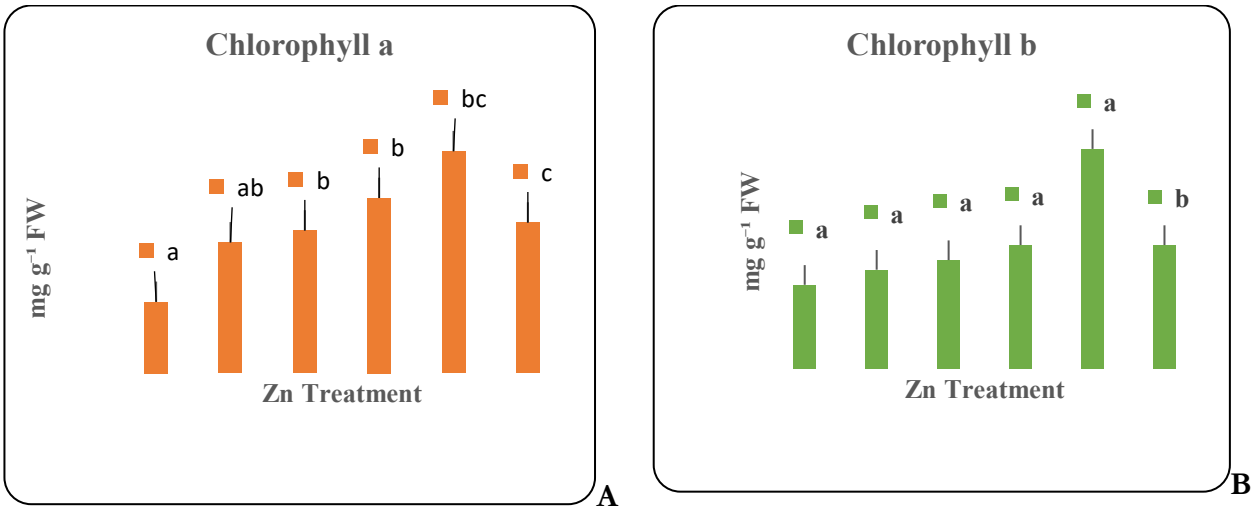
Foliar application of ZnO nanoparticles exhibited a dose-dependent enhancement of growth and biomass parameters in pearl millet. Lower doses (0.001–0.01 ppm) showed only slight enhancement, whereas moderate concentrations, specifically 1 ppm and 2 ppm, led to the most significant increases in plant length, leaf number, and biomass[31,32]. The treatment at a 2-ppm dose exhibited the most pronounced stimulatory effect, leading to increased shoot length as well as higher fresh and dry biomass. Additionally, this experiment demonstrated enhanced photosynthetic efficiency, reflected by an increase in chlorophyll content, which likely contributed to more effective nutrient utilization. However, a decrease in growth was observed at 10 ppm treatment that indicated potential phytotoxic effects at levels surpassing the ideal dosage (Table 1). The results finding indicate that zinc nanoparticles at 1–2 ppm significantly enhance vegetative growth, whereas higher concentrations (≥10 ppm) induce phytotoxic stress, leading to a pronounced decline in physiological performance.

Table 1. Morphological alteration of Pearl millet plant treated with ZnO nanoparticles.

Parameter	0.001ppm	0.01ppm	0.1ppm	1ppm	2ppm	10ppm	SEm ±	CV (%)
Root Length(cm)	5.3 ± 0.202^{a}	5±0.145^{a}	6.6±0.333^{a}	8±0.120^{b}	9±0.233^{c}	5.4±0.305^{c}	0.223	5.9
Shoot Length(cm)	6.333 ±0.509^{a}	9.466±0.658^{ab}	11.966±0.786^{bc}	12.899±0.702^{bc}	14.355±0.587^{bc}	12.03±0.428^{c}	0.611667	9.48
Leaf Fresh Weight(mg)	9.31 ± 0.627^{a}	8.83 ± 0.392^{a}	15.7 ± 1.411^{b}	13.94±1.167^{bc}	20.54±0.591^{cd}	17.033±0.969^{d}	0.8595	10.46
Stem Fresh Weight(mg)	13.706±0.600^{a}	12.433±0.283^{a}	19.986±0.961^{b}	21.566± 0.888^{b}	23.306±0.392^{b}	23.193 ± 0.822^{b}	0.657667	5.98
Root Fresh weight(mg)	12.42±1.173^{a}	8.4±0.599^{a}	15.013±1.071^{a}	17.45±1.493^{a}	14.9±0.609^{a}	18.413±0.666^{a}	0.935167	11.22
Leaf Dry Weight(mg)	1.232±0.144^{a}	1.479±0.107^{a}	2.123±0.196^{ab}	5.106±0.467^{b}	5.98±0.449^{c}	3.513±0.239^{c}	0.267	14.28
Stem Dry Weight (mg)	2.383±0.033^{a}	1.403±0.008^{a}	1.896±0.335^{ab}	7.54 ±0.540^{b}	8.126±0.545^{c}	4.43±0.660^{c}	0.3535	14.25
Root Dry Weight (mg)	2.473±0.082^{a}	1.323±0.069^{ab}	2.256±0.109^{ab}	7.733±0.554^{abc}	6.556±0.326^{bc}	4.256±0.331^{c}	0.245167	10.36
Leaf Number	5.285±0.606^{a}	5.857±0.404^{ab}	7.428±0.719^{bc}	7.857±0.553^{c}	8±0.755^{c}	7.857±0.633^{c}	0.611667	15.03

SEm ±: Standard Error of the Mean; each value is the mean ± SD of triplicate (n = 3); significant at $p < 0.05$ ($p \leq 0.05$)
CV represent Coefficient of Variance standardized measure of data dispersion in %.

3.2 Chlorophyll Content: Significant differences were observed in the photosynthetic pigment content of pearl millet under varying zinc treatments **(Fig. 1).** The levels of chlorophyll in the plants increased with the application of Zn up to 2 ppm, indicating a stimulatory effect. However, further increase in Zn concentration to 10 ppm resulted in a decrease in chlorophyll content, suggesting potential toxicity or inhibitory effects at higher levels [19, 31]. The chlorophyll content increased at the 2 ppm Zn treatment compared to the control, but decreased when the Zn concentration was further increased to 10 ppm. Carotenoid levels were also highest at this concentration, indicating an increased ability for photoprotection[24]. Plants in 10 ppm experienced a marked decrease in pigment levels, suggesting that higher concentrations of Zn inhibited chlorophyll metabolism[33]. At high concentrations, Zn likely induced oxidative stress and inhibited photosynthetic pigment production by increasing ROS, which is widely reported in nanoparticle toxicity studies[34].



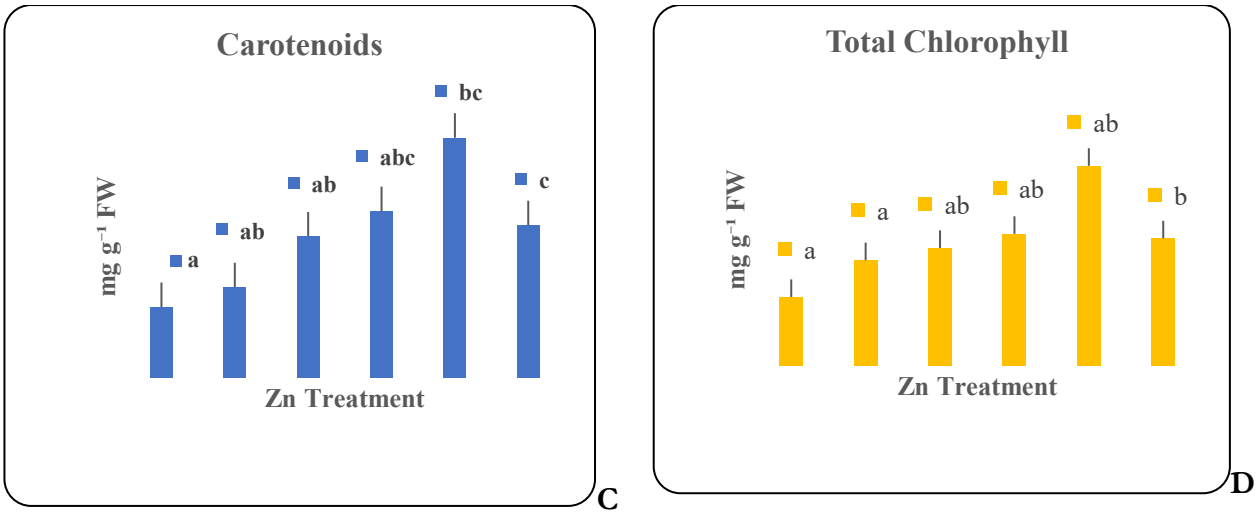


Figure 1. Effect of different Foliar Zinc concentration (0.001-10) on **A-** Chlorophyll a, **B-** Chlorophyll b, **C-** Carotenoid, **D-** Total Chlorophyll; Error show standard error of mean (± SE), Alphabets used (a-c) show significant difference ($p \leq 0.05$; by using Duncan's Multiple Range test in SPSS).

3.3 Antioxidant enzymes activities

3.3.1 Effect of Nanoparticles on Catalase and Peroxidase activity: Catalase and peroxidase activities in millet demonstrated notable variation as influenced by varying dosages of Zn nanoparticles. The activity of catalase showed an upward trend to the highest H_2O_2 decomposition, which occurred at 2 ppm. The activity of the plant transfected with 0.001 ppm showed a minor improvement and 0.01 was observed to be slightly less active. There was a decrease in activity of catalase at 10 ppm, suggesting less activity at a higher concentration of Zn. A similar pattern was observed for peroxidase activity, which increased with low Zn concentrations and plateaued at 2 ppm after the 1 ppm treatment. Minimal activity was recorded at 0.001 and 0.01 ppm Zn. At 10 ppm, peroxidase activity showed a slight decrease following exposure to Zn nanoparticles. This observation indicates that antioxidant activity tends to increase at moderate concentrations of Zn nanoparticles, while it diminishes at higher, potentially excessive concentrations.

3.3.2 Effect of Nanoparticles on SOD and APX activity: Influence of Nanoparticles on SOD and APX. (Fig. 2). The influence of nanoparticles on superoxide dismutase (SOD) and ascorbic acid peroxidase (APX) presented a gradual more enzyme activity that peaked at 2ppm, following a recorded steady increase in 0.001 and 0.01 until 1ppm. A slight decline was observed at 10ppm, suggesting that when high levels of Zn were applied, there was a significant decrease effect on the enzyme. Ascorbic acid peroxidase (APX) activity in millet leaves responded in a dose-dependent fashion following foliar application of ZnO nanoparticles. An incremental enzyme activity was observed at the lowest concentration (0.001ppm) with an increase corresponding with rising concentrations of ZnO nanoparticles until 1. APX activity was highest for 2ppm, demonstrating the greatest activation of the ascorbate-glutathione antioxidant system for this concentration.

3.3.3 Impact of Nanoparticles on H_2O_2 and Lipid Peroxidation: Pearl millet demonstrated a concentration-dependent shift in oxidation indicators after foliar treatment with zinc nanoparticles (ZnONPs). The increase of hydrogen peroxide (H_2O_2) grew constant with escalating concentration of ZnNPs suggesting a greater generation of ROS. At 0.001ppm, the lowest level of H_2O_2 was noted with little increment at 0.1 and 1ppm. The highest accumulation occurred at 2ppm demonstrating significant oxidative stress with notable increases of ZnNPs. However, the significant drop at 10ppm shows that the body's antioxidant defense systems or metabolic changes associated with development may have kicked in when ZnNP levels were too high. A same trend was seen for lipid peroxidation, quantified as malondialdehyde (MDA) concentration. The drop

at 10ppm that comes after that shows that ROS production has gone down, either because the cells have become used to stress or because their metabolism has slowed down. Additionally, the increased levels of H_2O_2 and MDA at medium to high ZnNP doses indicate the activation of oxidative stress and consequent membrane lipid peroxidation.

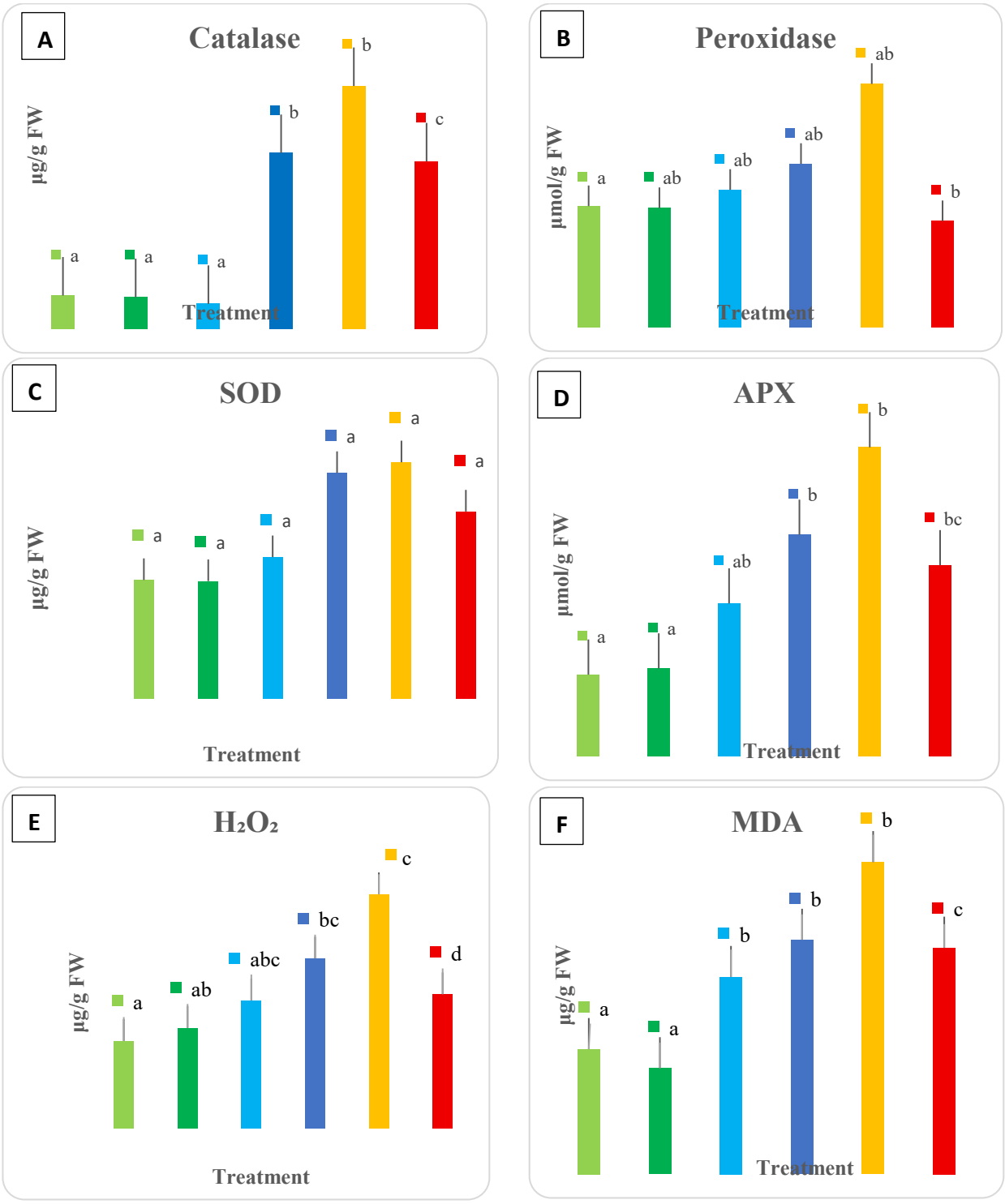


Figure 2. Effect of ZnO nanoparticle on antioxidant enzyme (A) Catalase, (B) Peroxidase, (C) SOD, (D) APX, (E) H_2O_2, (F) Lipid Peroxidase (MDA)

3.4 Yield attributes of Pearl Millet

ZnO nanoparticles markedly enhanced the productive characteristics of pearl millet in a concentration-dependent manner (Table 2). The quantity of tillers, panicle length, panicle and seed weight augmented by rising ZnO-NP concentration up to 2 ppm. At a dosage of 0.01 ppm, a marginal

enhancement was seen in the quantity of tillers and panicle weight (0.216 g), with no seed production occurring. A marginal enhancement was seen at 0.1 ppm, when seed production commenced (0.248 g plant^{-1}). Yield parameters under 1 ppm yielded notable increases in tiller number (7) and seed weight (1.648 g plant^{-1}). The yield parameters (including tillers (10), panicle length (10.8 cm), panicle weight (4.73 gm), and seed weight (3.697 g plant^{-1}) reached their maximum yield at the 2 ppm ZnO-NPs treatment level, suggesting a beneficial stimulatory effect of ZnO-NPs (at moderate concentrations) on reproductive development and subsequent grain filling in pearl millet. In contrast, with the 10-ppm treatment level, a toxicological effect from the nanoparticles may have induced decreased yield attributes due to decreased tillers (6) and decreased seed weight (1.069 g plant^{-1}). Overall, the findings clearly demonstrate that 2 ppm ZnO-NPs is the optimal dose for enhancing productivity in pearl millet, while higher concentrations negatively impact yield.

Table 2. Productive yields of pearl millets under zinc nanoparticle foliar treatments

Treatment	**No. of Tillers**	**Ear Head Length (cm)**	**Panicle Weight (g)**	**Seed Weight (g)**
0.001	3±0.2182^{a}	9.1±0.260^{a}	0.2133±0.0025^{a}	0
0.01	2±0.3779^{a}	10.5±0.297^{ab}	0.22±0.0063^{a}	0
0.1	3±0.218^{a}	13±0.308^{ab}	1.3488±0.0124^{b}	0.248±0.0116^{b}
1	7±0.308^{b}	10.16±0.288^{b}	2.07±0.0107^{c}	1.648±0.0125^{c}
2	10±0.487^{b}	10.8±0.246^{b}	4.73±0.0152^{d}	3.697±0.0127^{d}
10	6±0.308^{c}	10.01±0.218^{c}	0.85±0.0124^{e}	1.069±0.013^{e}

The mean ± SE of three replicates (n = 3) is shown for each value; $p < 0.05$ indicates significance.

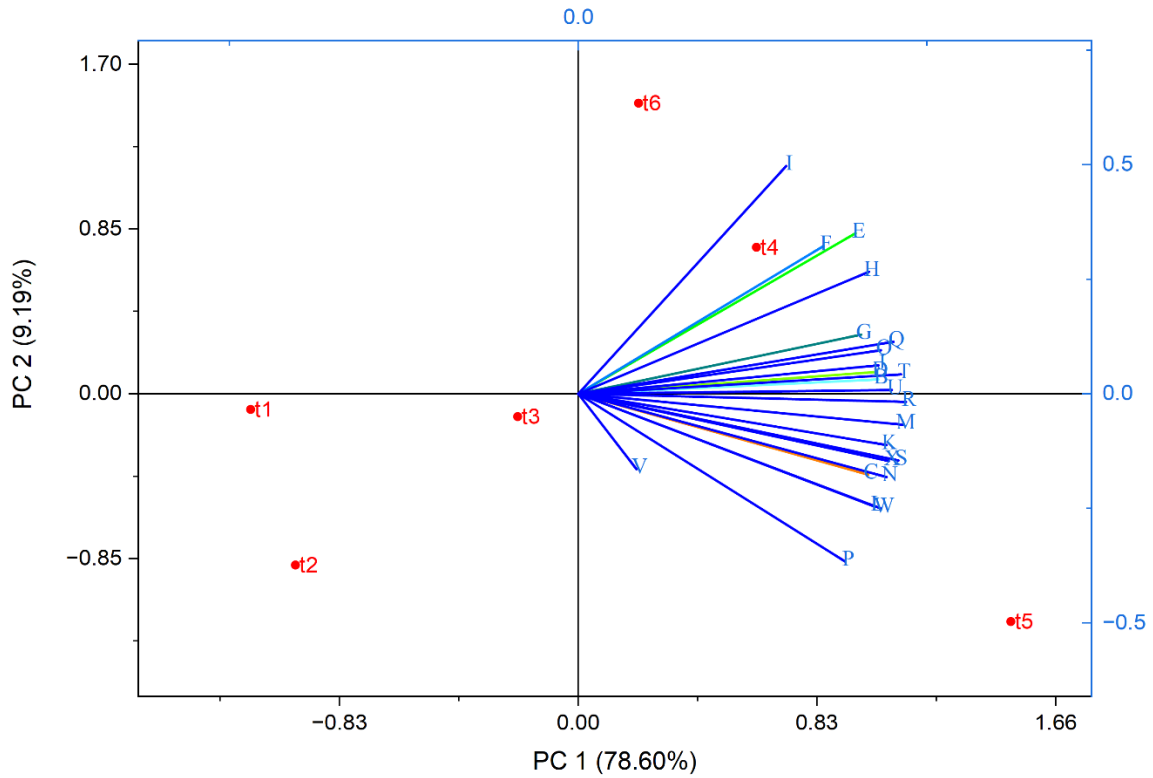


Figure 2. Principal component analysis (PCA) of morpho-physiological characteristics of pearl millet plants exposed to different foliar concentrations of ZnO nanoparticles. Variables included were: **B**-stem length; **C**-root length; **D**-leaf dry weight; **E**-stem dry weight; **F**-root dry weight; **G**-leaf fresh weight; **H**-stem fresh weight; **I**-root fresh weight; **J**-number of leaves; **K**-chlorophyll *a*; **L**-chlorophyll *b*; **M**-carotenoids; **N**-total chlorophyll; **O**-catalase (CAT); **P**-peroxidase (POD); **Q**-superoxide dismutase (SOD); **R**-ascorbate peroxidase (APX); **S**-

hydrogen peroxide (H_2O_2); **T**-lipid peroxidation (MDA); **U**-number of tillers; **V**-ear head length; **W**-panicle weight; and **X**-seed weight.

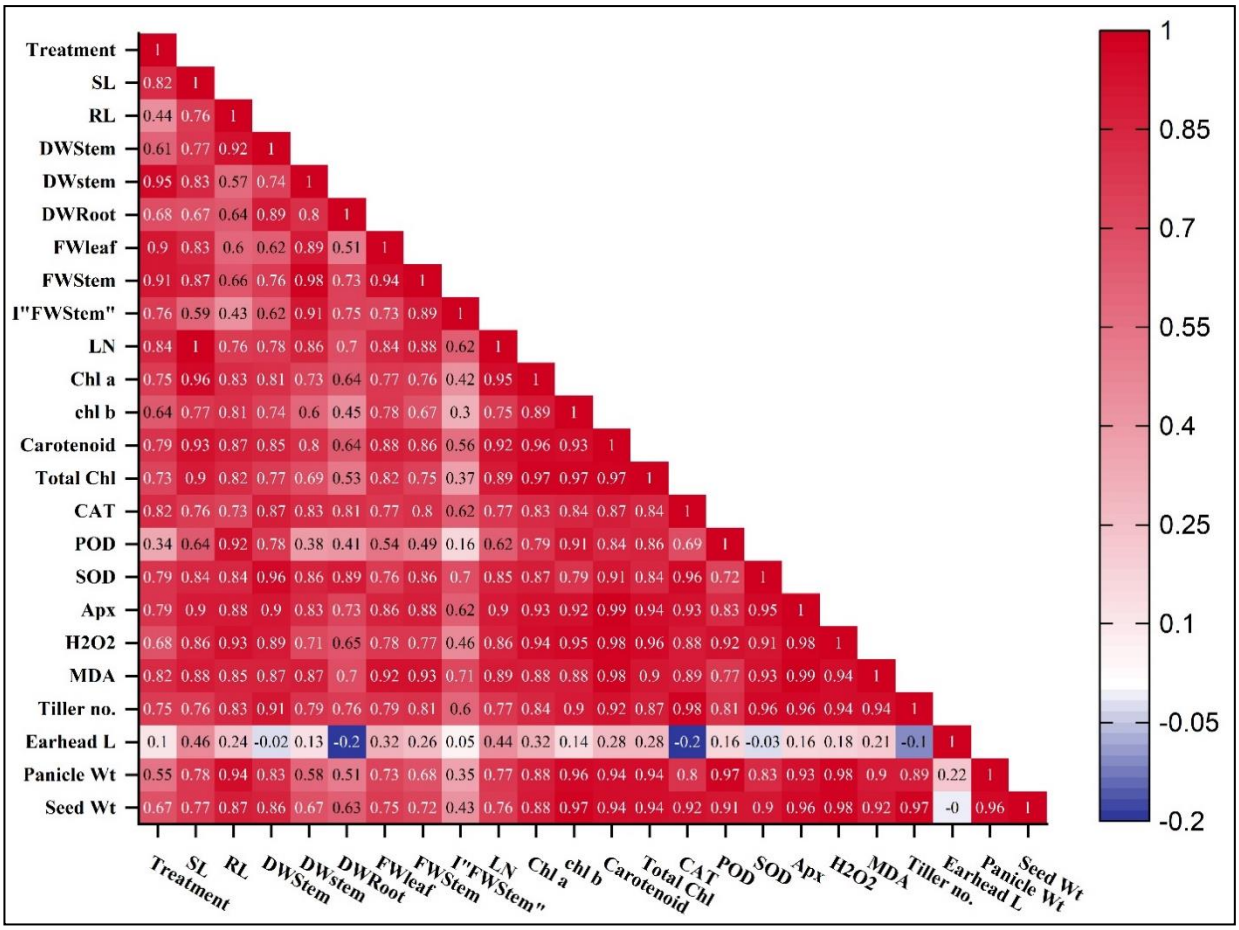


Figure 3. Correlation analysis among morpho-physiological and biochemical characteristics of pearl millet (*Pennisetum glaucum* L.) plants exposed to different foliar concentrations of zinc oxide nanoparticles (ZnO-NPs). Traits include shoot length (SL), root length (RL), stem dry weight (DW), fresh weight (FW), leaf number (LN), chlorophyll content (Chl), catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), and malondialdehyde (MDA; indicator of lipid peroxidation).

4. Conclusion

The present investigation clearly demonstrates that foliar application of zinc oxide nanoparticles (ZnO-NPs) exerts a pronounced dose-dependent influence on the growth, physiological performance, antioxidant defense system, and yield attributes of pearl millet. Among the tested concentrations, ZnO-NPs at 2 ppm emerged as the most effective treatment, providing an optimal balance between enhanced growth promotion and controlled oxidative stress. This concentration significantly improved vegetative growth parameters, chlorophyll biosynthesis, biomass accumulation, and yield-related traits such as tiller number, panicle weight, and grain weight. However, the concurrent increase in H_2O_2 and MDA at this concentration highlights the importance of closely monitoring stress indicators, particularly under field conditions. These findings demonstrate the considerable potential of nano-fertilization in promoting sustainable agriculture, especially in micronutrient-deficient soils. Nevertheless, the dose-dependent nature of plant responses underscores the necessity for precise optimization to achieve desirable outcomes. The marked upregulation of antioxidant enzymes, including superoxide dismutase, catalase, and peroxidase, at optimal ZnO-NP levels indicates strengthened antioxidative defense and improved cellular protection against reactive oxygen species. Conversely, higher ZnO-NP concentrations (10 ppm) resulted in reduced growth and photosynthetic efficiency, highlighting the onset of phytotoxic effects and underscoring the importance of dose optimization. These findings suggest that while ZnO nanoparticles possess superior bioavailability and efficiency compared to conventional zinc fertilizers,

their application must be carefully regulated to avoid adverse effects. Overall, the study underscores the potential of ZnO-NPs as an efficient nano-fertilizer for improving pearl millet productivity and stress tolerance. Judicious use of ZnO-NPs can contribute to sustainable micronutrient management and enhanced cereal production under zinc-deficient agricultural systems. Further studies on long-term environmental safety and field-scale validation are recommended.

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Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest: The authors declare that there are no conflicts of financial or personal interest.

Financial interest: The authors declare they have no financial interests.

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