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Improving biochemical traits and zinc bioavailability in wheat (*Triticum aestivum* L.) using zinc oxide nanoparticles

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Abstract

Zinc (Zn) is a vital micronutrient for both plants and humans, playing a key role in numerous enzymatic processes and metabolic functions. However, Zn deficiency is a widespread issue affecting over 50% of agricultural soils globally and nearly one-third of the global population, leading to stunted growth, increased susceptibility to infections, and significant economic impacts. To address Zn deficiency in wheat (*Triticum aestivum* L.), this study investigated the effects of zinc oxide nanoparticles (ZnO NPs) of varying sizes (35 nm, 40 nm, 95 nm) on Zn uptake, enzymatic activity, and phytic acid content. Conducted at the Indian Agricultural Research Institute, New Delhi, the experiment utilized a completely randomized design (CRD) with four treatments, including a control. The results indicated that smaller-sized ZnO NPs (35 nm) significantly enhanced superoxide dismutase (SOD) and catalase (CAT) activities, increasing them by up to 18% and 30%, respectively. Additionally, grain Zn content rose from 19.6 to 27.55 mg/kg, and phytic acid content decreased by up to 33% with 35 nm ZnO NP application. These findings suggest that smaller-sized ZnO NPs improve Zn bioavailability, nutrient uptake, and enzymatic activities in wheat, reducing the negative effects of Zn deficiency. The study highlights the potential of ZnO NPs as an effective strategy to enhance wheat yield and nutritional quality, thereby addressing Zn deficiency in crops and contributing to improved global health. Further research is recommended to optimize ZnO NP application and assess potential environmental impacts.

Keywords: Zinc Oxide Nanoparticles, Wheat, SOD, Catalase, Nutrient bioavailability, Zinc

Introduction

Zinc is a critical micronutrient for human health, regulating various enzymatic functions and metabolic processes (Maares *et al.*, 2020) [13]. It is essential for both plant growth and human nutrition; however, zinc deficiency in soils can limit plant development, reduce yields, and increase susceptibility to diseases. Studies have shown that over 50% of agricultural soils worldwide are deficient in zinc (Kopittke *et al.*, 2019) [11], and around one-third of the global population, particularly children and pregnant women, suffer from zinc deficiency (Aragie and Genanu, 2017) [3]. This deficiency in humans can lead to poor health outcomes, including stunted growth and increased vulnerability to infections (Sangeetha *et al.*, 2022) [19], as well as significant economic impacts, reducing GDP by up to 11% annually in some countries (Khan *et al.*, 2022) [10]. Nanotechnology offers innovative solutions to enhance nutrient management and crop productivity (Zain *et al.*, 2023) [23]. Recently, the application of ZnO NPs in various crops has gained attention for their potential to improve plant growth, nutrient uptake, and stress resistance (Zain *et al.*, 2023) [23]. Addressing zinc deficiency through soil and foliar applications of zinc oxide nanoparticles (ZnO NPs) could improve crop yields and nutritional quality, potentially benefiting global health and economies (Dimkpa and Bindraban, 2016) [7].

Wheat (*Triticum aestivum* L.) is the most widely grown cereal crop and serves as a staple food for over 50% of the global population. Wheat often suffers from zinc (Zn) deficiency, particularly in developing countries, leading to lower Zn content in populations that rely heavily on wheat as a staple food (Qaswar *et al.*, 2017) [17].

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Zinc fertilizers are vital for supplying essential micro-and macronutrients to plants and are one of the eight critical microelements required for proper growth, enzymatic activity, biomass accumulation, chlorophyll stability, cell division, and as a regulatory co-factor in protein synthesis (Shehzadi *et al.*, 2024) [20]. However, excessive zinc can cause phytotoxicity, leading to reduced chlorophyll content and nutritional imbalances. ZnO NPs, due to their small size, increased surface area, and high surface-to-volume ratio, possess unique physicochemical properties. Their application in soil or as foliar sprays can improve zinc bioavailability, enhance nutrient absorption efficiency, and reduce the negative effects of zinc deficiency in wheat crops. Traditional zinc fertilizers are less effective in neutral to alkaline soils due to limited zinc mobility in plants, resulting in unutilized zinc accumulation in soils and potential environmental risks (Elemike *et al.*, 2019) [8].

Despite their benefits, the impact of different sizes of ZnO-NPs on wheat, particularly regarding their application via foliar methods, requires further study. Understanding how the size of ZnO NPs affects their interaction with wheat crops biochemical agents is crucial for optimizing their effectiveness in enhancing superoxide dismutase (SOD) and catalase activity, zinc nutrient uptake, and phytic acid content in wheat.

2. Materials and Methods

2.1 Synthesis of Zinc-oxide nanoparticle

The synthesis of zinc oxide nanoparticles (ZnO NPs) was conducted following the procedures outlined in previous studies (Mahmood *et al.*, 2022) [14]. Reagent-grade chemicals such as $Zn(NO_3)_2{\cdot}6H_2O$, NaOH, ammonia, and oxalic acids were used in the preparation. To begin, 0.8 g of NaOH was dissolved in 200 mL of double-distilled water to create a 0.1 M NaOH solution. This solution was stirred using a magnetic stirrer at 400 RPM for 30 minutes, with the temperature maintained at 50°C. Separately, a 0.1 M $Zn(NO_3)_2{\cdot}6H_2O$ solution was prepared by dissolving 5.950 g of $Zn(NO_3)_2{\cdot}6H_2O$ in mL of double-distilled water, then stirred at 400 RPM for 2 hours at a temperature of 60200°C. To maintain a pH of 12, 4 drops of a 3% ammonia solution were added during stirring. Afterward, the 0.1 M NaOH solution was added dropwise to the $Zn(NO_3)_2{\cdot}6H_2O$ solution, allowing the temperature to drop to room temperature, followed by an additional 2 hours of stirring at 400 RPM. The resulting white precipitate was filtered, washed with ethanol, dried, and ground into a powder form, yielding ZnO-NPs (Gupta *et al.*, 2021) [9]. The zinc content of the powder was analyzed using UV-Visible spectroscopy (Evolution 300; Thermo Fisher, USA). The size of the ZnO-NPs was determined using transmission electron microscopy (TEM) (JEOL-1011, Japan; 100 KVA). For TEM analysis, a solution of ZnO-NPs powder and double-distilled water was applied to a carbon-coated grid, left to settle for 2-3 minutes, then washed. Excess water was drained off, and the samples were dried for 2 hours before size determination. The average sizes of the ZnO-NPs were 30, 40, and 95 nm. The synthesized ZnO-NPs of various sizes were then used to assess their effects on biochemical parameters and nutrient bio-availability in wheat crop.

2.2 Study Area

The study was conducted during the Rabi season of 2023 at MB4C plot, Indian Agricultural Research Institute, located at the research farm in New Delhi, India. Wheat variety HD-2967, were selected for the experiment. The site is situated at an elevation of 228.6 meters above mean sea level in the north western region of India, with geographical coordinates of 28° 37' N latitude and 77° 12' E longitude. This experimental location is within a subtropical semiarid region, receiving a total rainfall of 967 mm from November 2022 to April 2023 during the wheat growth period. The average relative humidity throughout the study was around 67.9%. The potting soil was sourced from a depth of 15 cm at the MB 4C research farm and was characterized as sandy loam with 55% sand, 32% silt, and 13% clay. The soil was first sun-dried, thoroughly mixed, sieved through a 2.0 mm mesh, and then filled into conical plastic pots (25 cm tall and 32 cm in inner diameter) at a rate of 10 kg per pot. ZnO-NPs were precisely weighed, dissolved in deionized water using a magnetic stirrer, and taken for spraying at crown root initiation and tillering stage.

2.3 Experimental Design

The experiment was accomplished with eight treatments: CK: (without Zn-NP), T_1: ZnO-NPs 35 nm (25 mg kg^{-1}), T_2: ZnO-NPs-40 nm (25 mg kg^{-1}), T_3: ZnO NPs-95 nm (25 mg kg^{-1}). The crop was grown using a completely randomized design (CRD). The pot soil had pH, organic carbon (OC), available nitrogen 253 kg (ha^{-1}), phosphorus (P), and potassium (K) 7.7, 0.49%, 253 (kg ha^{-1}), 10.21 (kg ha^{-1}) and 106 (kg ha^{-1}) respectively. During wheat sowing the Zn content of soil was 0.62 (mg kg^{-1}). Before sowing, half of the suggested amount of nitrogen (N) (0.52 g per pot) and the full amounts of P_2O_4 (0.43 g per pot), K_2O (0.4 g per pot), and S (0.31 g per pot) were applied. The nitrogen was given in three split applications first (0.26 g per pot) during sowing and 0.13 g per pot during crown root initiation and 0.13 g per pot during tillering stage.

2.4 Analysis of Superoxide dismutase and catalase

The leaves were collected at tillering stage and stored in an icebox at-80°C. CAT and SOD enzymatic activities were assessed based on the procedures outlined by Narwal *et al* (2009) [15] and Bradford (1976) [5], respectively.

2.5 Analysis of Phytic acid content in grains

To determine the phytic acid content in the grains, we followed the methods described by Vaintraub *et al.* (1988) [21] and Carneiro *et al.* (2002) [6]. The analysis was conducted using the Megazyme Phytic Acid Kit (CAT. No. K-PHYT, Lot No. 220224-2). Four standards with concentrations of 0, 0.5, 2.5, 5.0, and 7.5 mg L^{-1} were prepared to generate a calibration curve, which showed a high degree of accuracy ($R^2 = 0.996$). Absorbance measurements were taken at a wavelength of 655 nm using a UV-visible spectrophotometer (Evolution 300; Thermo Fisher, USA). Phytic acid content was calculated as phosphorus (P) (g 100 g^{-1}) divided by 0.282 (source: www.megazyme.com).

2.6 Statistical analysis

Statistical analysis was carried out in R software to determine the level of significance between varied size ZnO-NP application on wheat crop biochemical and nutrient bio-availability paramters. A two-way analysis of variance (ANOVA) and Duncan test was performed to assess the effects of varying size ZnO-NPs on crop biochemical parameters at 95% confidence level ($p<0.05$).

3. Results

3.1 Effect of various size ZnO-NP on biochemical attributes:

With application of varied size ZnO-NPs SOD content increased from 4% to 18% with ZnO-NP of small size (35 nm). Analysis of data revealed that SOD content varied significantly ($p<0.001$)

among varied size ZnO-NP application (Table. 1).

CAT content varied significantly with application of varied size ZnO-NPs (Table. 1). It was noticed that CAT content increased from 7% to 30% with application of different sized ZnO-NPs and maximum CAT content was observed with 35 nm ZnO-NPs application.

Table 1: Effect of varied size ZnO-NPs on biochemical and grain nutrient availability

Treatment	SOD (U g^{-1} FW)	CAT (U g^{-1} FW)	Grain Zn Nutrient (mg kg^{-1})	Phytic Acid (g 100g^{-1})
Ck	22.81^{d}	13.75^{d}	13.45^{d}	0.63^{a}
T$_1$	26.25^{a}	17.95^{a}	27.55^{a}	0.42^{d}
T$_2$	24.75^{b}	16.20^{b}	24.12^{b}	0.51^{c}
T$_3$	23.2^{c}	14.34^{c}	19.64^{c}	0.58^{b}
CD	1.38	0.55	3.49	0.49
SE (±)	0.27	0.12	1.38	0.25

Ck-Control
T$_1$-35 nm ZnO-NPs (25 mg kg^{-1})
T$_2$-40 nm ZnO-NPs (25 mg kg^{-1})
T$_3$-95 nm ZnO-NPs (25 mg kg^{-1})

3.2 Effect of various size ZnO-NPs on grain nutrient availability

Grain Zn content increased from 19.6 mg kg^{-1} to 27.55 mg kg^{-1} with application of varied size ZnO-NPs. Duncan test shows that grain Zn content varied significantly (*p*<0.001) with different sized ZnO-NPs and maximum grain zn content was observed in 35 nm ZnO-NPs application (Table 1).

Phytic acid content determines the bioavailability of micro-nutrient from grains. The current research showcases that phytic acid content in grain decreases from 7% to 33% with application of various size ZnO-NPs. Duncan test reveals that phytic acid content varied significantly (p<0.001) with application of varied size ZnO-NPs and maximum reduction is seen with 35 nm ZnO-NPs (Table 1).

4. Discussion

The application of ZnO nanoparticles (ZnO NPs) led to a significant increase in protein, proline, superoxide dismutase (SOD), and catalase (CAT) levels in plant leaves (Basit *et al.*, 2022) [4]. The use of nanoscale Zn enhances Zn concentration in plant leaves, which likely contributes to the observed improvements in proline, protein, SOD, and CAT levels. This is because Zn is an essential element for enzyme activation, stabilization, catalysis, supporting biochemical reactions, and structural protein synthesis (Naseer *et al.*, 2023) [16]. Further, Waqas Mazhar *et al*. (2022) [22] conducted an experiment on rice in which they did priming of rice seeds with ZnO-NPs and noticed that activities of SOD and CAT enhanced by 11% and 13% respectively. Song *et al*. (2021) conducted an experiment on rice and observed that the foliar application of zinc oxide nanoparticles (ZnO-NPs) increases the concentration of CAT and SOD in leaves. Our results pertaining to CAT and SOD supports their findings where these enzymes increase with application of varied size ZnO-NPs and maximum concentration is observed with 35 nm ZnO-NPs application. In previous studies it has been stated that the absorption of NPs is prominent with comparability of leave pore spaces and size of NPs (Basit *et al*, 2022) [4].

The increased zinc (Zn) concentration in rice grains may be attributed to enhanced Zn translocation, photosynthesis, enzymatic activities, nutrient assimilation, and the stimulation of gene expression. Zhang *et al*. (2021) [24] reported that applying various concentrations of ZnO-NPs foliarly to rice significantly increased Zn concentrations in grains. Similarly, Rizwan *et al*. (2019) [18] observed that priming wheat seeds with ZnO-NPs led to a linear increase in Zn concentration in different plant parts. Afzal *et al*. (2022) [1] found that using ZnO-NPs as a foliar spray on rice resulted in higher Zn accumulation in the shoots, roots, and leaves compared to the control. Our study corroborated with these findings and we also found that maximum Zn concentration is seen in wheat grains with 35 nm ZnO-NPs application.

Phytic acid, which is primarily present in crops as a form of phosphorus, accumulates throughout the growth period and constitutes 60 to 70% of the total phosphorus in plants. This naturally occurring compound binds with minerals like zinc, reducing its bioavailability for absorption by humans and animals (Kumar *et al.*, 2017) [12]. In this study, foliar applications of ZnO-NPs significantly (*p*<0.001) reduced phytic acid content in grains compared to the control. The decrease in phytic acid concentration enhances the bioavailability of minerals, thereby increasing Zn levels in plant parts (Table. 1). The unique physicochemical properties and higher surface area of ZnO-NPs improve the interaction between plant roots and Zn, which may explain the reduction in phytic acid content in wheat grains. Amat *et al*. (2022) [2] also suggested that lowering phytic acid levels in soybean grains can enhance mineral bioavailability. Our study also seen shown similar results with maximum reduction of phytic acid with smaller sized ZnO-NPs (35 nm).

5. Conclusion

In conclusion, the application of zinc oxide nanoparticles (ZnO-NPs) significantly enhances wheat crop performance by increasing zinc uptake, enzymatic activity, and reducing phytic acid content, particularly with smaller-sized nanoparticles (35 nm). These nanoparticles effectively improve the bioavailability of zinc and other micronutrients, contributing to better crop yields and nutritional quality. The study demonstrates the potential of using ZnO-NPs as a sustainable strategy to address zinc deficiency in crops, thereby benefiting both agricultural productivity and human health. Further research is needed to optimize their application and minimize any potential environmental impacts.

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