Research Article

Effect of Zinc Oxide Nanoparticles on the Growth and Germination of Wheat Plants *Triticum aestivum L*

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Abstract | This research aims to study the impact of Zinc oxide nanoparticles on Wheat Plants (*Triticum aestivum L.*) growth and germination. For this purpose, four treatments were arranged in pots with three replicates each and these treatments include a control group (without ZnO NPs), and three treatments with 100 mg (treatment 1), 200 mg (treatment 2) and 300 mg (treatment 3) of ZnO NPs per pot. Each pot was sown with 50 surface-sterilized wheat seeds at 5–10 cm depth and irrigated daily with distilled water. ZnO NPs were mixed into the top 5 cm of soil at the time of sowing and germination was observed after five days in the control group, while treatment 1 showed emergence on day 7, and treatments 2 and 3 showed delayed emergence after 10 and 20 days, respectively. Fertilizer was added on days 30 and 60 in accordance with standard agronomic practices. Spikes began forming by week 15, and the trial concluded at week 16. Growth performance and physiological indicators revealed that treatment 1 improved chlorophyll content, plant length, and spike yield (meaning 12 grains/spike), compared to 10 grains/spike in the control group. In contrast, treatments 2 and 3 exhibited reduced germination rates, leaf chlorosis, weak structure, and lower grain numbers (7–8 and 5–6 grains/spike, respectively). Soil Texture was analyzed at Al-Wisal Laboratory in Basra governorate to determine ZnO NP accumulation. The control group showed only trace levels of zinc, originating naturally from the soil itself, since no ZnO NPs were added. Treatment 1 presented limited ZnO translocation and favorable physiological outcomes, while higher concentrations resulted in excessive nanoparticle uptake and pronounced phytotoxic effects. These findings emphasize the dual nature of ZnO NPs which include beneficial effect at low concentrations, but harmful when over-applied, underscoring the importance of dose precision in agricultural nanotechnology.

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Introduction

Nanotechnology has emerged as a transformative force in modern agriculture, offering novel approaches to improve crop productivity, and sustainability, among the various nanomaterials explored for agricultural use, zinc oxide nanoparticles (ZnO NPs) have gained considerable attention due to their unique physicochemical properties, including a high surface-area-to-volume ratio, enhanced reactivity, and the ability to release micronutrients in a controlled and targeted manner (Pandya *et al*, 2024; Lahhob *et al*., 2025; Tarmooz *et al*., 2025). It has been highlighted that these characteristics enable ZnO NPs to interact effectively with plant tissues, positioning them as promising candidates for use as nano-fertilizers, zinc is an essential micronutrient for plants, playing a fundamental role in various physiological processes such as chlorophyll biosynthesis, enzyme activation, and stabilization of cellular membranes Minati and Mohammed-ameen (2020). demonstrated that the application of ZnO NPs significantly increased chlorophyll content, and photosynthetic activity in wheat (*Triticum aestivum L.*) under controlled environmental conditions, furthermore, Abbas and Alkhamisi (2025) have, showed that low concentrations of ZnO NPs enhanced seed germination, and seedling vigor in local Iraqi wheat varieties, reflecting their potential to improve early plant development in arid and semi-arid regions, despite these advantages, there is growing concern regarding the ecological risks associated with the excessive or unregulated use of ZnO NPs, reported that elevated concentrations of ZnO NPs adversely affect soil enzymatic functions, and microbial diversity, leading to the disruption of key nutrient cycles Ahmed *et al*. (2021).

Similarly, it has been emphasized the importance of evaluating not only the immediate agronomic benefits of nanoparticle use and also their long-term environmental impacts, particularly in soils already subjected to anthropogenic pressures, interactions between ZnO NPs and soil microbiota—especially fungi—have also been investigated in several regional. Al-hamidi and Yahya (2023) examined the antifungal effects of ZnO NPs on filamentous fungi isolated from contaminated soils in Thi-qar and identified significant growth inhibition in species including Aspergillus niger, aspergillus flavus, Rhizopus Stolonifer, and Rhizopus oryzae at higher nanoparticle concentrations, these findings align with the study of Hassan *et al*. (2016), who reported reduced fungal biodiversity in oil-polluted soils of southern Iraq, particularly noting the suppression of dominant fungal taxa such as aspergillus niger and aspergillus fumigatus following exposure to ZnO nanoparticles.

Despite the mounting evidence on the dual role of ZnO NPs in promoting plant growth, and affecting microbial communities, field-based studies remain limited in the Middle East, there is a lack of comprehensive research addressing the uptake, accumulation, and physiological impact of ZnO NPs on crops cultivated in the clay-loam soils of southern Iraq, the current study aims to bridge this knowledge gap by evaluating the agronomic performance, and biochemical responses of wheat plants subjected to three concentrations of ZnO NPs (100,200, and 300 mg per pot), alongside an untreated control group, the study was conducted over a 16-week period in an open greenhouse environment using natural clay soil collected from the Al-fuhud district in Al-nasiriyah, Dhi Qar, Iraq, it aimed to evaluate the agronomic and biochemical responses of wheat (*Triticum aestivum L.*) to three concentrations of zinc oxide nanoparticles (ZnO NPs)—100, 200, and 300 mg per pot—alongside an untreated control, key parameters assessed included germination rate, plant height, chlorophyll content, grain yield, and zinc accumulation in plant tissues, the goal was to provide region-specific insights into the safe and sustainable application of nanotechnology in agriculture under local environmental conditions, the primary objective of this study is to evaluate the phytotoxic effects of varying concentrations of zinc oxide nanoparticles (ZnO NPs) on wheat (*Triticum aestivum L.*), and also to investigate the environmental fate and mobility of ZnO nanoparticles in agricultural soil, assess the impact of ZnO nanoparticles on the growth and reproduction of dominant soil fungi under laboratory conditions, to quantify the bioaccumulation of ZnO nanoparticles in different parts of the wheat plant.

Materials and Methods

Experimental design

The study was carried out in an open greenhouse environment using natural clay soil sourced from Al-Fuhud district, al- nasiriyah, dhi qar, iraq.

Pot arrangement and treatments

Four treatment groups were established using pots (replicated three times each),
control group – no received ZnO NPs (only wheat seeds in untreated soil),
treated group 1 – received 100 mg ZnO NPs per pot,
treated group 2 – received 200 mg ZnO NPs per pot,
treated group 3 – received 300 mg ZnO NPs per pot.

Before sowing, each pot was filled with 50 wheat seeds that had been surface-sterilized using a 5% sodium hypochlorite (NaClO) solution for 5 minutes, then rinsed thoroughly 4–5 times with sterile distilled water and air-dried on filter paper, irrigation was carried out daily with distilled water, organic fertilizer added twice: once after 30 days, and again after 60 days of growth Zarcinas *et al*. (1987).

Sample collection and processing

After the growth period, plants were harvested and separated into roots, stems, and leaves. These parts were dried, ground, and digested using nitric acid for zinc content analysis. Soil samples were simultaneously extracted using hydrochloric acid. Filtration was performed using Büchner funnels and filter paper, samples were stored in a refrigerator until analysis [8].

Chlorophyll content analysis

The leaf samples underwent chlorophyll extraction using acetone before scientists measured their chlorophyll a, b and total chlorophyll content through UV-Visible spectrophotometry according to Goodwin (1976).

Zinc concentration measurement

All zinc concentrations in both soil and plant parts were quantified using an Automated Atomic Absorption Spectrophotometer (AAS), operated at Al-Wisal Analytical Laboratory, Basra governorate.

Results and Discussion

Effect of ZnO Nanoparticles on germination timing and germination percentage

The germination of wheat seeds began five days after planting (10/12/2024) in the control group, indicating optimal soil conditions without added zinc oxide nanoparticles, these results are consistent with the findings of Solanki and Laura. (2018) who reported that untreated control groups exhibited normal germination patterns, similarly, the control group in the study of Alsuwayyid (2022) also showed rapid germination without delay, confirming that the absence of ZnO NPs allows seeds to complete early activation processes efficiently. In the 100 mg ZnO NPs treatment group, germination was observed after seven days (12/12/2024), showing a slight delay but still within an acceptable biological range, this is in line with the results of Rai-Kalal and Jajoo (2021), who found that lower concentrations of ZnO NPs did not cause significant phytotoxic effects during the germination stage.

A comparable trend was reported by Alsuwayyid (2022), where their lowest nanoparticle concentration (2500 ppm) maintained full germination capacity (100%), indicating that moderate ZnO NP levels do not disrupt germination and may remain within the physiologically tolerated range. However, germination was significantly delayed in the 200 mg group (15/12/2024) and markedly inhibited in the 300 mg group, where seeds germinated only after twenty days (25/12/2024), this trend supports previous findings by Solanki and Laura (2018), who observed a strong dose-dependent delay in germination with increasing ZnO NP concentrations. This dose-dependent inhibition is also consistent with the findings of Alsuwayyid (2022), who recorded a substantial decline in germination at higher ZnO NP concentrations (10,000–15,000 ppm), accompanied by clear physiological stress responses and impaired radicle emergence. Furthermore, each pot contained 50 wheat seeds, in the control group, 44 seeds germinated, while the 100 mg group showed a slightly higher germination (49 seeds), the 200 mg group dropped to 35 successful germinations, and the 300 mg group showed only 29 germinated seeds, these results align with Rai-Kalal and Jajoo (2021), who attributed reduced germination rates at higher concentrations to potential interference with hormonal and enzymatic functions necessary for early plant development as shown in Table 1, also, statistical methods showed significant differences between concentrations, numbers of spikes, and number of grains. Similarly, Alsuwayyid (2022) documented reduced germination and early seedling vigor at elevated ZnO NP levels, which they associated with nanoparticle accumulation, oxidative stress, and disruptions in metabolic activity—factors that closely mirror the reductions observed in the 200 mg and 300 mg treatments in the present study.

Table 1: *Effect of ZnO NPs on average number of spikes and grains per spike in wheat (Triticum aestivum L).*

ZnO NPs Concentration	Average number of spikes	Average number of grains per spike
Control	49±0.577	11±1.000
100 mg	51±1.527	11±1.154
200 mg	36±1.527	8±0.577
300 mg	28±2.645	5±1.154

L.SD: 0.022 L.S.D: 0.000

Effect of ZnO NPs on vegetative growth and chlorophyll content

the plants grown in the control group showed a healthy appearance with sturdy stems and a clear richness in chlorophyll, in the 100 mg treatment the plants exhibited even better growth, with deeper green leaves and a noticeably larger leaf surface area, suggesting that at low concentrations ZnO NPs may function like supportive micronutrients similar to mild fertilizers Figure 1, this observation aligns with the findings of Rai-Kalal and Jajoo (2021) who reported that priming wheat seeds with 10 mg/L ZnO NPs improved seedling vigor, chlorophyll content, and general plant health through enhanced water uptake and increased enzymatic activity, similar improvements were reported by Ram (2023) who found that wheat varieties treated with 100 ppm ZnO NPs developed greener foliage, produced more tillers, and showed higher test weight which indicates that moderate exposure to nanoparticles can stimulate photosynthetic efficiency and support stronger structural development, likewise Pirzada (2021) demonstrated that low concentrations of ZnO NPs increased shoot length, fresh weight, and chlorophyll accumulation in wheat seedlings, attributing these improvements to higher auxin activity and better metabolic regulation which agrees with the improved leaf area and stronger greenness observed in the 100 mg treatment in the present study.

Figure 1: *Vegetative growth of wheat plants (Triticum aestivum L.) after 11 weeks under different ZnO nanoparticle concentrations: Control (no ZnO), Treatment 1 (100 mg), Treatment 2 (200 mg), and Treatment 3 (300 mg). All pots show varying degrees of leaf density and height.*

In contrast, higher concentrations (200 and 300 mg) led to visible chlorosis, shorter stem length, weak leaf structure, and fragile plants , Chlorophyll analysis confirmed a sharp decline in pigment levels in these groups, aligning with signs of oxidative stress and toxicity, these results are also supported by Solanki and Laura (2018), who documented that excessive levels of ZnO nanoparticles suppressed chlorophyll synthesis and plant vigor due to zinc-induced physiological stress as shown in Table 2 Also, statistical methods showed significant differences between concentrations and content of chlorophyll.

Table 2: *Effect of ZnO NPs on chlorophyll content in wheat (Triticum aestivum L.).*

ZnO NPs Concentration	Chlorophyll content
Control	1.160±0.001
100 mg	1.225±0.003
200 mg	1.092±0.001
300 mg	1.019±0.002

L.S.D: 0.00 L.S.D:0.167

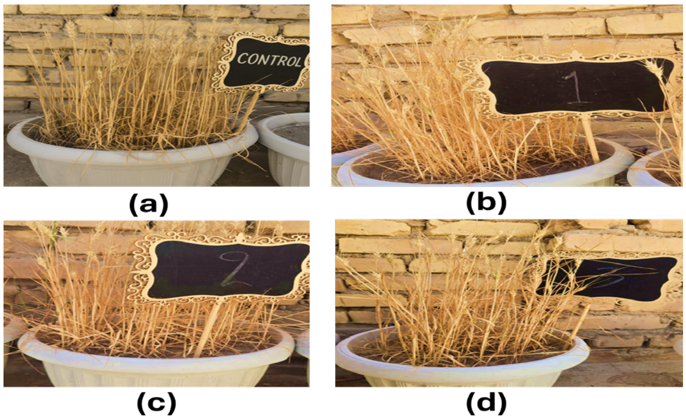

Figure 2: *Morphological appearance of wheat plants (Triticum aestivum L.) after 16 weeks of growth under different ZnO nanoparticle concentrations: (A) Control, (B) 100 mg ZnO NPs, (C) 200 mg ZnO NPs, (D) 300 mg ZnO NPs.*

Impact on reproductive development

The wheat plants in all groups produced their wheat spikes after 15 weeks of planting. The 100 mg group produced the most grains per spike at 12 while exceeding the control group's 10 grains per spike. The research of Rai-Kalal and Jajoo (2021) supports our findings because they discovered that ZnO nanoparticles at specific low concentrations boost grain priming efficiency and enhance nutrient absorption which results in superior grain yields under perfect growth conditions. The 200 mg group produced 7-8 grains per spike but the 300 mg group

Table 3: *Zinc concentration (mg/kg) in soil and different parts of plant wheat (Triticum aestivum L) under varying zno nanoparticle treatments.*

Sample	Control	100 mg ZnO	200 mg ZnO	300 mg ZnO
Soil	61.64±1.043	88.87±0.962	125.72±0.958	217.40±2.265
Plant	92.44±1.56	92.06±1.433	11.70±1.080	192.51±1.853
Root	34.14±2.213	92.45±1.524	115.28±1.225	193.27±1.550
Stem	87.54±1.526	72.48±1.645	135.17±2.122	203.37±2.148
Leaf	17.64±1.852	93.78±1.058	115.87±2.057	197.39±1.593
Grain	8.13±2.117	73.93±1.040	192.44±1.703	211.47±0.950

L.S.D: 0.042 L.S.D: 0.002

produced 5-6 grains per spike Figure 2. The study by Solanki and Laura (2018) showed that higher ZnO nanoparticle concentrations cause reproductive development problems which result in shorter shoots and roots and decreased chlorophyll content as signs of nanoparticle stress. The research shows that wheat yield performance decreases when ZnO NP concentrations exceed certain levels.

The study by Ram (2023) showed that wheat plants treated with 100 ppm ZnO nanoparticles developed more spikelets and produced more grains per spike but high nanoparticle concentrations caused reproductive problems. The research shows that wheat plants experience better yield traits when exposed to moderate nanoparticle levels but their grain development suffers from excessive nanoparticle exposure which creates physiological stress.

Zinc uptake in plant parts and soil residues

Elemental analysis revealed negligible zinc levels in the control plants aside from trace background content from the soil itself, In the 100 mg group minimal translocation of ZnO NPs was detected in roots and stems with no significant accumulation in edible parts ,This group also demonstrated the most favorable physiological response ,On the other hand plants from the 200 and 300 mg groups showed a high accumulation of zinc in roots stems leaves and even seeds which are consistent with symptoms of toxicity, These findings indicate that while ZnO NPs can enhance growth at low levels their accumulation at higher concentrations may pose health risks and reduce crop safety ,Similar results were observed by Rai-Kalal and Jajoo (2021) who reported that increased ZnO NP concentrations especially 300 mg/kg led to significant uptake and accumulation of zinc in wheat tissues particularly in leaves and seeds accompanied by visible signs of phytotoxicity such as chlorosis reduced biomass and poor seed development, This reinforces the conclusion that ZnO NPs exhibit dose-dependent toxicity with potential implications for food safety and plant health as shown in Table 3 ,Also statistical methods showed significant differences between concentrations soil and different parts of plant.

Comparable accumulation patterns were also documented by Alsuwayyid (2022) who found that high ZnO NP concentrations led to substantial zinc buildup in roots and limited but detectable translocation to shoots accompanied by pronounced ultrastructural damage such as mitochondrial swelling and cytoplasmic disruption Likewise Pirzada (2021) reported that increasing ZnO nanoparticle levels caused progressive zinc accumulation within wheat seedlings which corresponded with reduced shoot and root growth and elevated physiological stress markers further supporting the evidence that excessive ZnO NP uptake drives dose-dependent toxicity in wheat tissues.

Conclusions

The data obtained in the present study investigation advanced our knowledge of the role of ZnO Nanoparticle in agriculture environment and overall, low ZnO NP concentrations can enhance growth, but higher levels cause phytotoxic effects. These results emphasize the need for precise dosing to ensure crop safety and soil sustainability. Further long-term studies are recommended to evaluate environmental impacts and nanoparticle behavior in real field conditions.

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Novelty Statement

The novelty of this study lies in providing the first integrated assessment of zinc oxide nanoparticles (ZnO-NPs) behavior in agricultural soil in Thi-Qar Province under real environmental conditions, while simultaneously evaluating their phytotoxicity on wheat and their ecological impact on native soil fungi. This research uniquely combines soil analysis, plant response, and fungal diversity (A. niger, A. fumigatus, R. stolonifer) within a single experimental framework.

In addition, one of the fungal treatments employed a mixed fungal culture, allowing a novel comparison between individual fungal species and a combined microbial consortium in their ability to interact with, transform, or degrade ZnO-NPs. This mixed-inoculum approach has not been previously applied in local or regional studies and provides deeper insight into the collective microbial behavior toward nanoparticles under different carbon and nitrogen conditions.

Author's Contributions

The author carried out all stages of this study, including the design of the experimental work, collection and preparation of soil and fungal samples, synthesis and application of ZnO-NPs, cultivation and monitoring of wheat plants, and laboratory analyses of soil, plant, and fungal responses. The author also performed data processing, statistical analysis, interpretation of results, preparation of figures and tables, and writing and revision of the manuscript.

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Generative AI and AI-assisted technology statement
The author confirms that generative AI tools were used only in a limited manner for minor language polishing and improving clarity of expression. No AI systems were involved in generating data, analyzing results, interpreting findings, or drawing scientific conclusions. The author remains fully responsible for the academic content, methodology, results, and final interpretation presented in this manuscript.

Conflict of interest
The authors have no conflict of interest.

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