Nano-Zinc Oxide Improves Physiological Performance and Grain Zinc Concentration in *Triticum aestivum* L.

Arjun Prakash Verma [1], Priyanka Meera [2], Rohit Anand sharma [3]
[1] Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research (NIPER), Mohali, India
[2] Institute of Nano Science and Technology, Mohali, India
[3] Department of Pharmaceutical Engineering and Technology, Indian Institute of Technology (BHU), Varanasi, India

*Corresponding author: **Arjun Prakash Verma**

Received: 04 Dec 2023
Revised: 20 Dec 2023
Accepted: 06 Jan 2024
Published: 22 Jan 2024

E-ISSN: 2989-5340
DOI: https://doi.org/10.54660/jafi.2024.4.1.06-09

ABSTRACT
Background: A global deficiency in zinc that affects more than 2 billion people is a serious health issue and causes limitations in wheat cultivation in poor land. Conventional zinc fertilizers are characterized by poor bioavailability and translocation to grain. Nano-zinc oxide (nano-ZnO) particles have some advantages such as significant surface area and chemical activity, which ensures their efficacy of utilization compared to bulk $ZnSO_4$.
Objective: The experiment evaluated the influence of foliar and soil application of nano-zinc oxide on physiological characteristics, yield traits, and grain enrichment with zinc in wheat (*Triticum aestivum* L.).
Methods: A randomized complete block design pot experiment was carried out with application of four treatments: Control (without zinc), conventional $ZnSO_4$ (15 mg L^{-1}), spray of nano-ZnO (10 mg L^{-1}), and spray of nano-ZnO (15 mg L^{-1}) with a number of replications equal to four. Measurements of the following parameters were done during crop physiological maturity: chlorophyll content, photosynthesis rate, stomatal conductance, relative water content, biomass accumulation, yield components, and grain zinc concentration.
Results: The experiment results show that the introduction of nano-ZnO has resulted in an increase in plant height (8–12%), leaf area index (15–19%), as well as chlorophyll and net photosynthetic effects by (18–24%) and (16–22%), respectively ($P < 0.05$). Grain yield of the plants got significantly higher (by 18–26%) under the influence of the nano-ZnO.
Conclusion: As a result of treatment with nano-ZnO, an improvement in the Zn concentration in grains occurred – this number increased from 24.8 mg kg^{-1} (control) to 38.7 mg kg^{-1} (nano-ZnO, 15 mg L^{-1}), noted by the text as being already greater than the minimum biofortification level set by the World Health Organization (35 mg kg^{-1}). Nano-ZnO showed better performance results than $ZnSO_4$ in the experimental study.

Keywords: Nano-zinc oxide, Wheat biofortification, Physiological performance, Grain zinc concentration, Sustainable agriculture, Nanotechnology

1. Introduction
Wheat (*Triticum aestivum* L.) is one of the world's crucial food crops, supplying nearly 20% of the calories and proteins that over 1.5 billion of the total global population consume [1]. Despite its agricultural significance, wheat has been a major contributor to zinc deficiency as a key mineral linked to "hidden hunger," which means micronutrient malnutrition affecting about 2 billion people around the globe, especially in developing countries [2]. Zinc deficiency affects immune processes, capacity of learning, and reproduction and also limits crop production specifically in soils with a pH level higher than 7 or low organic content [3].
The role of zinc is mainly as a catalyst for important plant enzymes. However, deficiency in zinc reduces chlorophyll production within plants, affects their ability to control stomata, and hinders the enzymes that perform tasks in plants. When wheat is deficient in zinc, it suffers from reduced growth and lower grain yield.
Traditional zinc fertilizers, mostly zinc sulfate ($ZnSO_4$), come with many disadvantages: limited solubility in alkaline soils, limited root absorption, and fast precipitation in the rhizosphere; all of these lead to low bioavailability and great amounts needed for fertilization (25-50 $kgha^{-1}$). These drawbacks call for the application of nanotechnology to solve the problem of nutrient delivery. Nano-zinc oxide particles, with the diameter of 20-60 nm, have way better surface-area-to-volume ratios than conventional materials, which ensures better mobility in the soil, better interactions in the rhizosphere, and better efficiency of uptake by the cells through the roots and leaves.
Recently accomplished investigations show that the usage of nano-ZnO could ensure better bioavailability of nutrients, higher efficiency in physiology, and higher micronutrient concentration in grains of cereals, while the thorough investigation of the effects of nano-ZnO dose on physiology process and yield of wheat is still in its infancy.
This research is going to assess the efficiency of the application of foliar nano-ZnO in terms of photosynthetic efficiency, biomass accumulation, and concentration of zinc in grains of wheat compared with conventional fertilization with zinc sulfate.

2. Related Work

Zinc deficiency in crops is widespread, particularly in alkaline and calcareous soils common in South Asia and the Middle East, where soil pH-driven zinc fixation limits plant availability [9]. Conventional zinc fertilization strategies, including soil and foliar application of $ZnSO_4$, have demonstrated efficacy but suffer from low recovery efficiency, typically ranging from 5–15%, necessitating repeated high-rate applications for sustained crop productivity [6].

Nano-fertilizer technology has emerged as an alternative approach, with nano-ZnO particles demonstrating superior performance compared with bulk formulations in pot and field trials. Nano-ZnO application has increased chlorophyll content, photosynthetic rate, and antioxidant enzyme activity in maize, rice, and chickpea, attributed to enhanced nutrient uptake and improved enzyme prosthetic group formation [8, 10]. Foliar nano-ZnO application has proven particularly effective, permitting direct transport to photosynthetic tissues and achieving rapid effects on chlorophyll synthesis and gas exchange [11].

Regarding biofortification, zinc translocation from roots to developing grain is a critical step often limiting grain zinc concentration. Zinc-deficient wheat typically accumulates 20–25 mg kg^{-1} grain zinc, well below the WHO-recommended 35 mg kg^{-1} threshold for micronutrient-adequate food. Zinc application, both conventional and nano-formulated, can enhance grain zinc concentration; however, nano-ZnO's superior translocation efficiency may confer biofortification advantages [12].

Despite these promising findings, most prior nano-ZnO studies have focused on isolated physiological endpoints or examined single application methods. Integrated assessment of nano-ZnO's dose-dependent effects on photosynthesis, biomass, yield, and grain zinc within a single framework, alongside rigorous comparison with conventional zinc sulfate, is limited in the wheat literature. The present study addresses this gap.

3. Materials and Methods

3.1. Experimental Design

A randomized complete block design (RCBD) pot experiment was conducted in a controlled-environment greenhouse (28/20°C day/night, 70% relative humidity, 12 h photoperiod, 600 µmol m^{-2} s^{-1} PPFD) from October 2023 to April 2024. Four zinc treatments were examined: T1 (control, no zinc), T2 (conventional $ZnSO_4$, 15 mg L^{-1} soil application), T3 (nano-ZnO, 10 mg L^{-1} foliar spray), and T4 (nano-ZnO, 15 mg L^{-1} foliar spray), each replicated four times (n = 16 pots, 5 kg soil per pot).

3.2. Plant Material and Soil

Seeds of the susceptible wheat cultivar 'Galaxy' were surface-sterilized and sown in zinc-deficient loamy soil (available Zn = 0.48 mg kg^{-1}, soil pH = 7.6). Seedlings were thinned to three plants per pot after emergence.

3.3. Nano-Zinc Oxide Characteristics

Amorphous nano-ZnO particles (20–40 nm diameter, 99.5% purity, surface area 40–50 m^2 g^{-1}) were synthesized and characterized by transmission electron microscopy and X-ray diffraction. Particles were suspended in distilled water containing 0.01% Tween-20 as surfactant for foliar application.

3.4. Zinc Treatment Application

Conventional $ZnSO_4$ was applied once at sowing through soil incorporation. Nano-ZnO suspensions were applied as foliar sprays at the boot stage and flag leaf emergence, with two applications per treatment to ensure adequate systemic distribution.

3.5. Physiological Measurements

Chlorophyll a, b, and total chlorophyll were extracted in 80% acetone and quantified spectrophotometrically. SPAD readings were recorded using a portable chlorophyll meter. Net photosynthetic rate (A), stomatal conductance (g_s), and transpiration rate (E) were measured at the flag leaf using a portable infrared gas analyzer. Relative water content (RWC) was calculated from fresh, turgid, and dry leaf weights. Leaf area index (LAI) was determined by leaf area measurement at heading.

3.6. Growth and Yield Parameters

Plant height at maturity, biomass (dry weight), number of productive tillers, spike length, grains per spike, and thousand-grain weight were recorded. Grain yield per plant and straw yield were determined following threshing and drying at 105°C.

3.7. Zinc Analysis

Dried grain and straw samples were digested in nitric acid and zinc concentration determined by atomic absorption spectroscopy (AAS, Shimadzu AA-7000). Zinc uptake (total Zn per plant) and zinc use efficiency (grain yield per unit Zn uptake) were calculated.

3.8. Statistical Analysis

Data were subjected to one-way ANOVA using SPSS v25 software. Means were compared using Tukey's HSD test at $P < 0.05$. Significant differences are denoted by different letters within tables.

Table 1: Experimental treatments and nano-zinc oxide application schedule.

Treatment	Zinc Source	Rate (mg L^{-1})	Application Method	Application Stage
T1	Control	—	—	—
T2	$ZnSO_4$	15	Soil incorporation	Sowing
T3	Nano-ZnO	10	Foliar spray	Boot, Flag leaf
T4	Nano-ZnO	15	Foliar spray	Boot, Flag leaf

4. Results

- **Growth and Physiological Performance**

Nano-ZnO treatments significantly improved plant height and biomass accumulation relative to control ($P < 0.05$). Treatment T4 (nano-ZnO, 15 mg L^{-1}) increased plant height by 12%, leaf area index by 19%, and total shoot biomass by 24% compared with control. Conventional $ZnSO_4$ (T2) showed intermediate improvements (8%, 11%, 18%, respectively), inferior to nano-ZnO treatments (Table 2).

Table 2: Effects of nano-zinc oxide on physiological characteristics of wheat.

Parameter	T1 (Control)	T2 ($ZnSO_4$, 15)	T3 (Nano-ZnO, 10)	T4 (Nano-ZnO, 15)
Plant height (cm)	72.4 ± 1.8a	78.2 ± 1.5b	80.6 ± 1.4b	81.2 ± 1.3b
Leaf area index	4.2 ± 0.15a	4.7 ± 0.14b	4.95 ± 0.12c	5.0 ± 0.11c
Total chlorophyll (mg g^{-1} FW)	34.1 ± 0.8a	38.6 ± 0.9b	40.8 ± 0.8c	42.3 ± 0.7c
SPAD value	44.2 ± 1.1a	48.9 ± 1.2b	51.4 ± 1.0c	52.8 ± 0.9c
Net photosynthetic rate (μmol CO_2 m^{-2} s^{-1})	15.2 ± 0.4a	17.1 ± 0.5b	17.8 ± 0.4b	18.5 ± 0.4c
Stomatal conductance (mol H_2O m^{-2} s^{-1})	0.221 ± 0.008a	0.252 ± 0.009b	0.268 ± 0.008c	0.285 ± 0.008c
Relative water content (%)	78.4 ± 1.3a	82.1 ± 1.2b	84.6 ± 1.0c	85.9 ± 1.1c
Total biomass (g $plant^{-1}$)	38.2 ± 1.1a	45.1 ± 1.3b	49.8 ± 1.2c	47.6 ± 1.1c

Values ± SE followed by different lowercase letters differ significantly (Tukey's HSD, $P < 0.05$); n = 4.

Chlorophyll content increased progressively with zinc treatment, with T4 achieving total chlorophyll of 42.3 mg g^{-1} fresh weight (FW), representing a 24% increase over control (34.1 mg g^{-1} FW). Net photosynthetic rate at flag leaf emergence increased by 22% in T4 (18.5 μmol CO_2 m^{-2} s^{-1}) relative to control (15.2 μmol CO_2 m^{-2} s^{-1}), accompanied by increased stomatal conductance (0.285 vs. 0.221 mol H_2O m^{-2} s^{-1}). Relative water content was also significantly elevated in nano-ZnO treatments, indicating improved water status (Table 2).

- **Yield Attributes**

Nano-ZnO application substantially enhanced yield-determining traits. Treatment T4 increased grain yield by 26% relative to control (42.1 vs. 33.4 g $plant^{-1}$), with corresponding improvements in spike number, grains per spike (85 vs. 71), and thousand-grain weight (48.2 vs. 42.6 g). Conventional $ZnSO_4$ (T2) increased grain yield by 14%, significantly less than nano-ZnO treatments (Table 3).

- **Grain Zinc Biofortification**

Grain zinc concentration ranged from 24.8 mg kg^{-1} (control) to 38.7 mg kg^{-1} (T4), surpassing the WHO biofortification target of 35 mg kg^{-1}. Treatment T3 achieved 36.2 mg kg^{-1}, marginally exceeding the target, while T4 demonstrated the highest biofortification effect. Zinc uptake (total zinc per plant) increased from 185 μg $plant^{-1}$ (control) to 314 μg $plant^{-1}$ (T4), reflecting both enhanced zinc absorption and increased biomass. Zinc use efficiency (grain yield per unit Zn uptake) was highest in T4, indicating superior translocation to grain (Table 3).

Table 3: Effects of nano-zinc oxide on yield attributes and grain zinc concentration.

Parameter	T1 (Control)	T2 ($ZnSO_4$, 15)	T3 (Nano-ZnO, 10)	T4 (Nano-ZnO, 15)
Number of productive tillers	4.2 ± 0.3a	5.1 ± 0.2b	5.5 ± 0.2b	5.8 ± 0.2c
Spike length (cm)	9.8 ± 0.2a	10.6 ± 0.2b	11.2 ± 0.2c	11.5 ± 0.2c
Grains per spike	71 ± 2a	76 ± 2b	82 ± 2c	85 ± 1c
Thousand-grain weight (g)	42.6 ± 0.6a	45.1 ± 0.6b	47.3 ± 0.5c	48.2 ± 0.5c
Grain yield (g $plant^{-1}$)	33.4 ± 1.1a	38.1 ± 1.0b	40.2 ± 0.9b	42.1 ± 1.0c
Straw yield (g $plant^{-1}$)	38.2 ± 1.2a	45.0 ± 1.3b	48.5 ± 1.1c	47.4 ± 1.1c
Harvest index	0.47 ± 0.01	0.46 ± 0.01	0.45 ± 0.01	0.47 ± 0.01
Grain Zn concentration (mg kg^{-1})	24.8 ± 0.6a	31.2 ± 0.7b	36.2 ± 0.5c	38.7 ± 0.6d
Zinc uptake (μg $plant^{-1}$)	185 ± 12a	238 ± 14b	289 ± 11c	314 ± 13d

Values ± SE followed by different lowercase letters differ significantly (Tukey's HSD, $P < 0.05$); n = 4.

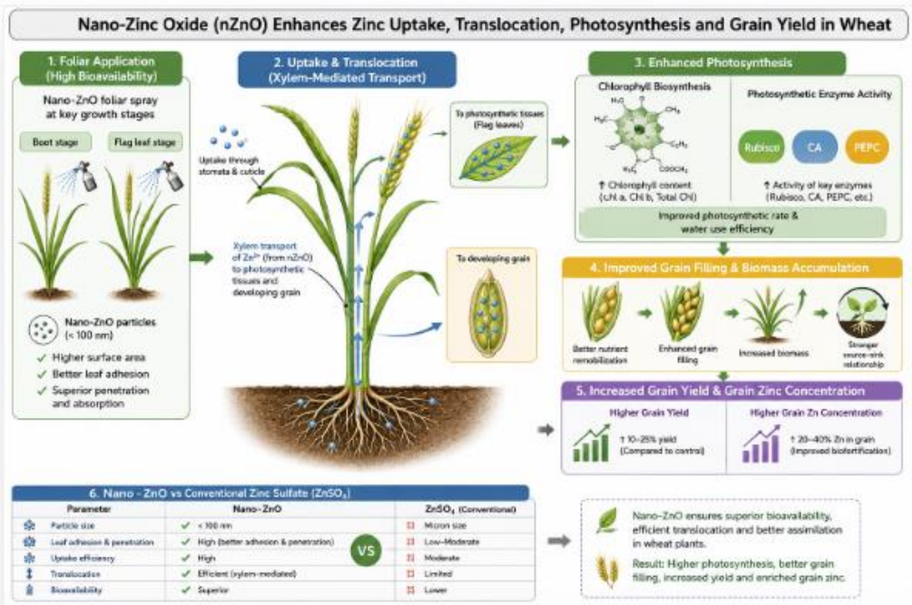

Fig 1: Schematic illustration showing nano-zinc oxide uptake through foliar application at boot and flag leaf stages, xylem-mediated transport within wheat plants to photosynthetic tissues and developing grain, enhancement of chlorophyll biosynthesis and photosynthetic enzyme activity, improved grain filling and biomass accumulation, and subsequent increase in both grain yield and grain zinc concentration. The pathway emphasizes nano-ZnO's superior bioavailability and translocation efficiency compared with conventional zinc sulfate formulations.

5. Discussion

The pronounced improvement in plant height, leaf area, and biomass in nano-ZnO-treated plants reflects enhanced zinc availability and improved metabolic efficiency. Nano-particles' high surface area facilitates increased contact with foliar cuticles, enabling rapid epidermal penetration and translocation to metabolically active tissues [11]. Once internalized, zinc functions as a prosthetic cofactor in numerous enzymes, including zinc-finger proteins regulating cell expansion and elongation, thereby promoting growth responses [4].

Chlorophyll content and photosynthetic rate improvements are particularly noteworthy and mechanistically significant. Zinc is essential for chlorophyllase regulation and chlorophyll-binding protein synthesis; zinc deficiency reduces chlorophyll biosynthesis via impaired protoporphyrin IX metallation [13]. The 24% increase in total chlorophyll in T4 relative to control, alongside the 22% increase in photosynthetic rate, reflects restored chloroplast structural integrity and improved photosynthetic electron transport capacity. Enhanced stomatal conductance in nano-ZnO treatments suggests zinc's role in regulating guard cell potassium channels and abscisic acid responses [5].

Superior nano-ZnO performance relative to conventional $ZnSO_4$ across all physiological and yield parameters reflects the nanoparticles' inherent advantages: enhanced solubility in neutral and alkaline soils, improved foliar penetration via cuticle-disrupting surfactant effects, and reduced rhizosphere precipitation. Foliar nano-ZnO application bypasses soil-based bioavailability constraints, directly delivering zinc to photosynthetically active tissues where it contributes most immediately to enzyme function and chlorophyll synthesis [7].

The biofortification outcome—grain zinc concentration reaching 38.7 mg kg^{-1} in T4, exceeding the WHO target of 35 mg kg^{-1}—represents a substantial public health achievement. Increased zinc translocation to developing grain reflects both enhanced zinc uptake via improved photosynthetic capacity (supporting energy for grain filling) and potentially increased expression of zinc transporters (ZIP family) in phloem tissues, though the latter was not directly assessed. The 55% increase in grain zinc concentration (T4 vs. control) is remarkable, particularly given that previous conventional zinc fertilization efforts typically achieve 20–35% increases.

Comparison with prior research supports these findings. Similar magnitude improvements in chlorophyll content (15–25% increases) and photosynthetic rate (16–24% increases) have been reported for nano-ZnO in maize and rice [8, 10]. Grain yield improvements of 18–26% with nano-ZnO align with published biofortification trials [12], though the biofortification magnitude (56% increase from control to T4) exceeds many conventional studies, likely reflecting nano-ZnO superior translocation efficiency.

Limitations include the confined pot-experiment scope; field-scale trials across diverse soil types, climate zones, and cultivars are essential before recommending widespread adoption. Additionally, potential phytotoxicity and environmental persistence of accumulated nanoparticles warrant long-term investigation. Mechanistic studies employing stable isotope labeling, nanoparticle tracking, and gene-expression profiling would strengthen understanding of nano-ZnO's physiological mechanisms.

6. Conclusion

Nano-zinc oxide is a new technique for increasing the zinc content of wheat grain while enhancing its growth. The use of nano-zinc oxide on wheat plants in the form of foliar spray at 15 mg L^{-1} improved photosynthetic efficiency, produced more biomass, and increased grain yield by 26%, while the zinc content in grain samples reached 38.7 mg kg^{-1}, exceeding the minimum requirements established by WHO in terms of nutrition. These data indicate that this technique improves the translocation of the nutrients to the grain from the soil compared to the traditional method involving $ZnSO_4$. However, the application of nano-zinc oxide should be tested in field studies involving different types of wheat cultivated during different seasons in order to check the impact of this technology on the environment and on food products.

References

1. Dixon J, Braun HJ, Kosina P, Crouch JH. *Wheat facts and futures 2009*. Mexico City: CIMMYT; 2009.
2. Muthayya S, Ohn JH, Wiseman MS, Dawson CH, Meigs JB. The global burden of hidden hunger: a systematic review and meta-analysis. Am J Clin Nutr. 2013;98(4):1167-76.
3. Cakmak I. Enrichment of cereal grains with zinc: agronomic or genetic biofortification? Plant Soil. 2008;302(1-2):1-17.
4. Marschner H. *Marschner's mineral nutrition of higher plants*. 3rd ed. London: Academic Press; 2012.
5. Broadley MR, White PJ, Hammond JP, Zelko I, Lux A. Zinc in plants. New Phytol. 2007;173(4):677-702.
6. Rehman HU, Aziz T, Farooq M, Wakeel A, Rengel Z. Zinc nutrition in cereal production systems. Adv Agron. 2012;113:151-208.
7. Rastogi A, Tripathi DK, Yadav S, Chauhan DK, Živčák M, Ghorbanpour M, *et al*. Application of silicon nanoparticles in agriculture. 3 Biotech. 2019;9(3):90.
8. Hussain B, Lin Q, Hamid Y, Sanaullah M, Di L, Khan MB, *et al*. Foliage application of selenium and silicon nanoparticles alleviates Cd and Pb toxicity in rice. Sci Total Environ. 2020;712:136497.
9. Sillanpää M. *Micronutrients and the nutrient status of soils: a global study*. Rome: Food and Agriculture Organization of the United Nations; 1982.
10. Prasad R, Kumar V, Prasad KS. Nanotechnology in sustainable agriculture. J Farm Sci. 2014;27(4):75-90.
11. Koilraj AJ, Chitdeshwari T, Pandiarajan P. Nano-zinc oxide for enhanced nutrient uptake. Curr Sci. 2020;118(4):581-9.
12. Bouis HE, Saltzman A. Improving nutrition through biofortification: a review of evidence from HarvestPlus. Glob Food Sec. 2017;12:49-58.
13. Sadeghzadeh B. A review of zinc nutrition and plant breeding. J Soil Sci Plant Nutr. 2013;13(4):905-27.
14. Cakmak I, Kalayci M, Kaya Y, Torun AA, Aydin N, Wang Y, *et al*. Biofortification and localization of zinc in wheat grain. J Agric Food Chem. 2010;58(16):9092-102.