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# EVALUATING YIELD AND QUALITY OF WHEAT GRAINS UNDER SINGLE AND INTEGRATED USE OF CHEMICAL AND NANO ZINC FERTILIZERS

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**Abstract**

Pakistani soils are generally deficient in zinc (Zn) and require fertilizer Zn to get optimum crop yield. Nanotechnology serves as a promising solution for sustainable agriculture. It has been advocated that the Zn oxide nanoparticles (ZnO-NPs) greatly enhance the efficiency of applied Zn, improve crop yield, and Zn content of produce compared to chemical Zn fertilizers. The effect of single and integrated application of different doses of chemical and nano-Zn on wheat growth, yield and grain Zn accumulation was studied in a field experiment arranged in a Randomized Complete Block Design with three repeats. The Zn treatments included, $T_1$: control (no Zn applied), $T_2$: 5.0 kg Zn $ha^{-1}$ through Zinc Sulphate, $T_3$: 2.5 kg Zn $ha^{-1}$ through Zinc oxide, $T_4$: 2.5 kg Zn $ha^{-1}$ through Zinc oxide nanoparticles, $T_5$: 1.25 kg Zn $ha^{-1}$ through Zinc oxide nanoparticles, $T_6$: 2.5 kg Zn $ha^{-1}$ through Zinc Sulphate + 2.5 kg Zn $ha^{-1}$ through Zinc oxide nanoparticles, and $T_7$: 1.25 kg Zn $ha^{-1}$ through Zinc Sulphate + 1.25 kg Zn $ha^{-1}$ through Zinc oxide nanoparticles. The results revealed that the application of ZnO-NPs at lower dose (1.25 kg Zn $ha^{-1}$) attained a maximum increase in various physiological parameters and plant Zn accumulation, such as spikelets $spike^{-1}$ (22.4) by 45.7%, grains $spike^{-1}$ (67.5) by 83.5%, weight of 1000 grains (52.9) by 78.7%, grain yield (5320 kg $ha^{-1}$) by 106.1%, straw yield (9719 kg $ha^{-1}$) by 88.5%, straw Zn content (23.7 mg $kg^{-1}$) by 72.5%, and grain Zn content (53.3 mg $kg^{-1}$) by 106.3% over no Zn application. However, maximum plant height (121.2 cm) and spike length (13.9 cm) were noted under integrated application of $ZnSO_4$ with ZnO-NPs at lower dose (1.25 kg Zn $ha^{-1}$, each). Zinc oxide nanoparticles, as an efficient and cost-effective source, could be applied over conventional Zn fertilizer to increase yield and alleviate grain Zn deficiency of wheat.

**Key words:** Zinc deficiency, Nanotechnology, Zinc sulphate, Zinc oxide nanoparticles, Zinc accumulation, Wheat.

## Introduction

Wheat (*Triticum aestivum* L.) is an important cereal crop of the world. The global wheat production is estimated to be around 800 million tons per annum. Historically, the global production of wheat along with rice and maize was 10% in 2000. However, between 2000 and 2020, the production of wheat, in comparison with maize and rice, decreased to 8% (Anon., 2023). According to a report by the United Nations, the global population has been projected to increase from around 8 billion to almost 10 billion (20%) by 2050 (UN, 2022). Given the severity of this issue, it is becoming a dire need of the day to enhance wheat production to feed this growing population (Albahri *et al.,* 2023).

Zinc (Zn) is one of the contributing micronutrients for the proper growth and development of plants (Cakmak & Kutman, 2018). In plants, Zn plays important roles in various enzyme processes, metabolic and redox reactions, synthesis of protein, carbohydrate metabolism, photosynthesis, and the conversion of sugar into starch. Additionally, it is involved in maintaining the integrity of membranes (Broadley *et al.,* 2012). Zinc also influences the activities of hydrogenase and carbonic anhydrase, ribosomal function stability, and cytochrome synthesis. For these reasons, Zn deficiency can restrict plant growth, cause diseases, reduction in crop yield and badly affect grain quality (Yadav *et al.,* 2023). In arid and semi-arid regions of the world, including Pakistan, Zn deficiency is prevalent, primarily attributed to increased Zn fixation and reduced Zn solubility (Rashid *et al.,* 2022). The optimum grain Zn content of wheat adequate for human nutrition should be about 45 mg $kg^{-1}$. However, wheat grains usually contain only around 20-35 mg Zn $kg^{-1}$ (Yadav *et al.,* 2023). To cope with this, Zn application through different fertilizer sources becomes indispensable to address its deficiency in soils and crops (Rehman *et al.,* 2020) and enhance its content in wheat grains (Rashid *et al.,* 2022). The efficiency of conventional Zn fertilizers is limited in those soils which contain low organic matter and have high soil pH and carbonate content (Rehman *et al.,* 2020). These conditions are common in Pakistani soils where a significant portion of applied Zn becomes unavailable to crop plants (Rashid *et al.,* 2022). The major reasons of Zn unavailability are alkalinity and calcareousness of soils which result in the fixation and adsorption of Zn, resulting in its reduced solubility (Abbas *et al.,* 2009). Moreover, soils with high proportion of sand, low organic matter, and high levels of phosphorus also exhibit Zn deficiency (Alloway, 2008).

Nanotechnology introduces nano-sized particles, such as nanofertilizers (NFs), nanopesticides, and nanocarriers for sustainable agriculture. These nanofertilizers enhance nutrient use efficiency due to their unique physicochemical properties and mode of action and hence decrease soil toxicity more effectively (Javed *et al.,* 2024). They have a tiny particle size and higher surface area and reactivity. As the name suggests, the particle size of the nanofertilizers ranges from 1-100 nm. Zinc oxide nanoparticles (ZnO-NPs) are considered ideal for crop Zn nutrition (Ditta & Arshad, 2016). Zinc nanofertilizer promotes the use efficiency of Zn, thereby enhancing the uptake in the specific plant parts (Al-Khayri *et al.,* 2023). As the deficiency of Zn is common in soils of Pakistan, the utilization of ZnO-NPs presents a potential solution as an effective source of Zn for enhancing growth and Zn content in cereal grains (Umar *et al.,* 2021). Nano-Zn fertilizers outweigh the use of conventional fertilizers as they improve Zn use efficiency and have positive environmental impacts (Umar *et al.,* 2021; Adil *et al.,* 2022). Zinc nanofertilizers have the potential to stimulate root development and elevate Zn levels in grains. The concentration of Zn in wheat grains can be increased by using ZnO-NPs since they are not affected by soil and environmental conditions that limit the efficiency of conventional fertilizers (Adrees *et al.,* 2021). Moreover, ZnO-NPs have proven to have higher effectiveness for fortification of grain Zn in wheat under normal as well as unfavorable conditions (Mazhar *et al.,* 2023). These ZnO-NPs may be recommended for biofortification of wheat, either used individually or in combination with conventional Zn sources, especially in Zn-deficient calcareous soils (Ahmad *et al.,* 2023). However, only a few studies have reported the potential impact of nano-Zn fertilizers on cereal yield or their grain Zn content (Umar *et al.,* 2021).

Keeping in view the facts given above, the present study was conducted with the hypothesis that zinc oxide nanoparticles, as an efficient and cost-effective source, can potentially supplement conventional Zn fertilization to enhance wheat yield and can address grain Zn deficiency. The objectives of the current study were to study the influence of single and integrated application of different doses of fertilizer-Zn and nano-Zn fertilizer on growth, yield, and Zn content of wheat grains.

## Material and Methods

**Physicochemical properties of experimental site:** A field experiment was performed at the experimental fields of the Nuclear Institute of Agriculture (NIA), Tandojam (25.414598N, 68.520897E), Pakistan. Soil samples were collected before fertilizer application and wheat sowing from the experimental field. The sampling was done using a stainless-steel augur at a depth of 0-20 cm. The samples were processed and analyzed for EC, pH, texture, organic matter, and available P, K, and Zn according to the standard procedures outlined by Ryan *et al*., (2001). The results suggested that the experimental soil had 17.5% sand, 57.5% silt, and 25% clay with an overall textural class of silt loam. Soil was non-saline (0.95 dS $m^{-1}$), medium alkaline (pH 7.81), low in organic matter content (0.67%), marginal in available P (6.0 mg $kg^{-1}$) and K (90 mg $kg^{-1}$), and low in available Zn (0.48 mg $kg^{-1}$).

**Experiment details:** The experiment was arranged in a Randomized Complete Block Design, consisting of seven treatments with three repeats. The treatments included the use of various sources of chemical and nano-Zn fertilizers, as T1: Control (No Zn applied), T2: 5.0 kg Zn $ha^{-1}$ through Zinc Sulphate, T3: 2.5 kg Zn $ha^{-1}$ through Zinc oxide, T4: 2.5 kg Zn $ha^{-1}$ through Zinc oxide nanoparticles, T5: 1.25 kg Zn $ha^{-1}$ through Zinc oxide nanoparticles, T6: 2.5 kg Zn $ha^{-1}$ through Zinc Sulphate + 2.5 kg Zn $ha^{-1}$ through Zinc oxide nanoparticles, T7: 1.25 kg Zn $ha^{-1}$ through Zinc Sulphate + 1.25 kg Zn $ha^{-1}$ through Zinc oxide nanoparticles. Pure analytical grade zinc sulphate ($ZnSO_4.H_2O$) and zinc oxide were used to administer the chemical Zn treatments. While Zinc oxide nanoparticles were developed through chemical synthesis (Buledi *et al.,* 2020) in the research lab of the National Center of Excellence in Analytical Chemistry (NCEAC), University of Sindh, Jamshoro. The size of each experimental unit was 9 $m^2$ (3 m x 3 m). The land was prepared following established practices and procedures. The sowing of wheat was done on November 16 during the Rabi season of 2022-23. Pure (99.9%) quality pre-basic seeds of wheat variety NIA-Shaheen were sown through the drilling method with a seed drill machine at the rate of 125 kg $ha^{-1}$. The distance between rows was kept 20 cm. A blanket dose of N, P, and K fertilizers was applied at 120-40-30 kg N-P-K $ha^{-1}$ through urea, DAP, and SOP, respectively. Full doses of P and K, and $^1/_3$ of N fertilizer were applied at sowing through the broadcasting method. The remaining amount of N fertilizer was applied in two equal splits at first and second irrigation. The Zn treatments were applied at first irrigation to avoid P-Zn antagonistic effect.

**Growth and yield parameters and Nutritional analysis:** During this study, 10 randomly selected plants were harvested from each experimental unit for recording various agronomic observations. The observations recorded included, plant height (cm), spike length (cm), spikelets per spike, number of grains per spike, weight of 1000 grains (g), grain yield (kg $ha^{-1}$), and straw yield (kg $ha^{-1}$). Zinc concentration in wheat straws and grains was determined. To quantify the concentration of Zn in plant, working standards of Zn followed by plant samples were analyzed on an atomic absorption spectrophotometer (Ryan *et al.,* 2001).

**Statistical analysis:** The data were subjected to ANOVA using Statistix ver. 8.1. The means of all treatments were compared using LSD test at alpha 0.05 ($p \leq 0.05$).

## Results

**Growth and yield parameters:** The application of Zn fertilizers significantly ($p \leq 0.05$) enhanced plant height of wheat. Maximum plant height (121.2 cm) was noted in response to 5.0 kg Zn $ha^{-1}$ (40.9% more) applied through the integration of ZnO-NPs (2.5 kg Zn $ha^{-1}$) and $ZnSO_4$ (2.5 kg Zn $ha^{-1}$), compared to the minimum plant height (86.0 cm) the control treatment (where no Zn was applied). The least relative increase in comparison to control was recorded in chemically applied Zn treatment through ZnO (14.8%) at 2.5 kg Zn $ha^{-1}$ with 98.8 cm. Chemically applied Zn through $ZnSO_4$ (5.0 kg Zn $ha^{-1}$) obtained a plant height of 121.0 cm with 40.7% increase in comparison to control. Application of ZnO-NPs at higher (2.5 kg $ha^{-1}$) and lower rates (1.25 kg $ha^{-1}$) showed a relative increase of 37.8% (118.6 cm) and 39.4% (119.9 cm) respectively. The integrated use of ZnO-NPs (1.25 kg Zn $ha^{-1}$) with $ZnSO_4$ (1.25 kg Zn $ha^{-1}$) at lower rate (2.5 kg Zn $ha^{-1}$) obtained an increase of 39.4% (119.9 cm) over control treatment (Table 1).

**Table 1. Effect of single and integrated applications of chemical and nano zinc fertilizers on growth and yield attributes of wheat.**

| Treatments | Plant height (cm) | Spike length (cm) | Spikelets spike$^{-1}$ | Number of grains spike$^{-1}$ | Weight of 1000 grains (g) | Grain yield (kg ha$^{-1}$) | Straw yield (kg ha$^{-1}$) |
|---|---|---|---|---|---|---|---|
| $T_1$ | 86.0 ± 0.53 c | 9.5 ± 0.35 d | 15.4 ± 0.15 d | 36.8 ± 0.12 e | 29.6 ± 0.46 e | 2581 ± 27.7 d | 5155 ± 112.3 d |
| $T_2$ | 121.0 ± 0.22 a | 13.6 ± 0.11 a | 21.1 ± 0.25 b | 64.4 ± 1.25 b | 50.3 ± 0.76 b | 4955 ± 183.6 ab | 8948 ± 145.2 b |
| $T_3$ | 98.8 ± 1.35 b | 11.2 ± 0.23 c | 17.4 ± 0.64 c | 47.8 ± 0.27 d | 39.9 ± 0.18 d | 3660 ± 60.8 c | 7011 ± 97.8 c |
| $T_4$ | 118.6 ± 0.14 a | 12.9 ± 0.17 b | 21.0 ± 0.09 b | 64.2 ± 0.20 b | 50.1 ± 0.52 b | 4927 ± 161.3 ab | 8702 ± 147.7 b |
| $T_5$ | 119.9 ± 1.33 a | 13.5 ± 0.13 ab | 22.4 ± 0.15 a | 67.5 ± 0.38 a | 52.9 ± 0.42 a | 5320 ± 224.2 a | 9719 ± 347.8 a |
| $T_6$ | 121.2 ± 0.58 a | 13.9 ± 0.15 a | 20.5 ± 0.18 b | 62.3 ± 0.41 c | 47.4 ± 0.92 c | 4715 ± 78.6 b | 8768 ± 119.2 b |
| $T_7$ | 119.9 ± 0.53 a | 13.5 ± 0.19 ab | 21.3 ± 0.27 b | 65.2 ± 0.50 b | 51.0 ± 0.91 ab | 5183 ± 176.4 a | 9254 ± 283.1 ab |
| **$LSD_{0.05}$** | **2.6521** | **0.6742** | **0.9863** | **1.7956** | **1.9581** | **394.42** | **555.81** |

Zinc fertilizer treatments include: $T_1$ = Control, no Zn application, $T_2$ = 5.0 kg Zn ha$^{-1}$ through $ZnSO_4$, $T_3$ = 2.5 kg Zn ha$^{-1}$ through ZnO, $T_4$ = 2.5 kg Zn ha$^{-1}$ through ZnO-NPs, $T_5$ = 1.25 kg Zn ha$^{-1}$ through ZnO-NPs, $T_6$ = 2.5 kg Zn ha$^{-1}$ through $ZnSO_4$ + 2.5 kg Zn ha$^{-1}$ through ZnO-NPs, $T_7$ = 1.25 kg Zn ha$^{-1}$ through $ZnSO_4$ + 1.25 kg Zn ha$^{-1}$ through ZnO-NPs. Different letters in the same column indicate significant difference by LSD at $p \leq 0.05$ and ± indicates the standard error (n=3)

Spike length of wheat varied from 9.5 to 13.88 cm across all treatments. The highest spike length was noted in the integrated application of $ZnSO_4$ with ZnO-NPs at higher dose (5.0 kg Zn ha$^{-1}$) with 2.5 kg Zn ha$^{-1}$ each. This treatment attained a 46.1% increase when compared with treatment of no Zn application which obtained the lowest spike length. A significant increase of 43.0%, 42.4%, and 41.8% over no Zn treatment was observed with $ZnSO_4$ alone (5.0 kg Zn ha$^{-1}$) (13.59 cm), lower dose of ZnO-NPs (13.53 cm), and integrated use of $ZnSO_4$ with ZnO-NPs at lower rate (13.47 cm), respectively. The least relative increase was observed in chemical Zn application through ZnO (17.9%) with 11.2 cm followed by ZnO-NPs (35.8%) at higher rate with 12.9 cm (Table 1).

The spikelets per spike were significantly ($p \leq 0.05$) improved by Zn treatments compared to control treatment. The sole and integrated application of Zn at lower rates acquired a maximum increase of 45.7% and 38.5% respectively over no Zn application treatment. The minimal increase was observed by using chemical Zn through bulk ZnO, showing a 13.0% increment. The integrated application of Zn at higher dose enhanced the number of spikelets spike$^{-1}$ by 33.3%. However, the number of spikelets spike$^{-1}$ were statistically alike under ZnO-NPs at higher dose (21.0) and $ZnSO_4$ (21.1) treatment with a relative increase of 36.6% and 37.0%, respectively, over control (Table 1).

The results related to the influence of various Zn sources on grains per spike are provided in Table 1. Statistically analyzed data suggested that the number of grains per spike were significantly ($p \leq 0.05$) enhanced with respect to Zn fertilizer application through various sources. The application of Zn through ZnO-NPs alone at lower dose recorded the maximum number of grains within a spike over control with 67.5 (83.5%) grains per spike. The integrated use of nano-Zn with inorganic Zn at lower dose showed a relative increase of 77.3% with 65.2 grains per spike when compared with control. Statistically similar results were noticed under $ZnSO_4$ and higher dose of ZnO-NPs (2.5 kg Zn ha$^{-1}$) with 64.4 (75.2%) and 64.2 (74.7%) grains per spike, respectively. The integrated application of $ZnSO_4$ with ZnO-NPs at higher rate produced 62.3 grains spike$^{-1}$ (69.4%). While an increment of 29.9% (47.8 grains per spike) over control was recorded in bulk ZnO treatment.

The weight of 1000 grains was significantly ($p \leq 0.05$) improved by supplementing Zn nutrition. The singly applied ZnO-NPs at lower dose (1.25 kg Zn ha$^{-1}$) performed the best of all treatments with an increase in weight of 1000 grains (52.9 g) of 78.7% over control. Similarly, the integrated application of Zn through $ZnSO_4$ with ZnO-NPs at lower dose, produced a 1000-grain-weight of 51.0 g (72.3%). The lowest increase over control was obtained from chemical Zn treatment through ZnO (39.9 g) followed by the integrated application of chemical with nano-Zn at higher rate (47.4 g) with 34.7% and 60.0%, respectively. Moreover, Zn application as ZnO-NPs at higher dose and conventional Zn application through $ZnSO_4$ were statistically similar (Table 1).

The application of Zn, either, as $ZnSO_4$ or ZnO showed a substantial ($p \leq 0.05$) increase in the grain yield over no Zn application treatment. However, the former treatment was superior to the latter. A two-fold increase was the maximum, recorded with ZnO-NPs (5320 kg ha$^{-1}$) followed by the integrated application of $ZnSO_4$ with ZnO-NPs (5183 kg ha$^{-1}$) at lower rates respectively over control (2581 kg ha$^{-1}$). A substantial decrease in grain yield was noted with the increasing rate of ZnO-NPs. A grain yield of 4927 kg ha$^{-1}$ (90.9%) was recorded under ZnO-NPs at higher dose. The integrated application of ZnO-NPs with $ZnSO_4$ at higher rate showed a relative increase of 82.7% (4715 kg ha$^{-1}$). Chemical Zn treatment under $ZnSO_4$ (5.0 kg Zn ha$^{-1}$) yielded 4955 kg ha$^{-1}$ (92.0%). Moreover, the least relative increase over control was 41.8%, with bulk the ZnO treatment (3660 kg ha$^{-1}$) depicted in Table 1.

Straw yield of wheat varied with respect to Zn application treatments as shown in Table 1. The higher and lower doses ZnO-NPs showed an increase of 88.5% (9719 kg ha$^{-1}$), and 68.8% (8702 kg ha$^{-1}$) in straw yield respectively, when compared to control (5155 kg ha$^{-1}$). However, the former treatment achieved the maximum straw yield over all treatments. A straw yield of 8948 kg ha$^{-1}$ (73.6%), and 7011 kg ha$^{-1}$ (36.0%) was obtained in the chemical Zn applications of $ZnSO_4$ and ZnO, respectively. While a relative increase of 70.1% (8768 kg ha$^{-1}$), and 79.5% (9254 kg ha$^{-1}$) compared to control, was recorded under higher and lower doses of integrated applications of chemical with nano-Zn fertilizer, respectively.

**Zinc content of wheat:** The data showed that straw Zn accumulation was ($p \leq 0.05$) enhanced by Zn application treatments over control. Numerically, ZnO-NPs at lower dose was the treatment to obtain the maximum straw Zn content (23.7 mg kg$^{-1}$) with a relative increase of 72.5% over no Zn applied treatment. The higher dose of ZnO-NPs acquired a straw Zn content of 22.5 mg $kg^{-1}$ with 63.8% increase over control. Notably, with the increasing integrated dose, Zn accumulation in straw was decreased. Integrated use resulted in a 64.3% (22.5 mg $kg^{-1}$) and 58.9% (21.8 mg $kg^{-1}$) increase at lower and higher dose respectively, when compared to control. Chemical Zn application at higher rate, obtained a straw Zn content of 22.4 mg $kg^{-1}$ (63.3%), and at lower rate, 18.5 mg $kg^{-1}$ (34.9%) (Fig. 1A).

The results suggested that Zn content in grains was significantly ($p \leq 0.05$) higher under Zn applied treatments compared to no Zn application. In comparison to control (25.8 mg $kg^{-1}$), grain Zn concentration under ZnO-NPs at lower rate was two folds higher (53.3 mg $kg^{-1}$). The results revealed that at higher rate of ZnO-NPs, the grain Zn enrichment decreased to 51.4 mg $kg^{-1}$ (98.7%). The quality of wheat grains was also enhanced by conventional Zn sources. However, 5.0 kg Zn $ha^{-1}$ through $ZnSO_4$ proved to be superior by obtaining a grain Zn content of 51.1 mg $kg^{-1}$ than 2.5 kg Zn $ha^{-1}$ through ZnO (36.9 mg $kg^{-1}$), an increase was of 97.7% and 42.8%, respectively over control. A 90.6% increase (49.3 mg $kg^{-1}$) and a two-fold increase (51.9 mg $kg^{-1}$) over no Zn application was recorded in the integrated applications of Zn at higher and lower rates, respectively (Fig. 1B).

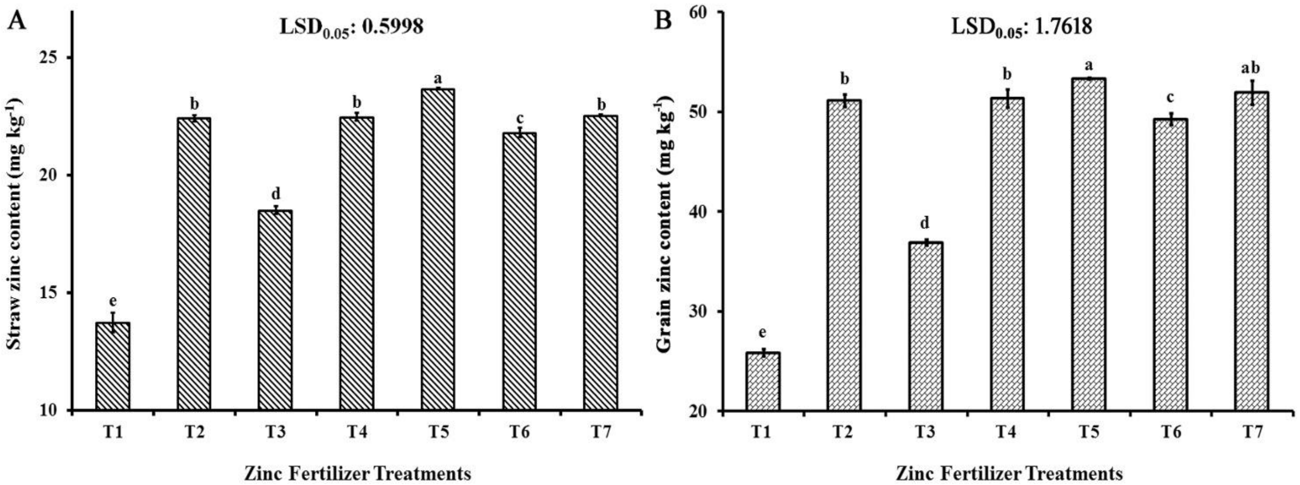


Fig. 1. A, Effect of single and integrated applications of chemical and nano zinc fertilizers on straw Zn content of wheat; B, Effect of single and integrated applications of chemical and nano zinc fertilizers on grain Zn content of wheat. (Zinc fertilizer treatments include: $T_1$ = Control, no Zn application, $T_2$ = 5.0 kg Zn $ha^{-1}$ through $ZnSO_4$, $T_3$ = 2.5 kg Zn $ha^{-1}$ through ZnO, $T_4$ = 2.5 kg Zn $ha^{-1}$ through ZnO-NPs, $T_5$ = 1.25 kg Zn $ha^{-1}$ through ZnO-NPs, $T_6$ = 2.5 kg Zn $ha^{-1}$ through $ZnSO_4$ + 2.5 kg Zn $ha^{-1}$ through ZnO-NPs, $T_7$ = 1.25 kg Zn $ha^{-1}$ through $ZnSO_4$ + 1.25 kg Zn $ha^{-1}$ through ZnO-NPs).

## Discussion

About 40% of the world population relies on wheat as a staple food (Singh *et al.,* 2023a). However, wheat grains contain low Zn content which falls short of meeting the optimum requirement of Zn for humans (Cakmak & Kutman, 2018). Zinc plays crucial roles in plant nutrition as well as protection from environmental stresses. It increases overall plant productivity by enhancing major nutrients uptake (Abbas *et al.,* 2009) and helps plants thrive in multiple stress conditions, including salinity (Adil *et al.,* 2022; Mazhar *et al.,* 2023), and heavy metal stress (Hussain *et al.,* 2018). Moreover, Zn deficiency represents a significant micronutrient disorder affecting crop plants and exerting adverse effects on agricultural output (Rashid *et al.,* 2022). The commonly used chemical fertilizers promise an increase in crop production, however; the imbalance and overuse of those fertilizers have led to some unfavorable impacts on environmental quality and soil productivity which is a major concern in present-day agriculture (Nongbet *et al.,* 2022).

Given the present circumstances, there is a strong need for the implementation of novel fertilizer technologies that can ensure the production of nutrient-enriched crops (Mazhar *et al.,* 2023). Nanoparticles possess some unique physicochemical properties which make them superior to their conventional counterparts (Mustafa *et al.,* 2024). Utilization of nanofertilizers is a well-established technique to enhance crop growth and yield. Additionally, nanofertilizers are available in the soil for longer periods of time for plant uptake, release nutrients slowly, and enhance the uptake efficiency in targeted plant parts (Al-Khayri *et al.,* 2023). The application of ZnO-NPs improves plant growth, reduces abiotic stresses, improve photosynthetic parameters, and maintains the metabolism of carbohydrates (Liu *et al.,* 2022). Moreover, the application of ZnO-NPs ameliorates the negative effects of drought and reactive oxygen species by improving the growth, physiology and antioxidant defense in wheat (Kausar *et al.,* 2023).

This field experiment was conducted on a Zn deficient soil (0.48 mg Zn $kg^{-1}$). The analysis of Zn was carried out through AB-DTPA method, and according to this method, the critical limit of Zn is 1.0 mg $kg^{-1}$ (Ryan *et al.,* 2001). In this study, the application of Zn, either through ZnO-NPs alone or its integrated use with $ZnSO_4$, substantially improved the agronomic and yield parameters of wheat over control treatment which performed the least of all treatments. However, among Zn sources, the application of ZnO-NPs was superior to $ZnSO_4$ which performed better than the other Zn fertilizer source, bulk ZnO. Moreover, the grain yield was higher in the treatments receiving ZnO-NPs when compared with the rest of the treatments. However, singly applied ZnO-NPs at lower rate gave the maximum grain yield by outyielding all other treatments. The past studies are in accordance with the results of this study as ZnO-NPs significantly increased grain yield of wheat when compared with its conventional counterparts (Jalal *et al.,* 2023; Mazhar *et al.,* 2023). The findings of this study are further corroborated by Al-Salama *et al.* (2024) who found that the application of ZnO-NPs increased plant height, weight of 1000 grains, and straw yield of wheat. The findings of current investigation are further supported that nano-ZnO had the potential to overcome the fixation of Zn in alkaline and carbonate rich soils leading to improved productivity of wheat (Ahmad *et al.,* 2023). The application of Zn nanofertilizer through foliar significantly improved wheat yield when the research was conducted particularly in similar soil and climatic conditions (Singh *et al.,* 2023b). Zinc oxide nanoparticles improved plant height, spike length, and grain yield of wheat. Similar results were observed in a study by Adil *et al*., (2022).

Zinc oxide nanoparticles have a positive impact on grain yield which is a main determinant of the productivity of wheat (Yadav *et al.,* 2023). This may be due to the several benefits associated with ZnO-NPs. Nano-ZnO has a positive impact on increasing the rate of photosynthesis and reducing reactive oxygen species. They have higher use efficiency due to relatively smaller size and greater surface area showcasing their potential to outperform the conventional Zn sources. Their greater surface to volume ratio makes them highly reactive and easily accessible to plants (Seleiman *et al.,* 2023). The other possible reasons ZnO-NPs out classed conventional Zn salt, $ZnSO_4$, because ZnO-NPs are smaller sized, highly stable and slowly soluble resulting in their persistence in soil for a longer duration instead of moving in different directions and thereby causing the unfavorable impacts on the environment. On the other hand, $ZnSO_4$ has a lower surface area, and rapid solubility which leads to its loss in various directions and thus being unavailable to plants (Du *et al.,* 2019). Moreover, chemical Zn applied through bulk ZnO was the least responsive among Zn applied treatments. This showed that the efficiency of ZnO is lesser than that of other Zn sources. Umar *et al*., (2021) further discussed that chemical ZnO in soil was relatively less available to plants therefore nano-ZnO improved plant growth than bulk ZnO. In this study, ZnO-NPs significantly altered the growth of wheat. However, its higher rate decreased the yield parameters of wheat. This response is aligned with a study by Du *et al*., (2019) who found that the grain yield of wheat substantially decreased under the higher dose of nano-ZnO. At lower concentrations of nano-ZnO, proper germination, increased yield, and biomass was observed. While at increased doses, a substantial reduction in the productivity along with toxicity of ZnO-NPs within plant tissues was observed in various crops (Mazumder *et al.,* 2020). This concept is further supported by Ahmed *et al*., (2023) who reported that ZnO-NPs may negatively affect the growth and quality of produce of tomato plants when applied at higher levels. Therefore, choosing the right dose of Zn is very important if the concerned source is ZnO-NPs.

The singly applied ZnO-NPs in comparison with its integrated use with $ZnSO_4$ improved all the agronomic parameters of wheat except for plant height and spike length which responded positively to the integrated application of ZnO-NPs with $ZnSO_4$ at higher dose. The results of the current study corroborate the findings obtained by Ahmad *et al*., (2023). They suggested that ZnO-NPs, when combined with conventional $ZnSO_4$, obtained the maximum spike length over ZnO-NPs alone. However, for the yield attributes, sole ZnO-NPs was far better. Application of ZnO-NP in comparison with various other Zn sources was found to be better when applied in maize crop (Umar *et al.,* 2021).

The utilization of ZnO-NPs is an effective way for improving the quality of wheat grains by enriching Zn content (Adrees *et al.,* 2021). Zinc oxide nanoparticles significantly enhanced Zn concentrations of the straw and grain of wheat. Maximum straw Zn content was obtained through ZnO-NPs and integration of ZnO-NPs with $ZnSO_4$ at lower doses, respectively. Minimum straw Zn content was observed where no Zn was applied. Likewise, a similar pattern of treatments was noticed for grain Zn concentration. The results of this study are further supported by Jalal *et al*., (2023). They determined that Zn recovery was maximum in both straw and grains under the lower dose of ZnO-NPs. Higher rates of ZnO-NPs failed to store the amount of Zn as high as accumulated by lower doses as in the case of this study's findings. One of the consequences of higher concentrations of ZnO-NPs is the toxicity of Zn nanoparticles in wheat crop (Lee *et al.,* 2020).

The findings of the current study are consistent with Yadav *et al*., (2023) who reported that straw and grain Zn concentrations were higher in the treatment of ZnO-NPs over $ZnSO_4$. This phenomenon may be attributed to improved translocation of Zn towards the reproductive part of the plant, due to higher efficiency of ZnO-NPs compared to chemical $ZnSO_4$ (Lateef *et al.,* 2016). The results of the current study also corroborate with the previous findings related to wheat Zn accumulation. Zinc content of wheat was significantly enhanced through ZnO-NPs (Yadav *et al.,* 2023). Moreover, in crops other than wheat, particularly maize, the application of nano-Zn increased grain Zn content when studied under the local conditions (Naseer *et al.,* 2024).

In terms of plant Zn accumulation, similar to its response in agronomic parameters, bulk ZnO treatment proved to be least beneficial among the rest of the Zn treatments. In the case of integrated applications of ZnO-NPs with $ZnSO_4$, similar trend as yield parameters was observed. Integrated use at lower dose performed better than all treatments except for singly applied ZnO-NPs at lower rate. However, straw and grain Zn content decreased with increasing integrated application dose. These results are aligned to an earlier study involving the integrated application of ZnO-NPs with $ZnSO_4$. Combined applications of chemical and nano-Zn fertilizers did not further improve Zn use efficiency in wheat in comparison to sole nano-Zn. It is suggested that integrated use of $ZnSO_4$ and ZnO-NPs could be effective for the biological yield, and not for wheat Zn enrichment (Ahmad *et al.,* 2023).

## Conclusion

Zinc oxide nanoparticles were proved to be more efficient, either at higher or lower dose, in improving growth, yield, and Zn content of wheat grains over chemical Zn fertilizer source ($ZnSO_4$). A thorough understanding of Zn nano-fertilizer such as soil interactions, their retention and availability, and economics should be further investigated under field conditions.

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