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Research Article

COMPARATIVE EFFICACY OF Zn-EDTA AND ZnSO₄ IN MITIGATING ZINC DEFICIENCY IN WHEAT FOR SUSTAINABLE PRODUCTION

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ABSTRACT

Zinc (Zn) deficiency in soils and crops poses a significant challenge to sustainable wheat production and human nutrition worldwide. Agronomic biofortification, enhancing cereal crops with zinc, is a rapid and effective strategy to address this issue. The present field study evaluated two zinc sources, zinc sulfate (ZnSO₄·H₂O, 33% Zn) and chelated zinc (Zn-EDTA, 5% Zn), for their effectiveness in enhancing yield and Zn concentration in Zn-responsive wheat varieties (Anmol-91, TD-1, NIA-Sarang, and NIA-Amber). The Zn treatments included: a control (no Zn), 13.2 kg Zn ha⁻¹ as ZnSO₄·H₂O, and 0.125 and 0.25 kg Zn ha⁻¹ as Zn-EDTA. Treatments were arranged in a randomized complete block split-plot design with three replications. Zinc application significantly improved wheat growth and yield traits compared to the control. However, no significant interaction was observed between treatments and varieties for agronomic parameters. Application of 0.25 kg Zn ha⁻¹ as Zn-EDTA significantly increased plant height (by 4%), spike length (31%), number of spikelets per spike (7%), 1000-grain weight (12%), straw yield (10%), grain yield (10%), Zn concentration (16%), the phytic acid-to-zinc molar ratio (PA:Zn) (46%), and total absorbable zinc (TAZ) (87%) compared to the control. Furthermore, it significantly reduced phytic acid concentration in wheat grains by 37%. Soil Zn concentration also increased significantly ($P < 0.05$), with the highest increase observed at 13.2 kg ha⁻¹ ZnSO₄ (125%) and the lowest with Zn-EDTA (95%) compared to the control. Among the wheat varieties evaluated, Anmol-91, NIA-Amber, and NIA-Sarang demonstrated the best performance.

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INTRODUCTION

Zinc (Zn) deficiency is a major global concern, particularly affecting human health through cereal-based diets that are low in zinc (Zhang et al., 2012; Sher et al., 2022). It is a leading cause of hidden hunger, a form of malnutrition caused by insufficient intake of essential micronutrients,

resulting in serious health consequences, especially in developing countries (Wang et al, 2019; Yokokawa et al., 2024). More than 30% of people worldwide suffer from zinc deficiency (Rashid et al., 2025), which is associated with stunted growth, weakened immune function, impaired cognitive development, and increased

vulnerability to infectious diseases (Shahzad et al., 2014; Yokokawa et al., 2024). This deficiency disproportionately affects women and children in low- and middle-income countries (WHO, 2020). Globally, zinc deficiency ranks as the fourth most critical micronutrient deficiency and significantly contributes to the disease burden in low-income regions (Cakmak, 2008; Saleem et al., 2015). A Japanese study reported marginal zinc deficiency in 46% of men and 38.4% of women, largely attributed to dietary habits (Yokokawa et al., 2024). Similarly, in Pakistan, the prevalence of zinc deficiency is alarming, with over 40% of adult women and 18.6% of children under the age of five classified as severely deficient (NNS, 2018).

Globally, wheat cultivated in Zn-deficient regions such as India, Turkey, and parts of China consistently exhibits low Zn content (Cakmak and Kutman, 2018). This soil deficiency is closely linked to Zn deficiency in plants, significantly affecting crop yields and human nutrition (Alloway, 2009). Studies show that approximately 70% of Pakistani soils are deficient in zinc, contributing to reduced crop productivity and widespread malnutrition (Imtiaz et al., 2010). In Sindh, widespread soil Zn deficiency poses a major regional challenge (Mari et al., 2006). In such areas, poor soil nutrient profiles, particularly high alkalinity and calcareous conditions, further exacerbate the problem (Abbas et al., 2009). Although wheat is a staple food, its grains have low zinc content, particularly when grown in zinc-deficient soils (Cakmak and Kutman, 2018).

Zinc deficiency disrupts vital plant processes, including enzymatic activity and protein synthesis, leading to stunted growth and reduced yields (Akram et al., 2017). As a result, crops, especially wheat, tend to accumulate suboptimal Zn levels, typically around 20-35 mg/kg, which is well below the recommended 45 mg/kg for human health (Xia et al., 2020). This nutritional gap underscores the urgent need for biofortification of staple crops.

Agronomic biofortification of wheat has emerged as a promising, low-cost, and effective strategy to enhance Zn levels in grains and combat zinc deficiency in human diets (Shahzad et al., 2014; Singh et al., 2023). Biofortification aims to increase the levels of micronutrients in the edible parts of plants through a combination of agronomic practices and genetic enhancements (Cakmak, 2008; Zulfiqar et al., 2020). This approach involves the application of zinc fertilizers to crops, particularly in nutrient-stressed soils, to improve the bioavailability of micronutrients (Cakmak and

Kutman, 2018; Zhao et al., 2018, 2019).

Chelated Zn (Zn-EDTA) has demonstrated greater efficiency than ZnSO₄ in enhancing zinc diffusion, grain Zn concentration, and bio-accessibility, particularly in calcareous soils. The chelated form improves Zn uptake by minimizing nutrient loss in high-pH or Zn-deficient soils, thereby ensuring better plant availability (Liu et al., 2012). Unlike ZnSO₄, Zn-EDTA remains soluble and is more readily absorbed by plant roots, resulting in improved Zn uptake and translocation within crops (Zhao et al., 2019). Studies have shown that Zn-EDTA increases grain Zn concentration and bio-accessibility more effectively than inorganic Zn sources, especially in wheat (Cakmak and Kutman, 2018). Moreover, its higher diffusion rate in the soil enhances Zn mobility and reduces fixation losses (Alloway, 2008). Comparative research on Zn sources has revealed that Zn-EDTA significantly improves plant Zn status, yield, and nutritional quality, making it a preferred choice for biofortification in Zn-deficient soils (Liu et al., 2012; Singh et al., 2023).

Biofortification enhances Zn accumulation in crops, with Zn fertilization effectively increasing grain Zn levels (Shahzad et al., 2014; Zulfiqar et al., 2020). Agronomic biofortification not only improves plant growth and nutrient uptake but also contributes to food security by addressing widespread Zn deficiencies (Cakmak and Kutman, 2018; Singh et al., 2023). Enhancing Zn content in wheat through chelation offers a sustainable strategy to combat hidden hunger (Cakmak, 2008; Shakoor et al., 2023).

The present study aims to evaluate the most effective Zn application methods and sources for improving wheat grain yield and Zn content. Based on previous findings, it is hypothesized that chelated Zn forms, such as Zn-EDTA, will enhance Zn bioavailability, leading to significant improvements in both yield and grain Zn concentration. This study provides valuable insights into wheat biofortification strategies and contributes to combating global Zn deficiencies by identifying optimal application rates and the most effective Zn sources. The findings could have far-reaching implications for improving food security and public health by increasing the nutritional quality of wheat, a vital staple crop worldwide.

MATERIALS AND METHODS

Experimental location

The field experiment was conducted during the Rabi season at the Agricultural Research Centre, Tando Jam, Sindh, Pakistan (25°25'27.0"N, 68°32'39.9"E).

Physico-chemical properties of the experimental soil

Before sowing, a composite soil sample (0-5 cm depth) was collected using a stainless-steel auger. The analyses were performed following the procedures described by Estefan et al. (2013). Soil pH and electrical conductivity (EC) were measured using Senso Direct Con 110 and 100 meters (Lovibond, Germany). Soil texture was determined by the hydrometer method, organic matter content was estimated using the Walkley-Black wet oxidation method, and lime content was assessed by reacting 1 g of soil with 10 ml of 1 N HCl overnight, followed by titration with 1 N NaOH. Extractable zinc (Zn) was determined using the DTPA extraction method as described by Estefan et al. (2013).

Experimental design, varieties, and treatments

The experiment was laid out in a randomized complete split-plot design, with wheat varieties assigned to the main plots and zinc application rates to the sub-plots. Each treatment was replicated three times. A total area of 517.5 m² (23 m × 22.5 m) was prepared for the study and divided into 48 equal units, with each net plot measuring 6 m² (2 m × 3 m). Four wheat varieties, viz. Anmol-91, NIA-Amber, NIA-Sarang, and TD-1 were cultivated. Two sources of zinc fertilizer were used: Zinc Sulfate (ZnSO₄, 33% Zn) and Zinc Chelate (Zn-EDTA, 5% Zn). These were applied at four different rates: 0 and 13.2 kg Zn ha⁻¹ for ZnSO₄, and 0.125 and 0.250 kg Zn ha⁻¹ for Zn-EDTA.

Crop husbandry, land preparation, and fertilizer schedule

The land was meticulously prepared through two tractor ploughings, followed by leveling and thorough soaking to achieve optimal soil moisture (Nawaz et al., 2015). Wheat seeds were sown manually using hand drilling at a rate of 125 kg ha⁻¹ with a consistent row spacing of 20 cm (Nawaz et al., 2015; Zulfiqar et al., 2020). Fertilization involved the application of 120 kg N ha⁻¹, 60 kg P₂O₅ ha⁻¹, and 60 kg K₂O ha⁻¹ using urea, DAP, and SOP at sowing. One-third of the nitrogen was applied during seedbed preparation, while the remaining two-thirds were applied with irrigation water between the tillering

and booting stages. This schedule ensured effective nutrient uptake throughout the crop growth period and minimized nutrient loss (Kumar et al., 2023).

Agronomic observations

Crop harvesting was performed manually once the plants reached full maturity. Each replication plot was harvested using a 0.5 m² wooden frame to estimate yield. The harvested plants were manually threshed and separated into grain and straw, which were weighed using a digital scale. At harvest, five plants from each replicated plot were randomly selected to record data on plant height, number of tillers per plant, spike length, and 1000-grain weight (Nawaz et al., 2015; Iqbal et al., 2017).

Zinc and phytic acid determination in wheat grain

Grain samples were oven-dried at 70°C for 48 h, following the method described by Esfandiari et al. (2016). After drying, the grains were ground into a fine powder using a Geepas GCG289 grinder. Zinc concentration in the grains was determined using a wet digestion technique followed by analysis with an atomic absorption spectrophotometer (AAS). For this purpose, 1.0 g of dried and ground grain was digested overnight with a nitric acid-perchloric acid mixture (2:1). The digest was then heated at 150°C for 1 h and at 235°C for 30 min. After cooling, the mixture was filtered, diluted to 50 ml with deionized water, and analyzed for zinc using an AAS (NovAA-400, Analytik Jena, Germany), following the procedure outlined by Estefan et al. (2013).

Phytic acid (PA) concentration was determined using the method developed by Haug and Lantzsch (1983), as outlined by Bughio et al. (2021). A 0.05 g sample of wheat flour was mixed with 10 ml of 0.2 N HCl and shaken for 2 h. A portion of the extract was reacted with a ferric solution, heated, and then mixed with 2 ml of Bipyridine (C₁₀H₈N₂) solution. Sodium phytate standards (0-30 ppm PA-P) were prepared for calibration. Absorbance was recorded at 510 nm using a UV-Vis spectrophotometer. The phytic acid concentration (g kg⁻¹) in the samples was calculated using the formula:

$$\text{PA concentration (g/kg)} = \frac{\text{Concentration from the spectrophotometer}}{0.282} \times 200$$

Phytic acid-to-zinc molar ratio

The phytic acid-to-zinc molar ratio, a qualitative parameter for zinc bioavailability, was calculated using the formula delineated by (Imran et al., 2015; Saha et al., 2017):

$$\text{PA: Zn molar ratio} = \frac{(\text{PA content in mg/kg}/660)}{\text{Zn content in mg/kg}/65}$$

Total daily absorbed zinc, a quantitative parameter, was estimated using the Trivariate model (Liu et al., 2017; Wang et al., 2019).

$$TAZ = 0.5 \times 65 \times 100 [AMAX + TDZ + KR \times (1 + TDP/KP) - \{(AMAX + TDZ + KR \times (1 + TDP/KP))\}^2 - 4 \times AMAX \times TDZ]^{1/2}$$

Where TAZ = total daily absorbed Zn (mg Zn Day⁻¹), AMAX = maximum Zn absorption (0.091), TDZ = total daily dietary Zn (mmol Zn Day⁻¹), KR = equilibrium dissociation constant of the Zn receptor binding reaction (0.680), TDP = total daily dietary PA (mmol phytic acid day⁻¹), and K_p = equilibrium dissociation constant of the Zn-PA binding reaction (0.033).

Statistical analysis

All agronomic and laboratory data were subjected to analysis of variance (ANOVA) using Statistix version 8.1 (Analytical Software, 2006). A two-way ANOVA was performed, and treatment means were compared using Tukey's test at a significance level of $P < 0.05$.

RESULTS

Soil analysis before planting

The experimental soil was analyzed prior to planting to assess its physicochemical properties. At a depth of 0-15 cm, the soil was classified as silt loam in texture, non-saline (EC: 1.00 ± 0.2 dS m⁻¹), slightly alkaline (pH: 7.96 ± 0.1), moderately calcareous ($11.0 \pm 0.3\%$), low in organic matter ($0.75 \pm 0.1\%$), and deficient in DTPA-

extractable zinc (0.42 ± 0.1 mg kg⁻¹).

Evaluation of wheat yield characteristics

The application of ZnSO₄ and Zn-EDTA significantly enhanced wheat yield traits compared to the control, highlighting the essential role of zinc as a micronutrient. The results demonstrated that Zn-EDTA was particularly effective in maximizing yield and boosting overall productivity.

As shown in Table 1, the application of zinc substantially improved plant growth and yield parameters across wheat varieties. The maximum plant height was observed in Anmol-91 (95.70 ± 0.74 cm), NIA-Amber (95.35 ± 0.59 cm), and NIA-Sarang (95.58 ± 0.50 cm), while the lowest was recorded in TD-1 (72.11 ± 0.62 cm). Spike length was also highest in Anmol-91 (9.90 ± 0.18 cm), NIA-Amber (9.48 ± 0.12 cm), and NIA-Sarang (9.64 ± 0.14 cm), with the shortest in TD-1 (8.99 ± 0.18 cm). The number of spikelets per spike was significantly greater in Anmol-91 (19.58 ± 0.20) and NIA-Sarang (19.47 ± 0.22), followed by NIA-Amber (18.93 ± 0.18) and TD-1 (18.69 ± 0.16).

These findings confirm the critical role of zinc in enhancing both vegetative and reproductive traits, with Zn-EDTA emerging as the most effective treatment for improving wheat productivity.

Table 1. Effect of different sources and application rates of soil-applied zinc on the yield characteristics of selected wheat varieties.

Varieties	Plant height (cm)	Spikes length (cm)	Number of spikelets per spike
Zn 0	87.65 ± 2.17^b	8.88 ± 0.18^b	18.36 ± 0.09^b
Zn 13.2 (ZnSO ₄)	90.80 ± 2.14^a	9.63 ± 0.08^{ab}	19.30 ± 0.13^a
Zn 0.125 (EDTA)	89.82 ± 2.21^a	9.47 ± 0.13^b	19.37 ± 0.13^a
Zn 0.250(EDTA)	90.48 ± 2.25^a	9.94 ± 0.11^a	19.63 ± 0.12^a
Zinc fertilization impact on wheat varieties			
Anmol-91	95.70 ± 0.74^a	9.90 ± 0.18^a	19.58 ± 0.20^a
NIA-Amber	95.35 ± 0.59^a	9.48 ± 0.12^a	18.93 ± 0.18^b
TD-1	72.11 ± 0.62^b	8.99 ± 0.18^b	18.69 ± 0.16^b
NIA-Sarang	95.58 ± 0.50^a	9.64 ± 0.14^a	19.47 ± 0.22^a

Each value in a cell is presented as Mean \pm SE (n = 3); means followed by different letters indicate significant differences ($P < 0.05$).

Table 2 shows that Zn fertilization levels and wheat varieties significantly influenced wheat yield characteristics, although their interaction was not significant. The highest 1000-grain weight values were observed with 13.2 kg ha⁻¹ Zn (as ZnSO₄) and 0.25 kg ha⁻¹ Zn (as EDTA). Similarly, the highest straw and grain

yields were recorded with 0.25 kg ha⁻¹ Zn (EDTA). Among the varieties, Anmol-91 and NIA-Sarang exhibited the highest yield-related traits. Notably, Zn application at 0.25 kg ha⁻¹ (EDTA) resulted in significant increases in 1000-grain weight, straw yield, and grain yield, with greater increments than other treatments.

Furthermore, 13.2 kg ha⁻¹ Zn (ZnSO₄) increased the 1000-grain weight.

Zinc application significantly enhanced 1000-grain weight, straw yield, and grain yield across all wheat varieties. Anmol-91 recorded the highest 1000-grain weight (44.57 ± 0.44 g) and straw yield (4840.7 ± 54.7 kg/ha), while TD-1 had the lowest values (43.76 ± 1.01 g for 1000-grain weight and 4727.7 ± 79.06 kg/ha for

straw yield). For grain yield, Anmol-91 again performed best (4830.6 ± 54.03 kg/ha), whereas TD-1 recorded the lowest yield (4717.3 ± 79.2 kg/ha). Zinc application at 0.25 kg ha⁻¹ (EDTA) proved more effective in improving yield metrics than other levels, including 13.2 kg ha⁻¹ Zn (ZnSO₄) as shown in Table 2. However, no significant (P > 0.05) variation was observed in straw and grain yield or Zn application × variety interaction.

Table 2. Effect of different soil zinc sources and application rates on the yield of selected wheat varieties.

Treatments	1000 grain weight(g)	Straw yield (kg ha ⁻¹)	Grain yield (kg ha ⁻¹)
Impact of Soil Zinc application (kg ha ⁻¹)			
Zn 0	41.06 ± 0.36 ^C	4560.25 ± 24.67 ^C	4549.4 ± 24.75 ^C
Zn 13.2 (ZnSO ₄)	45.78 ± 0.37 ^A	4722.19 ± 30.53 ^{BC}	4712.3 ± 30.21 ^B
Zn 0.125 (EDTA)	43.77 ± 0.42 ^B	4854.22 ± 27.70 ^B	4840.9 ± 26.88 ^B
Zn 0.250 (EDTA)	46.07 ± 0.63 ^A	5024.56 ± 32.83 ^A	5017.7 ± 32.63 ^A
Zinc fertilization impact on wheat varieties			
Varieties			
Anmol-91	44.57 ± 0.44	4840.7 ± 54.70	4830.6 ± 54.03
NIA-Amber	43.88 ± 0.67	4770.4 ± 72.09	4761.5 ± 72.11
TD-1	43.76 ± 1.01	4727.7 ± 79.06	4717.3 ± 79.20
NIA-Sarang	44.38 ± 0.94	4822.5 ± 46.98	4810.9 ± 47.47

Each value in a cell is presented as Mean ± SE (n = 3); means followed by different letters indicate significant differences among them (P < 0.05).

Enhanced zinc concentration, bioavailability, and nutritional quality in wheat varieties

Zinc fertilization and its interaction with wheat varieties significantly affected both Zn and phytic acid (PA) concentrations in wheat grains. Zn application increased the grain Zn concentration from 40.31 to 46.64 mg/kg (Table 3) and reduced PA content from 7.82 to 6.76 g/kg (Table 3). The highest Zn concentration and the lowest PA levels were observed with the application of 0.25 kg Zn (as Zn-EDTA) per hectare. Among the tested varieties, Anmol-91 and NIA Sarang recorded the highest Zn concentrations (Table 3), while TD-1 exhibited the lowest PA content.

Zinc concentration in grains

The highest Zn concentrations were observed in Anmol-91 (44.72 ± 0.83 mg/kg), NIA-Sarang (44.16 ± 0.99 mg/kg), and NIA-Amber (43.49 ± 0.80 mg/kg) (Table 3). The lowest Zn concentration was found in TD-1 (42.58 ± 0.59 mg/kg). Significant differences (P < 0.05) in grain Zn concentrations were noted among the varieties, with Anmol-91 outperforming TD-1. However, the interaction between Zn application and variety was not significant. The optimal Zn-EDTA dose was 0.25 kg/ha (Table 3).

Phytic acid to zinc molar ratio

The greatest reduction in the phytic acid to zinc molar ratio was recorded in TD-1 (15.81 ± 1.37), while the highest ratios were observed in Anmol-91 (17.43 ± 1.18), NIA-Sarang (17.20 ± 1.35), and NIA-Amber (16.72 ± 1.42) (Table 3). A significant reduction (P < 0.05) in the PA/Zn molar ratio was observed among the wheat varieties, with TD-1 showing the most favorable ratio. However, the interaction effect of Zn fertilization was not significant. The optimal Zn-EDTA dose for improving this ratio was 0.25 kg/ha, as shown in Table 3.

Total daily absorbed zinc (mg Zn day⁻¹)

Zinc application significantly increased total daily absorbed Zn (TAZ) compared to the control, with the highest values observed at 0.25 kg Zn/ha (Zn-EDTA), particularly in two varieties. The highest TAZ values were found in TD-1 (1.94 ± 0.16 mg/day) and NIA-Amber (1.83 ± 0.15 mg/day), while the lowest were recorded in Anmol-91 (1.71 ± 0.11 mg/day) and NIA-Sarang (1.76 ± 0.14 mg/day). Significant differences (P < 0.05) in TAZ were observed among the varieties, but the interaction with Zn application was not significant (Table 3).

Table 3. Effect of soil-applied zinc sources and application rates on zinc concentration (mg kg^{-1}), phytic acid to zinc molar ratio, and total daily absorbed zinc (mg Zn day^{-1}) in selected wheat varieties.

Treatments	Impact of Soil Zn application (kg ha^{-1})		
	Zinc concentration (mg kg^{-1})	Phytic acid to Zinc molar ratio	Total daily absorbed Zn (mg Zn Day^{-1})
Zn 0	40.31 \pm 0.20 ^C	22.43 \pm 0.28 ^A	1.27 \pm 0.02 ^D
Zn 13.2 (ZnSO_4)	43.18 \pm 0.32 ^B	19.49 \pm 0.32 ^B	1.46 \pm 0.02 ^C
Zn 0.125 (EDTA)	44.81 \pm 0.32 ^B	13.24 \pm 0.20 ^C	2.14 \pm 0.03 ^B
Zn 0.250 (EDTA)	46.64 \pm 0.46 ^A	12.02 \pm 0.20 ^D	2.37 \pm 0.04 ^A
Varieties	Zinc fertilization impact on wheat varieties		
Anmol-91	44.72 \pm 0.83 ^A	17.43 \pm 1.18 ^A	1.71 \pm 0.11 ^B
NIA-Amber	43.49 \pm 0.80 ^{AB}	16.72 \pm 1.42 ^{AB}	1.83 \pm 0.15 ^{AB}
TD-1	42.58 \pm 0.59 ^B	15.81 \pm 1.37 ^B	1.94 \pm 0.16 ^A
NIA-Sarang	44.16 \pm 0.99 ^{AB}	17.20 \pm 1.35 ^A	1.76 \pm 0.14 ^B

Each value in a cell is presented as Mean \pm SE (n = 3); means followed by different letters indicate significant differences among them ($P < 0.05$).

Phytic acid concentration in grain (g kg^{-1})

Plants treated with different Zn fertilizer sources exhibited a significant reduction in phytic acid content in wheat grains compared to the control. The phytic acid concentration in grain was highest in two varieties, Anmol-91 and NIA-Sarang (7.58 ± 0.41 and 7.58 ± 0.41), with the highest concentration recorded at 7.82 ± 0.37 g

kg^{-1} . The lowest phytic acid concentration was found in the TD-1 wheat variety (6.76 ± 0.45). A significant variation ($P < 0.05$) in grain phytic acid content was observed among wheat varieties and their interaction with Zn applications, with the TD-1 + Zn 0.250 (EDTA) treatment showing the lowest phytic acid concentration, as shown in Table 4.

Table 4. Effect of soil Zn application sources and rates on phytic acid concentration (g kg^{-1}) in selected wheat varieties.

Varieties	Soil Zn application (kg ha^{-1})				Mean of Varieties
	Zn 0	Zn 13.2 (ZnSO_4)	Zn 0.125 (EDTA)	Zn 0.250 (EDTA)	
Anmol-91	9.30 \pm 0.20 ^a	8.95 \pm 0.34 ^a	6.64 \pm 0.25 ^{bc}	6.38 \pm 0.04 ^{cd}	7.82 \pm 0.37 ^A
NIA-Amber	9.05 \pm 0.30 ^a	8.84 \pm 0.21 ^a	5.74 \pm 0.03 ^{cde}	5.47 \pm 0.09 ^{de}	7.27 \pm 0.46 ^B
TD-1	9.08 \pm 0.35 ^a	7.49 \pm 0.03 ^b	5.52 \pm 0.17 ^{de}	4.93 \pm 0.15 ^e	6.76 \pm 0.45 ^C
NIA-Sarang	9.25 \pm 0.20 ^a	8.88 \pm 0.11 ^a	6.20 \pm 0.05 ^{cd}	5.99 \pm 0.04 ^{cd}	7.58 \pm 0.41 ^{AB}
Mean of soil Zn applications	9.17 \pm 0.08 ^A	8.54 \pm 0.13 ^B	6.02 \pm 0.09 ^C	5.70 \pm 0.11 ^C	

Each value in a cell is expressed as Mean \pm SE (n = 3). Means followed by different letters indicate significant differences ($P < 0.05$).

Extractable zinc concentration

The results of extractable zinc concentration in the plots of wheat varieties with different sources and rates of Zn are presented in Table 5. The soil zinc content was significantly affected by soil Zn applications ($P < 0.05$). The Zn-fertilized plots exhibited a higher zinc concentration in the soil compared to the plots where no Zn was applied. The highest zinc buildup was observed in plots where Zn was applied at 13.2 kg ha^{-1} (ZnSO_4).

There was no significant variation in Zn concentration in the plots where different wheat varieties were grown with varying Zn fertilization ($P > 0.05$). However, there was a significant ($P < 0.05$) interaction between Zn applications and wheat varieties for zinc concentration in the soil. The plots where Zn was applied to Anmol-91 at 13.2 kg ha^{-1} (ZnSO_4) showed the highest concentration of Zn, while the lowest concentration was found in Anmol-91 plots with 0 kg Zn ha^{-1} .

Table 5. Effect of soil zinc application sources and rates on extractable zinc concentration in post-harvest soil.

Varieties	Soil Zn application (kg ha ⁻¹)				Mean of Varieties
	Zn 0	Zn 13.2 (ZnSO ₄)	Zn 0.125 (EDTA)	Zn 0.250 (EDTA)	
Anmol-91	0.34 ± 0.01 ^g	0.96 ± 0.02 ^a	0.54 ± 0.02 ^{de}	0.72 ± 0.01 ^c	0.64 ± 0.07
NIA-Amber	0.43 ± 0.01 ^{efg}	0.89 ± 0.03 ^{ab}	0.51 ± 0.01 ^{def}	0.75 ± 0.01 ^c	0.65 ± 0.06
TD-1	0.44 ± 0.02 ^{efg}	0.81 ± 0.03 ^{bc}	0.53 ± 0.01 ^{de}	0.88 ± 0.03 ^{ab}	0.67 ± 0.06
NIA-Sarang	0.40 ± 0.01 ^{fg}	0.92 ± 0.04 ^{ab}	0.57 ± 0.02 ^d	0.75 ± 0.02 ^c	0.66 ± 0.06
Mean of soil Zn applications	0.40 ± 0.01 ^D	0.90 ± 0.01 ^A	0.54 ± 0.01 ^C	0.78 ± 0.01 ^B	

Each value in a cell represents the mean ± SE (n = 3); means followed by different letters indicate significant differences from each other (P < 0.05).

DISCUSSION

Agronomic performance

The application of Zn has shown significant improvements in key wheat yield parameters, emphasizing its essential role as a micronutrient in enhancing crop productivity. Zinc supplementation has been widely reported to boost wheat yields and improve grain quality, with similar findings documented globally (Nawaz et al., 2015; Zulfiqar et al., 2020; Yokokawa et al., 2024). In the present study, the use of Zn fertilizers, particularly Zn-EDTA, resulted in marked increases in wheat yield, grain weight, and overall productivity. The experiment evaluated the effects of different zinc concentrations and sources, including ZnSO₄ and Zn-EDTA, on wheat growth and development. These results are consistent with previous research supporting the role of Zn in improving crop yield and quality (Wang et al., 2019; Singh and Sandhu, 2020).

Chelated fertilizers, such as Zn-EDTA, are more effective than traditional inorganic forms like ZnSO₄ because they minimize nutrient losses due to oxidation, precipitation, and leaching, thereby increasing zinc availability for plant uptake. Studies have demonstrated that Zn-EDTA is more efficient in enhancing zinc biofortification in wheat, even at lower application rates (Zhao et al., 2018, 2019). Chelated zinc compounds, stabilized by organic ligands, resist oxidation and immobilization, significantly improving zinc uptake and plant absorption efficiency (Liu et al., 2012). For example, Zn-EDTA applied at only 20% of the ZnSO₄ rate exhibited comparable effectiveness, highlighting its superior stability in calcareous soils (Zhao et al., 2019). This is especially important in high-pH soils, where zinc availability is typically limited.

Zinc fertilization and yield

The statistical analysis of agronomic data revealed a significant improvement in wheat yield and quality following the application of Zn from various sources.

Specifically, the application of Zn-EDTA at 0.25 kg ha⁻¹ resulted in increases in plant height (4%), number of spikelets per spike (7%), spike length (31%), 1000-grain weight (12%), straw yield (10%), and grain yield (10%) compared to the control. These increases in zinc-related metrics were statistically significant, with both Zn-EDTA and ZnSO₄ outperforming the control. These results are consistent with the findings of (Liu et al., 2012; Kumar et al., 2023), who also reported improvements in wheat productivity with zinc fertilization, highlighting the essential role of zinc in improving crop yields.

Moreover, the beneficial effects of zinc on wheat production have been well documented both in Pakistan and internationally (Nawaz et al., 2015; Zhao et al., 2019; Singh and Sandhu, 2020). Zinc fertilization enhanced both biological and grain yields of wheat, and the findings of this study reinforce the view that zinc availability is crucial for improving wheat productivity. The application of Zn, particularly in the form of Zn-EDTA, significantly impacted the performance of wheat varieties, with the highest yields observed in those treated with Zn-EDTA. These findings align with those of Zhao et al. (2019), who reported similar enhancements in grain weight and yield following zinc application.

Zinc bioavailability and phytic acid reduction

The application of zinc positively influenced several key parameters, including zinc concentration, TAZ, and the PA:Zn molar ratio. Wheat treated with Zn-EDTA showed the highest zinc content, with cultivars Anmol-91 and Sarang exhibiting similar trends. Zinc application increased grain zinc concentration by 16%, reduced PA content by 37%, and decreased the PA:Zn molar ratio by 46%. Moreover, TAZ increased by 87% in wheat grains. The reduction in PA, a known inhibitor of zinc absorption, is particularly significant, as it suggests enhanced zinc bioavailability for human nutrition. These findings are

consistent with those of Cakmak and Kutman (2018), and Zulfiqar et al. (2020), who highlighted the importance of lowering PA levels to improve zinc bioavailability.

Soil zinc dynamics

Extractable soil zinc concentrations were significantly enhanced by zinc fertilization ($P < 0.05$), with the highest accumulation observed at an application rate of $13.2 \text{ kg ha}^{-1} \text{ ZnSO}_4$, especially in the Anmol-91 cultivar (Zhao et al., 2019). Although differences among wheat varieties were not statistically significant on their own, the significant interaction ($P < 0.05$) between zinc application rate and genotype emphasizes the crucial role of varietal traits in zinc uptake, a topic of ongoing debate in recent research (Nawaz et al., 2015; Singh and Sandhu, 2020). These findings challenge the traditional view that soil zinc levels are determined solely by fertilizer input, highlighting the need for genotype-specific zinc management strategies to optimize biofortification efforts.

Zinc and nutritional quality

An increased zinc concentration was positively correlated with higher protein content in wheat grains, underlining the role of Zn in protein biosynthesis (Alloway, 2009; Cakmak and Kutman, 2018). The activation of enzymes involved in chlorophyll biosynthesis and other metabolic processes likely contributed to the observed improvements in wheat quality and yield. These findings align with earlier studies by Alloway (2008) and Cakmak (2008), who attributed enhanced plant health and productivity to zinc's involvement in enzyme activation and chlorophyll formation.

The results of this study also enhance our understanding of the mechanisms behind zinc accumulation in wheat grains. As noted by Aciksoz et al. (2011), zinc accumulation in plant tissues is often associated with improved growth, which is further supported by the increased zinc concentration in grains under favorable growth conditions facilitated by zinc application. The ability of wheat plants to accumulate more zinc in their grains can be attributed to enhanced overall growth and metabolic activity, as also reported by Ghasemi et al. (2013). This is particularly important for biofortification strategies aimed at improving the nutritional quality of staple crops like wheat.

Zinc fertilization had a notable impact on wheat varieties, with Anmol-91, NIA-Amber, and NIA-Sarang exhibiting the highest grain zinc concentrations when treated with Zn-EDTA. This highlights the importance of selecting suitable wheat varieties to maximize the benefits of zinc

fertilization, as different genotypes vary in their ability to absorb and accumulate zinc. The superior performance of these varieties is consistent with findings by Zulfiqar et al. (2020), who reported that zinc fertilization increased both grain yield and zinc content across various wheat genotypes, reinforcing the potential of zinc biofortification in improving wheat nutrition.

Comparison of zinc sources

Compared to ZnSO_4 , Zn-EDTA proved to be a more efficient and stable source of zinc, particularly when applied in a banded manner in the soil (Zhao et al., 2019). Zn availability is low in calcareous soils; EDTA's effectiveness in enhancing grain Zn in wheat (Wang et al., 2019). This method enhanced zinc distribution and uptake, resulting in higher zinc concentrations in wheat grains (Alloway, 2009). In contrast, the banded application of ZnSO_4 limited zinc availability to a localized soil zone, reducing root interception and uptake (Liu et al., 2017). This limitation often resulted in reduced wheat growth and yield, as seen in the present study where ZnSO_4 banding led to lower zinc uptake and potential toxicity. These findings are consistent with (Wang et al., 2019; Zhao et al., 2019), who reported that Zn-EDTA was more effective than ZnSO_4 in enhancing zinc bioavailability and grain zinc concentration, particularly in high-pH soils.

CONCLUSION

This field experiment demonstrated that applying Zn-EDTA at 0.250 kg ha^{-1} significantly enhanced wheat growth, yield, zinc concentration, and bioavailability while reducing phytic acid content. The varieties Anmol-91, NIA-Amber, and NIA-Sarang performed exceptionally well. Zn-EDTA was more effective than other sources of ZnSO_4 , improving both nutrient uptake and overall crop productivity. Agronomic biofortification, a simple yet often overlooked approach, offers farmers an effective solution to enhance crop nutrition. Advances in specialty fertilizers, such as sulphates and chelates, are improving nutrient use efficiency and the nutritional quality of wheat. Future research should prioritize biofortification as a strategy to combat hidden hunger.

AUTHORS' CONTRIBUTIONS

MAS and SMB designed the study and conducted the experiments; MAS and ND jointly collected, arranged and analyzed the data; MAS drafted the manuscript; AJ reviewed and proofread the final version of the paper.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUSTAINABLE DEVELOPMENT GOALS TARGETED

SDG 2: Zero Hunger

SDG 12: Responsible Consumption and Production

SDG 13: Climate Action

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