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ZINC AND COPPER INTERACTION AND WHEAT RESPONSE IN RELATION TO THEIR CO-APPLICATION

FAIZAN ARAIN[1†], ZIA-UL-HASSAN[1*†], NAHEED AKHTER TALPUR[1], JAVARIA AFZAL ARAIN[2], INZAMAM ALI JAMALI[3] AND NIZAMUDDIN DEPAR[2]

[1]Department of Soil Science, Sindh Agriculture University, Tandojam, Pakistan
[2]Soil & Environmental Sciences Division, Nuclear Institute of Agriculture, Tandojam, Pakistan
[3]Department of Soil Science and Plant Nutrition, Erciyes University, Kayseri, Turkey
[†]These authors contributed equally to this work and share first authorship.

*Corresponding author's email: zhnshah@gmail.com

Abstract

Wheat response to adequate zinc (Zn) and copper (Cu) nutrition has been widely reported in Pakistan, due to their soil deficiency, however, their interactive effect has not been well established. We evaluated wheat (*cv*. MSH-14) response to co-application of Zn and Cu following a randomized complete block design with three repeats. Eight treatments were involved: a control with no Zn or Cu, three Zn levels @ 2.5, 5.0, and 7.5 kg ha^{-1} applied alone, or each combined with 2.5 kg Cu ha^{-1}, and a sole Cu treatment @ 2.5 kg ha^{-1}. The crop also received a blanket fertilizer dose @ 150-90-00 kg N-P-K ha^{-1}. Co-application of Zn and Cu (5.0-2.5 kg ha^{-1}) significantly ($p<0.001$) enhanced wheat growth, yield, nutrient concentration and uptake, and grain-to-nutrient efficiency ratio (GNER), when compared to other treatments. The Zn-Cu interaction varied with Zn application rate. Co-application of Zn at low or high doses @ 2.5 or 7.5 kg ha^{-1} along with 2.5 kg Cu ha^{-1} led to antagonism, as the observed GNER was lower than the predicted additive response. Balanced Zn-Cu co-application at an optimal ratio is critical for maximizing wheat grain yield, as their interaction may shift between synergism or antagonism depending on application rate.

Key words: Wheat, Zinc, Copper, Co-application, Grain-to-nutrient efficiency ratio, Interaction, Antagonism, Synergism

Introduction

Wheat (*Triticum aestivum* L.) is one of the significant staple food crops worldwide with annual production of around 789 million tons. The annual wheat consumption for 2023-24 was ~796 million metric tons, surpassing the overall production (Anon., 2023). Based on a United Nations report, it is projected that the worldwide population will rise from approximately 8.2 billion in 2024 to nearly 10 billion (21.95%) by the year 2050 (Anon., 2024). Given the severity of this issue, it is crucial to increase the production and yield of wheat in order to deal with the hunger to be caused by this expanding population (Albahri *et al.*, 2023).

In Pakistan, wheat is grown on ~9.0 million ha area as an important food crop. Its production is ~28 million tons with 3.1 Mg ha^{-1} average yield. However, the wheat production target for the country has not been met with the estimated production standing at more than 29 million tons, falling short of the targeted production (Anon., 2024).

Plants need essential nutrients in the balanced amounts and proper forms at right time, including macro and micronutrients both, that are crucial for crop nutrition and greatly impact the achievement of increased yields (Arif *et al.*, 2006). Zinc (Zn) is recognized as a crucial micronutrient for growth, development, and reproduction of crops (Ali *et al.*, 2021; Memon *et al.*, 2024). In plants, Zn is essential for numerous enzymatic functions, metabolic and redox processes, protein synthesis, as well as nitrogen (N) and carbohydrate-metabolism, photosynthesis, and sugar transformation to starch. Furthermore, Zn is crucial for protein and auxin metabolism and for preserving membrane integrity (Broadley *et al.*, 2012). In addition, Zn plays vital roles in enhancing plant tolerance to various abiotic stresses by stabilizing cellular structures and supporting antioxidant defense mechanisms (Jamali & Zia-ul-Hassan, 2025).

Copper (Cu) is another crucial micronutrient for crop growth as it enhances tillering and the viability of pollen. It is a vital component of different enzyme systems. These enzymes are essential for photosynthesis, respiration, and lignin synthesis, and the activity of these enzymes is mainly dependent on Cu availability. Copper is also an integral component of regulatory proteins and is involved in processes such as photosynthetic electron transport, metabolism of cell wall, hormonal signals, and respiration (Alloway, 2008).

Soil micronutrient deficiency not only restricts productivity of crops but also their nutritional value, ultimately affecting human health (Adal & Mulat, 2024). Similarly, Cu deficiency is also recognized as a significant factor limiting crop yield, food quality, and human health (Alloway, 2008).

The availability of Zn and Cu is often higher in acidic soils (Rashid *et al.*, 2022), and commonly noted restricted in alkaline-calcareous soils, including Pakistan (Imtiaz *et al.*, 2010; Rashid *et al.*, 2022; Jamali *et al.*, 2025). Reports indicate that Zn deficiency affects 51% of cultivated sites

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in the country (Rashid *et al.,* 2022) while Cu deficiency is said to be very limited and localized (Imtiaz *et al.,* 2010).

Nutrient interactions in plants are evaluated by observing responses to growth and variations in the concentration of nutrients. A combination of two nutrients that leads to a yield increase greater than when either nutrient is applied alone signifies a positive (synergistic) interaction; conversely, a yield decrease indicates a negative (antagonistic) interaction. If there is no change, it suggests that there is no interaction. These interactions can be quite intricate, as the presence of different nutrients can simultaneously influence one another, resulting in deficiencies, toxicities, or altered growth responses. Therefore, it's crucial to comprehend how specific nutrients interact with one another (Fageria, 2001). Understanding the mechanisms behind nutrient interactions in cropping systems is crucial for applying nutrients at the optimal time, ensuring that nutrient release aligns with crop uptake. This approach is thought to support sustainable agricultural practices and improve food security (Ayamba *et al.*, 2023).

The Zn–Cu interaction differs with soil type, organic matter status, and pH. All these factors affect the nutrient bioavailability and uptake by plants (Qiu *et al.*, 2016; Hartemink & Barrow, 2023). For that reason, the presence of Zn and Cu at higher concentrations may reduce the plant availability of each other (Imtiaz *et al.*, 2003). However, research on nutrient interactions is limited because studies are typically conducted on a small number of crops, specific types of nutrients, certain soil types and in particular climates (Rietra *et al.*, 2017).

Significant effect of Zn and Cu nutrition on wheat have been reported in Pakistan suggesting that the adequate supply of these micronutrients, along with other necessary macronutrients, can be highly beneficial for wheat production (Rashid *et al.*, 2022). Nonetheless, co-application effect of Zn and Cu has not been properly established in terms of their interaction (Isakova *et al.*, 2022). The effectiveness of Cu fertilization varies in different studies, with some showing no negative or positive impacts. Therefore, it is crucial to supply plants with Zn and Cu fertilizers to cope with their deficiencies in alkaline-calcareous soils and properly understand their interaction.

The interaction of Zn and Cu remained the subject of many studies previously reported in literature (Imtiaz *et al.*, 2003; Rietra *et al.*, 2017; Isakova *et al.*, 2022). Nonetheless, the subject of most of these studies was individual nutrient responses or simple co-application effects. These studies did not explore structured dose-response under calcareous soils. Use of efficiency-based models for the interpretation of interaction behavior in quest of integrating yield and nutrient dynamics is highly scarce. For these reasons, it was indispensable to clearly understand the response of graded Zn levels integrated with a fixed dose of Cu under alkaline-calcareous soils.

We conducted this field study by hypothesizing that the Zn and Cu co-application can increase wheat yield by enhancing their concentration. Balanced Zn–Cu co-application would produce synergistic yield enhancement, whereas imbalanced ratios would result in antagonism. This study aimed to evaluate wheat yield and Zn and Cu relations, along with their nature of interaction as affected by their single and integrated application.

Materials and Methods

Experimental site: Field study was conducted at the Research Farm of Nuclear Institute of Agriculture (NIA), Tandojam, Sindh, Pakistan (25.414598N, 68.520897E).

Soil sampling, processing, and analysis: Soil sampling was done prior to the application of fertilizer before wheat sowing. The samples were brought to the laboratory for processing and analysis of physicochemical properties of soil, i.e. texture, electrical conductivity (EC), pH, organic matter, and ABDTPA extractable P, Zn, and Cu following Ryan *et al*., (2001).

Experimental design and treatment plan: The study was conducted using a Randomized Complete Block Design (RCBD), which included eight different treatments, each repeated three times. The treatments involved varying doses of chemical Zn and Cu fertilizers, viz: $T_{1:}$ control (no Zn or Cu applied), $T_{2:}$ 2.5 kg Zn ha^{-1}, $T_{3:}$ 2.5 kg Zn ha^{-1} + 2.5 kg Cu ha^{-1}, $T_{4:}$ 5.0 kg Zn ha^{-1}, $T_{5:}$ 5.0 kg Zn ha^{-1} + 2.5 kg Cu ha^{-1}, $T_{6:}$ 7.5 kg Zn ha^{-1}, $T_{7:}$ 7.5 kg Zn ha^{-1} + 2.5 kg Cu ha^{-1}, and $T_{8:}$ 2.5 kg Cu ha^{-1}. The rationale for choosing a fixed Cu dose (2.5 kg ha^{-1}) across treatments is because of regional fertilizer recommendations and soil critical limits (~0.19 mg kg^{-1} AB-DTPA extractable Cu).

Fertilizer source and application method: Pure analytical grade (≥99% purity) sulfates of Zn ($ZnSO_4.7H_2O$) and Cu ($CuSO_4.5H_2O$), with 22% Zn and 25% Cu, respectively, were utilized to apply the fertilizer treatments of Zn and Cu. Zinc and copper were soil-applied at first irrigation and incorporated into the soil; no foliar application was performed.

Seed source: The seeds for the wheat variety MSH-14 were sourced from NIA Tandojam.

Execution of the field study: A total area of 360 m^2 was designated for the experiment and subdivided into 24 equal experimental units based on the research plan. Each plot measured 15 m^2 (3.0 m x 5.0 m). The land preparation was conducted in accordance with established methodologies and protocols. Wheat was sown in November during the Rabi season of 2023-24. Pure seeds of the wheat variety MSH-14 were sown by drilling @125 kg ha^{-1}. Row spacing was maintained at 20 cm. A blanket application of nitrogen (N) and phosphorus (P) was administered at 150-90 kg N-P ha^{-1} using urea and diammonium phosphate (DAP). Full dose of P with ½ N was applied during land preparation by broadcasting. The remaining N was applied with Zn and Cu treatments at the first irrigation. Additionally, crop was irrigated as per its need, totaling four irrigations throughout its growth period. Continuous monitoring of the crop was carried out up to the harvesting stage. Weeds, diseases, and insect pests were managed according to standard practice.

Collation of data: Ten randomly chosen plants were collected to determine nutrient concentration and record plant height, spike length, number of spikelets per spike, grains per spike, seed index (1000-grains weight), and yield of grains and straw.

Plant analysis: Plants were properly washed with distilled water to remove dust and contaminants, and air-dried for about two days, prior to oven-drying (Heraeus B5050 F) at 70 °C for 48 hours. Once all moisture was removed, the samples were cut into smaller pieces using stainless steel scissors, and ground to powder in a grinder (IKA MF10 basic). Grain and straw Zn and Cu concentration was determined through a 1:5 perchloric acid-nitric acid ($HClO_4$-HNO_3) diacid wet digestion method (Rashid, 1986). Plant Zn and Cu concentration was assessed by preparing working standards and analyzing alongside the plant samples on atomic absorption spectrophotometer (Ryan *et al.*, 2001).

Calculation of grain-to-nutrient efficiency ratios (GNER, %): The grain-to-nutrient efficiency ratio (GNER) was calculated to evaluate interaction effects between Zn and Cu. The predicted additive response was computed as:

Predicted response = (Yield under Zn alone + Yield under Cu alone − Yield under control).

The actual combined response was the observed yield under Zn + Cu application.

The GNER (%) was calculated as:

$$\text{GNER (\%)} = \frac{\text{Actual combined response} - \text{Predicted response}}{\text{Predicted response}} \times 100$$

The additive prediction model assumes independent nutrient effects and interprets biological rather than statistical interaction. Positive and negative GNER values indicate synergistic and antagonistic interactions, respectively, where the observed combined response is higher or lower than the predicted additive response.

Determining zinc and copper interaction: The interaction between Zn and Cu was determined by calculating the predicted GNER for the treatments where these nutrients were co applied at lower dose, i.e. T3: 2.5 kg Zn and 2.5 kg Cu ha^{-1} (by adding the actual GNER of T2 and T8), balanced dose, i.e. T5: 5.0 kg Zn and 2.5 kg Cu ha^{-1} (by adding the actual GNER of T4 and T8) and higher dose, i.e. T7: 7.5 kg Zn and 2.5 kg Cu ha^{-1} (by adding the actual GNER of T6 and T8). The Zn and Cu interaction was termed "Antagonistic", "Synergistic" or "Neutral" if the actual GNER was lower, higher, or equal when compared to the predicted GNER.

Interpretation of zinc and copper interaction: The antagonism highlights competition and hinderance because of Zn and Cu co-application for their plant uptake and utilization and calls for their balanced application and better management. The synergism points out positive and beneficial relationship of Zn and Cu integrated application for their plant uptake and utilization. The neutral effect due to Zn and Cu co-application advocates that both these nutrients act independently and neither help nor hinder each other in their uptake and utilization by the plants.

Statistical analysis: Analysis of variance (ANOVA) was performed on Statistix version 8.1. Means were separated by employing Honestly Significant Difference (HSD) test (alpha 0.05).

Results

Physicochemical properties of the experimental soil: The soil of experimental area was analyzed to assess key characteristics. The laboratory analysis (Table 1) revealed that soil was composed of a silt-loam texture (Sand: 17.5%, Silt: 57.5%, Clay: 25%). The soil was free from salinity (EC: 0.95 dS m^{-1}), moderately-alkaline (pH: 7.81), and deficient in organic matter (0.67%). Additionally, the soil showed marginal levels of AB-DTPA extractable phosphorus (6.0 mg kg^{-1}) along with a low concentration of ABDTPA-extractable Zn (0.48 mg kg^{-1}) and Cu (0.19 mg kg^{-1}), as reported earlier for calcareous soils.

Table 1. Physico-chemical properties of the experimental soil before the start of study.

Parameter			Result
Texture	Sand (%)	17.5	Silt loam
	Silt (%)	57.5	
	Clay (%)	25	
EC (dS m^{-1})			0.95
pH			7.81
Organic matter (%)			0.67
ABDTPA Phosphorus (mg kg^{-1})			6.0
ABDTPA Zinc (mg kg^{-1})			0.48
ABDTPA Copper (mg kg^{-1})			0.19

Growth and yield traits of wheat: The results revealed that plant height was substantially increased by both individual and combined (interactive) applications of Zn and Cu (Table 2). Plant height was minimum (88.3 cm) where no Zn or Cu was applied, while Zn + Cu combinations outperformed Zn-alone treatments by an average of 1.9%. It was maximum (98.0 cm) in integrated application of 5.0 kg Zn + 2.5 kg Cu ha^{-1}, relatively 10.9% increase over control, and 4.5% compared to Zn-alone (5.0 kg ha^{-1}) (93.7 cm). The application of sole Zn at higher rate achieved a plant height of (96.1 cm), while its co-application with Cu at (7.5 kg Zn + 2.5 kg Cu ha^{-1}) obtained (94.5 cm) of plant height, suggesting a synergistic effect of Zn and Cu at moderate Zn doses. Notably, Cu alone (2.5 kg ha^{-1}) (91.5 cm) marginally surpassed the control but underperformed compared to Zn-inclusive treatments. Intermediate plant heights were recorded at lower application rate of Zn and its combination with Cu with (89.7 cm) and (92.3 cm), respectively. These results imply that balanced Zn-Cu fertilization optimizes growth more effectively than single applications.

The application of Zn and Cu significantly influenced spike length, with distinct responses observed across treatments (Table 2). The highest spike length was noticed under integrated Zn and Cu (5.0 kg Zn ha^{-1} + 2.5 kg Cu ha^{-1}), where spike length reached (13.23 cm) a 37.4% increase over control, demonstrating a clear synergistic effect between Zn and Cu. The control (no Zn or Cu) produced the minimum spike length (9.63 cm). However, a spike length of (12.07 cm) was observed at the higher rate of single Zn application (7.5 kg Zn ha^{-1}). Interestingly, Cu alone (10.38 cm) had a marginal effect compared to the control, while except integration of Zn with Cu at higher rate and sole Zn at higher rate (7.0 kg ha^{-1}), the rest of the treatments (9.63–10.97 cm) were non-significant ($p>0.05$). These findings suggest that combination of Zn with Cu at optimal levels (5.0 kg Zn/ha + 2.5 kg Cu ha^{-1}) maximizes spike length over other single and integrated Zn and Cu applications.

The number of spikelets per spike were non-significant among treatments ($p>0.05$), except the balanced integrated application of (5.0 kg Zn ha^{-1} + 2.5 kg Cu ha^{-1}) which produced the maximum spikelets per spike of wheat plants (22.8), as given in Table 2. All other treatments showed statistically similar results ranging from (18.1 to 20.5), regardless of Zn or Cu application alone or in combination. Notably, the synergistic effect of moderate Zn (5.0 kg) integrated with Cu (2.5 kg) significantly enhanced spikelets per spike.

Grains per spike were enhanced with the application of Zn and Zn + Cu (Table 2). The maximum grains per spike (62.7) were observed in the moderate integrated dose of Zn + Cu, representing a 46% increase over the control (42.9), and an 18.5% over sole Zn nutrition at 5.0 kg ha^{-1} (52.9). The lower doses (2.5 kg) of Zn and Zn + Cu produced comparable results (49.8, 49.3 grains spike^{-1}), while higher doses (7.5 kg Zn + 2.5 kg Cu ha^{-1}) acquired lower grains per spike than (5.0 kg Zn kg ha^{-1}) (53.8–57.5). Copper alone (2.5 kg ha^{-1}) showed minimal benefit (47.8) but synergized with Zn at optimal doses.

The seed index of wheat was significantly improved across Zn and Cu fertilizer treatments (Table 2). The control (no Zn/Cu) recorded the lowest seed index (30.9 g). While, the integrated fertilization of 5.0 kg Zn + 2.5 kg Cu ha^{-1} achieved maximum seed index (48.8 g), showing a 57.9% increase over control. The sole Zn applications (5.0-7.5 kg ha^{-1}) also enhanced seed index up to (38.6 g) and (45.2 g), respectively, with an increase of 25% and 46.3% over control. However, the rest of the Cu and Zn applications further enhanced seed index, except at the highest Zn rate integrated with Cu, where the seed index (42.3 g) was 6.4% lower than (7.5 kg Zn) (45.2 g), suggesting a potential threshold for Zn-Cu synergy.

Table 2. Plant growth traits of wheat as influenced by single and integrated applications of zinc and copper at different rates.

Zn - Cu (kg ha^{-1})	Plant height (cm)	Spike length (cm)	Number of spikelets per spike	Number of grains per spike	Seed index (g)
0.0 - 0.0	88.3 E	9.63 C	18.1 B	42.9 E	30.9 F
2.5 - 0.0	89.7 DE	10.31 C	19.8 B	49.8 CD	34.3 DEF
2.5 - 2.5	92.3 BCDE	10.17 C	19.3 B	49.3 CD	36.3 DE
5.0 - 0.0	93.7 BCD	10.37 C	18.8 B	52.9 C	38.6 CD
5.0 - 2.5	98.0 A	13.23 A	22.8 A	62.7 A	48.8 A
7.5 - 0.0	96.1 AB	12.07 AB	20.5 AB	57.5 B	45.2 AB
7.5 - 2.5	94.5 ABC	10.97 BC	19.1 B	53.8 BC	42.3 BC
0.0 - 2.5	91.5 CDE	10.38 C	18.9 B	47.8 D	32.6 EF
SEm (±)	0.85	0.28	0.49	0.92	0.92
CV (%)	1.58	4.37	4.33	3.04	4.10
$HSD_{0.05}$	4.2308	1.3726	2.4501	4.5662	4.5658

Table 3. Grain and straw yield of wheat as influenced by single and integrated applications of zinc and copper at different rates.

Zn – Cu (kg ha^{-1})	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})
0.0 - 0.0	3683 E	6732 D
2.5 - 0.0	4297 CD	7448 C
2.5 - 2.5	4320 CD	7883 B
5.0 - 0.0	4387 CD	7957 B
5.0 - 2.5	5059 A	8561 A
7.5 - 0.0	4830 AB	8488 A
7.5 - 2.5	4643 BC	8382 A
0.0 - 2.5	4163 D	7032 D
SEm (±)	76.25	62.08
CV (%)	2.99	1.38
$HSD_{0.05}$	380.35	309.68

The application of single and integrated doses of Zn Cu improved grain yield over control (3683 kg ha^{-1}) (no Zn/Cu) (Table 3). The maximum grain yield (5059 kg ha^{-1}) was achieved with co-application of (5.0 kg Zn + 2.5 kg Cu ha^{-1}), representing a 37.3% increase over the control. The sole Zn applications (2.5, 5.0, and 7.5 kg Zn ha^{-1}) showed substantial enhancements in grain yield (4297–4830 kg ha^{-1}). However, the integrated effect of Zn and Cu was dependent on application rates. While (5.0 kg Zn + 2.5 kg Cu ha^{-1}) acquired the highest yield (5059 kg ha^{-1}), a 15.3% increase over the sole Zn application treatment (5.0 kg ha^{-1}). The integrated supply of Zn and Cu at the lowest rates showed small, but meaningful increases in grain yield (4320 kg ha^{-1}). At the highest rate of integrated application, it was noticed that grain yield was reduced (4643 kg ha^{-1}) by 3.9% compared to singly applied Zn at the highest dose.

The results showed that straw yield varied across Zn and Zn + Cu treatments (Table 3). The control (no Zn/Cu) obtained the minimum straw yield (6,732 kg ha^{-1}), while the combined supply of Zn and Cu consistently outperformed Zn-alone applications. The maximum yield was observed in (5.0 kg Zn + 2.5 kg Cu ha^{-1}) (8561 kg ha^{-1}), then Zn supply at highest dose (8,488 kg ha^{-1}) followed by integrated supply of Zn and Cu at higher doses (8,382 kg ha^{-1}), proving synergistic effects of Zn and Cu at moderate Zn doses. Notably, Cu alone (7,032 kg ha^{-1}) showed minimal relative enhancement over the control, whereas all Zn-alone treatments increased yields by 10.6% (7,448 kg ha^{-1}), 18.2% (7,957 kg ha^{-1}), and 26.1% (8,488 kg ha^{-1}), respectively.

Zinc and copper concentration of wheat: Grain Zn concentration was significantly enhanced by Zn and Zn + Cu treatments (*$p<0.05$*) (Table 4). The control (no Zn/Cu) and sole Cu treatment showed the least Zn concentration (14.1 and 14.7 mg kg^{-1}, respectively). However, Zn applications alone linearly increased grain Zn concentration, with (23.9 mg kg^{-1}), (29.2 mg kg^{-1}), and (32.2 mg kg^{-1}), respectively, demonstrating a dose-dependent response. Co-application of Zn and Cu further enhanced Zn concentration where the lower combined dose achieved (28.8 mg kg^{-1}). While the maximum grain Zn content (35.6 mg kg^{-1}) was recorded under integrated application at moderate doses. However, Zn application at higher dose (7.5 kg Zn + 2.5 kg Cu ha^{-1}) achieved a Zn content of (33.8 mg kg^{-1}), which was lower than Zn applied at lower dose (5.0 kg Zn + 2.5 kg Cu ha^{-1}).

Straw Zn concentration was considerably increased as affected by Zn and Cu treatments (Table 4). The control (no Zn/Cu) recorded the Zn concentration of (9.8 mg kg^{-1}), while Cu-alone application (2.5 kg ha^{-1}) further reduced Zn levels to (7.5 mg kg^{-1}). The maximum Zn concentration (23.6 mg kg^{-1}), a 141% increase over control was observed with combined moderate doses of Zn and Cu, highlighting synergistic effects. Other Zn+Cu combinations also enhanced Zn concentrations (17.8 and 21.5 mg kg^{-1}) but were less effective than moderate integrated dose of Zn and Cu. Notably, higher Zn doses (7.5 kg ha^{-1}) with or without Cu showed low straw Zn concentration compared to (5.0 kg Zn + 2.5 kg Cu ha^{-1}), suggesting a balanced rate for Zn-Cu co-application.

The data showed that grain Cu concentration was substantially altered by Zn and Cu fertilizer treatments (Table 4). The control showed the lowest Cu concentration (3.4 mg kg^{-1}), while moderate supply of Zn+Cu obtained (12.4 mg kg^{-1}) grain Cu concentration, a 3.6-fold increase over control and 9% higher than sole Cu fertilizer treatment (11.4 mg kg^{-1}), showing a positive interactive effect of moderate Zn-Cu co-application. Other Cu-supplemented treatments also increased grain Cu concentration up to (10.9 mg kg^{-1}), though the integrated dose at higher rates of Zn and Cu (7.5 kg + 2.5 kg ha^{-1}) showed a 12% decrease in Cu concentration compared to the balanced rate mentioned above. Zn-alone treatments achieved a grain Cu content of (3.8–4.2 mg kg^{-1}), which remained statistically indistinguishable from the control (3.4 mg kg^{-1}).

The results demonstrated that straw Cu concentration of wheat was influenced by Cu application or its combination with Zn (Table 4). Compared to the control (4.4 mg kg^{-1}), all Cu-treated applications showed substantially higher Cu concentration, with the maximum concentration (16.4 mg kg^{-1}) observed in sole Cu treatment (2.5 kg ha^{-1}), showing a 3.7-fold increase over the control. The integrated Zn + Cu applications at moderate and higher dose also enhanced Cu concentration, though to a lesser extent than Cu alone. While integrated Zn and Cu application at lower dose reached (10.0 mg kg^{-1}). Notably, Cu concentration decreased by 12% (from 14.9 to 13.1 mg kg^{-1}) as the rate of Zn varied under fixed Cu (2.5 kg ha^{-1}), suggesting a potential antagonistic effect of higher Zn levels. Treatments with Zn alone showed minimal Cu enrichment (4.7–5.5 mg kg^{-1}).

Zinc and copper uptake of wheat: The results demonstrated that Zn and combined Zn + Cu applications significantly enhanced grain Zn uptake compared to the control (0.0519 kg ha^{-1}) (Table 5). The highest uptake was recorded with (5.0 kg Zn + 2.5 kg Cu ha^{-1}) (0.1804 kg ha^{-1}), marking a 3.4-fold increase over control and a 40% increase over sole Zn application (5.0 kg ha^{-1}) (0.1283 kg ha^{-1}). Combined treatments at lower (2.5 kg Zn + 2.5 kg Cu ha^{-1}) and higher (7.5 kg Zn + 2.5 kg Cu ha^{-1}) doses also showed substantial increases (2.3- and 3.0-fold over control, respectively). The sole Zn treatments exhibited a dose-dependent increase, with maximum at (7.5 kg Zn ha^{-1}) (0.1556 kg ha^{-1}). The Cu-alone treatment (0.0613 kg ha^{-1}) had minimal role in enhancing grain Zn uptake of wheat.

The results pertaining to straw Zn uptake are furnished in Table 5, revealing that Zn uptake in straw was significantly influenced by Zn and Zn + Cu applications. The control (no Zn/Cu) recorded the Zn uptake in straw at (0.0660 kg ha^{-1}), while sole Cu treatment (2.5 kg ha^{-1}) further reduced uptake (0.0528 kg ha^{-1}). Maximum uptake was observed in (5.0 kg Zn + 2.5 kg Cu ha^{-1}) (0.2017 kg ha^{-1}), representing a 3.1-fold increase over control and a 34% increase over (5.0 kg Zn ha^{-1}) (0.1504 kg ha^{-1}). Combined Zn + Cu treatments at lower (2.5 kg Zn + 2.5 kg Cu ha^{-1}) and higher (7.5 kg Zn + 2.5 kg Cu ha^{-1}) doses also enhanced Zn uptake (2.1- and 2.7-fold over control, respectively). The single applications of Zn treatments (2.5 to 7.5 kg ha^{-1}) exhibited dose-dependent increases, with maximum at (7.5 kg Zn ha^{-1}) (0.1804 kg ha^{-1}).

Total Zn uptake by wheat varied significantly across treatments ($p < 0.05$), reflecting the influence of Zn and Zn + Cu applications (Table 5). The control (no Zn/Cu) and Cu-only treatment (2.5 kg Cu ha^{-1}) showed the lowest Zn uptake (0.1179 and 0.1141 kg ha^{-1}, respectively). The highest Zn uptake observed at balanced nutrition of 5.0 kg Zn + 2.5 kg Cu ha^{-1} (0.3820 kg ha^{-1}), representing a 3.2-fold increase over the control and 37% over Zn-alone treatment (5.0 kg ha^{-1}) (0.2787 kg ha^{-1}). Furthermore, the integrated Zn and Cu treatments at lower (2.5 kg Zn + 2.5 kg Cu ha^{-1}) and higher (7.5 kg Zn + 2.5 kg Cu ha^{-1}) doses also enhanced uptake (2.2- and 2.8-fold over control, respectively). Solo Zn treatments exhibited dose-dependent increases, with maximum at (7.5 kg Zn ha^{-1}) (0.3360 kg ha^{-1}).

Grain copper uptake varied significantly across Cu-alone and Zn + Cu treatments (Table 5). The control (no Zn/Cu) showed the lowest grain Cu uptake (0.0124 kg ha^{-1}), while the integrated Zn + Cu treatments consistently outperformed for leftover treatments. The co-nutrition of Zn and Cu at (5.0 kg Zn + 2.5 kg Cu ha^{-1}) achieved maximum Cu uptake (0.0625 kg ha^{-1}), a 5.0-fold increase over control and 31% higher than sole Cu treatment at (2.5 kg ha^{-1}) (0.0475 kg ha^{-1}). Similarly, (2.5 kg Zn + 2.5 kg Cu ha^{-1}) and (7.5 kg Zn + 2.5 kg Cu ha^{-1}) recorded substantial grain Cu uptake (0.0433 and 0.0504 kg ha^{-1}, respectively). Among Zn-alone treatments, (7.5 kg Zn ha^{-1}) showed minimal Cu uptake (0.0203 kg ha^{-1}).

Straw Cu uptake varied significantly with Cu and Zn + Cu applications (Table 5). The control showed the lowest Cu uptake in straw (0.0294 kg ha^{-1}), while integrated treatments Zn + Cu enhanced Cu uptake. Notably, (5.0 kg Zn + 2.5 kg Cu ha^{-1}) achieved the maximum Cu uptake (0.1273 kg ha^{-1}), outperforming control by 333% and exceeding Cu-alone treatment (0.1153 kg ha^{-1}) by 10%. Similarly, (2.5 kg Zn + 2.5 kg Cu ha^{-1}) and (7.5 kg Zn + 2.5 kg Cu ha^{-1}) recorded substantial Cu uptake (0.0791 and 0.1101 kg ha^{-1}, respectively). Lower and moderate Zn doses without Cu (2.5 and 5.0 kg Zn ha^{-1}) (0.0355, 0.0377 kg ha^{-1}) were non-significant ($p > 0.05$), However, the higher dose of Zn (7.5 kg ha^{-1}) contributed minimally to straw Cu uptake (0.0465 kg ha^{-1}).

Total Cu uptake in wheat responded distinctly to Cu and Zn + Cu treatments (Table 5). The control (no Zn/Cu) showed the lowest Cu uptake (0.0418 kg ha^{-1}). The maximum Cu uptake was observed at balanced application of 5.0 kg Zn ha^{-1} + 2.5 kg Cu ha^{-1} (0.1898 kg ha^{-1}), representing a 354% increase over the control and 16% over Cu-alone treatment 2.5 kg ha^{-1} (0.1627 kg ha^{-1}). Furthermore, combined Zn + Cu treatments at lower (2.5 kg Zn ha^{-1} + 2.5 kg Cu ha^{-1}) and higher (7.5 kg Zn ha^{-1} + 2.5 kg Cu ha^{-1}) doses also enhanced Cu uptake (0.1225-0.1605 kg ha^{-1}). In contrast, sole Zn treatment 7.5 kg Zn ha^{-1} (0.0668 kg ha^{-1}) had minimal impact, while 2.5 and 5.0 kg Zn kg ha^{-1} (0.0517, 0.0538 kg ha^{-1}) were found non-significant ($p > 0.05$).

Table 4. Zinc and copper concentration of wheat as influenced by single and integrated applications of zinc and copper at different rates.

Zn – Cu (kg ha^{-1})	Zinc concentration (mg kg^{-1})		Copper concentration (mg kg^{-1})	
	Grain	Straw	Grain	Straw
0.0 - 0.0	14.1 E	9.8 E	3.4 D	4.4 E
2.5 - 0.0	23.9 D	15.8 D	3.8 D	4.8 E
2.5 - 2.5	28.8 C	17.8 C	10.0 C	10.0 D
5.0 - 0.0	29.2 C	18.9 C	3.7 D	4.7 E
5.0 - 2.5	35.6 A	23.6 A	12.4 A	14.9 B
7.5 - 0.0	32.2 B	21.3 B	4.2 D	5.5 E
7.5 - 2.5	33.8 AB	21.5 B	10.9 BC	13.1 C
0.0 - 2.5	14.7 E	7.5 F	11.4 AB	16.4 A
SEm (±)	0.39	0.29	0.27	0.30
CV (%)	2.57	2.90	6.28	5.71
$HSD_{0.05}$	1.9687	1.4226	1.3489	1.5163

Table 5. Zinc and copper uptake of wheat as influenced by their single and integrated applications at different rates.

Zn - Cu (kg ha^{-1})	Zinc uptake (kg ha^{-1})			Copper uptake (kg ha^{-1})		
	Grain	Straw	Total	Grain	Straw	Total
0.0 - 0.0	0.0519 E	0.0660 E	0.1179 E	0.0124 D	0.0294 E	0.0418 E
2.5 - 0.0	0.1026 D	0.1179 D	0.2205 D	0.0162 CD	0.0355 DE	0.0517 DE
2.5 - 2.5	0.1245 C	0.1404 C	0.2649 C	0.0433 B	0.0791 C	0.1225 C
5.0 - 0.0	0.1283 C	0.1504 C	0.2787 C	0.0161 CD	0.0377 DE	0.0538 DE
5.0 - 2.5	0.1804 A	0.2017 A	0.3820 A	0.0625 A	0.1273 A	0.1898 A
7.5 - 0.0	0.1556 B	0.1804 B	0.3360 B	0.0203 C	0.0465 D	0.0668 D
7.5 - 2.5	0.1571 B	0.1803 B	0.3374 B	0.0504 B	0.1101 C	0.1605 B
0.0 - 2.5	0.0613 E	0.0528 E	0.1141 E	0.0475 B	0.1153 B	0.1627 B
SEm (±)	0.0036	0.0028	0.0055	0.0015	0.0023	0.0034
CV (%)	5.16	3.52	3.72	7.93	5.58	5.49
$HSD_{0.05}$	0.0179	0.0138	0.0274	0.0076	0.0117	0.0168

Grain-to-nutrient efficiency ratio (%): Grain to nutrient efficiency ratio (GNER) varied significantly ($p < 0.05$) across Zn and Cu treatments (Fig. 1). The control (no Zn/Cu) showed no efficiency (0.0%), while standalone Cu (2.5 kg Cu ha^{-1}) achieved a modest 13.1%. Zn-only treatments improved efficiency progressively with increasing rates (2.5 kg Zn ha^{-1}) at 16.8%, (5.0 kg Zn ha^{-1}) at 19.3%, and (7.5 kg Zn ha^{-1}) peaking at 31.2%. However, the maximum GNER was recorded in Zn + Cu combination at (5.0 kg Zn + 2.5 kg Cu ha^{-1}) which reached 37.7%, outperforming all other treatments. Notably, Zn + Cu integrated application enhanced efficiency at lower Zn levels (2.5 kg Zn + 2.5 kg Cu ha^{-1}) (17.5%) against (2.5 kg Zn ha^{-1}) (16.8%). While Zn + Cu application at (7.5 kg Zn + 2.5 kg Cu ha^{-1}) recorded a GNER of 26.1% against (7.5 kg Zn ha^{-1}) which obtained 31.2% GNER, suggesting a synergistic effect at moderate Zn + Cu doses (5.0 kg + 2.5 kg ha^{-1}) but potential antagonism at excess.

Zinc and copper interaction analysis: The results suggested that the interaction of Zn with Cu may vary depending upon the rate of Zn application (Fig. 2). At the lower and higher application rates of Zn (2.5 and 7.5 kg ha^{-1}, respectively), the interaction of Zn with Cu (2.5 kg ha^{-1}) was antagonistic as evident from the negative GNER (-12.41% and -18.1%, respectively), since it was lower for actual (17.3% and 26.1%, respectively) than the predicted (29.7% and 44.2%, respectively). This suggests that when Zn was applied at lower or higher rates with Cu, their combined effect was less than their expected additive effect. This could indicate competition between Zn and Cu for uptake or utilization at their imbalanced ratios.

However, at the proper ratio and balanced application rate of Zn (5.0 kg ha^{-1}) and Cu (2.5 kg ha^{-1}) their interaction was synergistic as evident from the positive grain to nutrient efficiency ratio (+5.21%), since it was higher for actual (37.4%) than the predicted (32.1%). This suggests a positive interaction, meaning that Zn and Cu together at this level improved nutrient uptake or efficiency.

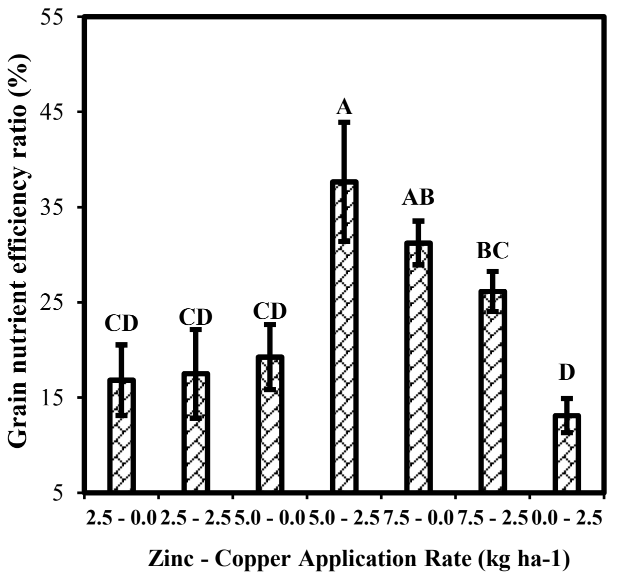

Fig. 1. Grain-to-nutrient efficiency ratio of wheat in relation to single and integrated application of zinc and copper at different rates. Error bars represent ± standard error of the mean based on three replications. CV (%) = 18.73; HSD (0.05) = 3.093.

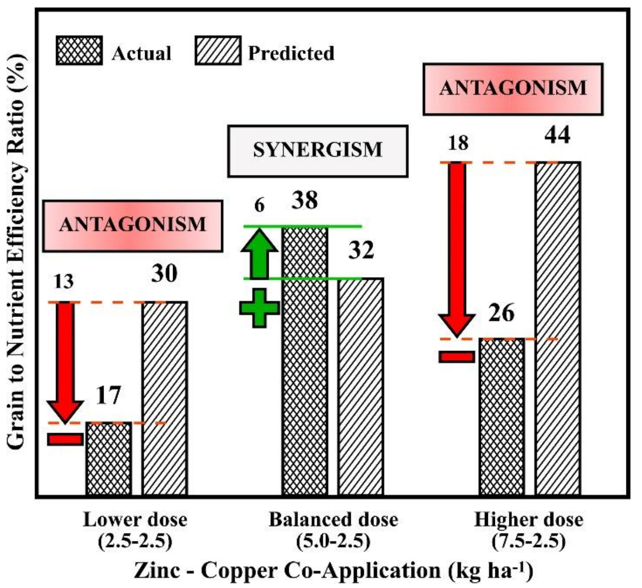

Fig. 2. Nature of zinc and copper interaction in response to their co-application at lower, balance and higher application rates in wheat.

Relationship of wheat grain yield with some key traits: The grain yield of wheat was correlated with GNER, and the grain concentration of Zn and Cu through Scatter Plot Matrix (SPLOM). A very strong positive correlation (0.9393) suggested that higher grain nutrient efficiency is closely linked to higher grain yield (Fig. 3). Wheat grain yield was strongly correlated with grain Zn concentration (0.8704) which indicated that higher grain yield is associated with greater Zn concentration in grains. This suggests that higher-yielding wheat varieties may also have improved Zn accumulation, which is beneficial for nutritional quality. However, a weaker correlation (0.3921) compared to Zn suggested that grain yield is not as strongly influenced by Cu concentration as it is by Zn. This may imply that Zn plays a more crucial role in supporting grain yield than Cu. For Zn and Cu interaction, the relatively low correlation (0.2571) suggests that grain Zn and Cu concentrations are not strongly linked. This indicates that these two micronutrients may be regulated independently in wheat grains, possibly influenced by different genetic or environmental factors. Keeping the Zn biofortification potential in mind, given the high correlation between the concentration and yield of grains, breeding programs that aim for both high-yielding and nutritionally enhanced wheat may achieve success without needing to make trade-offs between wheat grain yield and Zn content.

Discussion

The present study focused on the interactive effects of Zn and Cu on the performance of wheat under Zn and Cu-deficient environments. Compared to the control (no Zn/Cu application), which showed the poorest performance, the application of Zn either alone at varying doses or in combination with Cu significantly enhanced wheat agronomic parameters and yield. Among the individual Zn and Cu treatments, the integrated balanced nutrition of 5.0 kg Zn ha^{-1} along with 2.5 kg Cu ha^{-1} indicated a synergistic effect, resulting in the highest grain yield, improved Zn and Cu concentrations in grains, and optimal grain-to-nutrient efficiency.

It is evident that this best integration leads to a balanced supply of Zn and Cu. A balanced Zn supply positively affects enzyme activation, protein synthesis, and growth processes, while a balanced supply of Cu plays critical functions in photosynthetic electron transport and redox metabolism (Broadley *et al*., 2012). Hence, Zn-Cu co-application at moderate rates likely maintained their primary physiological functions and resulted in a synergistic response, while the reverse was true at their lower and higher application rates. Hence, the synergism noted highlights a balanced Zn-Cu ratio under the tested soil conditions.

These findings highlight the importance of balanced Zn and Cu supplementation for maximizing wheat productivity in nutrient-deficient soils. Numerous workers reported that the combination of zinc and copper fertilization can improve wheat yield, with optimal levels of both micronutrients leading to optimum filling of grains and better seed quality (Ali *et al*., 2021). A study by Bhowmik *et al*., (2022) demonstrated that balanced zinc and copper application resulted in a higher grain yield and better nutrient uptake, particularly in zinc-deficient soils, a common problem in many wheat-growing regions. The synergistic effect of Zn and Cu applications enhanced wheat growth through improved photosynthetic efficiency and nutrient assimilation.

In addition to wheat, the interaction of zinc and copper has also been studied in other grain crops. For instance, in maize, combined applications of zinc and copper led to increased biomass production and improved nitrogen assimilation (Dai *et al*., 2021). Similarly, in barley, the synergistic effect of zinc and copper contributed to better resistance to biotic and abiotic stresses, as well as improved yield (Rashid *et al*., 2022). These results suggest that balanced Zn–Cu fertilization may have potential to increase productivity in other grain crops under similar soil and environmental conditions.

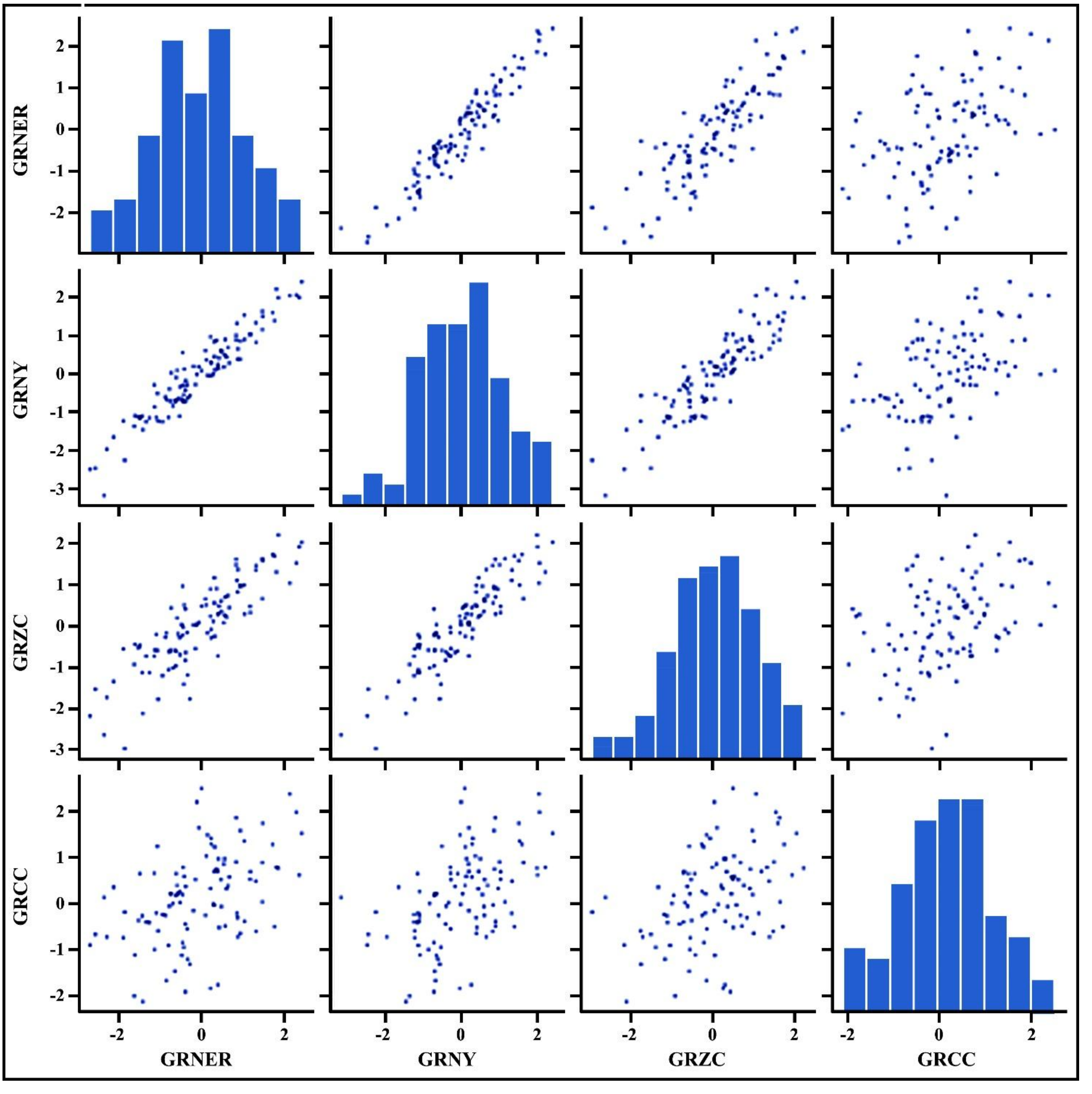

Fig. 3. Relationship of wheat grain yield with some key traits in relation to single and integrated application of zinc and copper at different rates.

It was observed that a considerable enhancement in yield and quality of wheat grains was achieved with Zn and Cu over their respective controls (Qane *et al.*, 2024), likely because of a variety of benefits related to Zn and Cu nutrition. A balanced supply of Zn and Cu to soil system is vital for optimal crop productivity, particularly in grain crops like wheat, where micronutrient deficiency can significantly reduce yield and quality (Marschner, 2012).

Zinc and copper are both involved in enzyme systems that are essential for photosynthesis, protein synthesis, and the regulation of plant stress responses (Aydin *et al.*, 2021). For instance, Zn is very crucial for chlorophyll synthesis, while Cu is involved in the respiratory enzymes as a key component. Thus, an adequate supply of both nutrients supports improved metabolic functions and enhanced growth potential in wheat (Yue *et al.*, 2020).

This study highlighted that the co-application of Zn and Cu significantly influenced wheat growth. However, a higher combined rate of Zn + Cu (7.5 kg + 2.5 kg ha^{-1}) exhibited an antagonistic response, which may be associated with nutrient imbalance. Excess Zn or Cu beyond critical levels may induce phytotoxicity under calcareous conditions. Zn toxicity may occur when soil solution concentrations exceed safe limits, while Cu toxicity can impair root growth and chlorosis (Alloway, 2008; Marschner, 2012). These findings align with Rahman *et al.*, (2022), who reported that excessive Zn application with Cu can lead to toxicity, impairing plant health and reducing yields, particularly in phosphorus-deficient conditions. Interestingly, even a lower combined rate of Zn + Cu (2.5 kg + 2.5 kg ha^{-1}) showed antagonistic effects, suggesting that the ratio of these micronutrients is critical. This observation is supported by Alloway (2008), who emphasized that balanced Zn and Cu application enhances enzyme activity and grain filling in cereals, whereas insufficient or imbalanced doses can negatively impact yield.

The antagonistic interaction between Zn and Cu at lower and higher Zn doses may be related to their competitive uptake at the root level. It is known that Zn is mainly transported by members of the Zrt- and Irt-like protein (ZIP) family. Many of these proteins show overlapping substrate specificity and can transport multiple divalent metal ions. These shared transport pathways may enhance competition between Zn and Cu under imbalance supply, limit their uptake efficiency, and lead to antagonism (Robe *et al*., 2025). Similar Zn–Cu interaction responses have been reported in South Asian and other wheat-growing regions, where balanced micronutrient supply enhanced yield, while imbalanced application resulted in antagonistic trends (Rashid *et al*., 2022; Qane *et al*., 2024; Isakova *et al*., 2022). These comparisons indicate that Zn–Cu interaction patterns are context-dependent and influenced by soil properties and nutrient management strategy.

From a practical standpoint, balanced Zn–Cu co-application at recommended rates may improve wheat productivity in micronutrient-deficient calcareous soils. However, economic feasibility and cost–benefit considerations should be evaluated under local input and market conditions before broader farmer-level recommendations are made.

Conclusions

The interaction of Zn at lower (2.5 kg Zn ha^{-1}) and higher (7.5 kg Zn ha^{-1}) application dose with Cu (2.5 kg Cu ha^{-1}) resulted in antagonistic responses and reduced nutrient efficiency. However, at a balanced ratio (5.0 kg Zn and 2.5 kg Cu ha^{-1}), the nutrients acted synergistically, enhancing yield beyond the predicted additive effect. These findings indicate that balanced Zn-Cu management is critical, and that interaction outcomes depend on application rate. Zn-Cu interactions are not always additive and may shift between synergistic and antagonistic responses depending on nutrient balance. The observed interaction patterns were influenced by soil and management conditions. As this study was conducted under a single-season, single-location design with one wheat variety and a fixed Cu level; interpretations are confined to these experimental conditions. Future research should evaluate Zn-Cu interactions across diverse soil types, chelated forms, and long-term residual effects on soil health.

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