






Comparative Performance of Nano-ZnO and Nano-Fe₂O₃ Fertilizers on Wheat Yield and Biofortification

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Submitted: 11 April 2026

Revised: 5 June 2026

Accepted: 12 June 2026

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Keywords: Cereal, Conventional fertilizers, Nanoparticle, Nutrient use efficiency, Growth traits, Yield traits.

How to cite this paper: A. H. K. Enayat, F. H. Awlqadr, M. N. Saeed, and M. Waqar, "Comparative performance of nano-ZnO and nano-Fe₂O₃ fertilizers on wheat yield and biofortification", KJAR, vol. 11, no. 1, pp: 193-206, Jun 2026, doi: [10.24017/science.2026.1.13](https://doi.org/10.24017/science.2026.1.13)



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Abstract: Micronutrient deficiencies, particularly zinc (Zn) and iron (Fe), significantly limit wheat productivity and grain nutritional quality in calcareous soils, contributing to hidden hunger in populations heavily dependent on cereal-based diets. This study aimed to evaluate the effectiveness of nano-based micronutrient fertilizers compared to conventional sources in improving wheat growth, yield, biofortification, and nutrient use efficiency under semi-arid conditions. A field experiment was conducted in the Sharazoor Plain, Halabja, using a randomized complete block design with four treatments: control, conventional zinc sulfate (ZnSO₄) + iron sulfate (FeSO₄), zinc oxide nanoparticles (ZnO), and combined zinc oxide nanoparticles + iron oxide nanoparticles (ZnO + Fe₂O₃). The soil composition consisted of clay loam with a mild alkalinity (pH 7.6) and contained a total calcium carbonate level of 19.8%. It exhibited a deficiency in readily available Zn and Fe, as indicated by diethylenetriaminepentaacetic acid extractable concentrations of Fe and Zn measuring 0.39 mg kg⁻¹ and 0.63 mg kg⁻¹, respectively. Nanofertilizer treatments significantly enhanced plant growth parameters, with the combined nano-ZnO + nano-Fe₂O₃ treatment producing the highest plant height (93.67 cm), tiller density (413.33 m⁻²), and chlorophyll index (SPAD 50.93). Yield components were markedly improved, resulting in a maximum grain yield of 5.53 Mg ha⁻¹, representing a 39% increase over the control. Grain quality was also enhanced, with protein content increasing to 13.30%, while Zn and Fe concentrations reached 48.83 and 51.17 mg kg⁻¹, respectively. Overall, nano-based zinc and iron fertilizers, particularly the combined ZnO + Fe₂O₃ treatment, significantly improved wheat growth, yield, grain protein, and micronutrient concentrations, highlighting their potential for sustainable biofortification and enhanced nutrient use efficiency in micronutrient-deficient soils.

1. Introduction

Wheat (*Triticum aestivum* L.) is a vital cereal crop that defines world food security, contributing a significant portion of dietary calories, protein and micronutrients for several billion people across the planet. Although substantial advances have been made in crop management and breeding, the problem of micronutrient deficiency particularly zinc (Zn) and iron (Fe) still remains as a serious agronomic and nutritional concern. Deficiency of Zn and Fe in soils restricts uptake of the elements by plants, decreases photosynthetic ability, stunts growth as well as yield and grain nutritional quality [1]. Such deficiencies also enhance the "hidden hunger", a type of malnutrition in which the food provides

calorie, but poor essential minerals to sufficient human bodies that mainly rely on wheat for cognitive development and immune system [1, 2].

Zn and Fe are important trace elements for regulating the physiological processes of plants. Zinc is a cofactor of various enzymes, and an element that supports protein synthesis, hormone regulation, membrane stability, while Fe is essential for photosynthesis and respiration of plants. The Fe involves chlorophyll synthesis and electron transport in photo-synthesis, respiratory metabolism. Limited availability of Zn and Fe in calcareous and alkaline soils present in several wheat growing areas precludes their uptake, resulting into stunted growth, chlorosis and yield loss [3]. Much conventional Zn and Fe sulfate supplementation is characterized by a low nutrient use efficiency as a result of fixation, precipitation, and immobilization in soil; high losses; environmentally hazardous risks; and ultimately limited bioefficacy under field conditions. Recent advances in agricultural nanotechnology offer great promise as new approaches for relieving micronutrient deficiencies in crops [3].

Nanofertilizers, including zinc oxide nanoparticles (nano-ZnO) and iron oxide nanoparticles (nano-Fe₂O₃), possess special physicochemical properties due to large surface area, controlled release capability, further improved solubility in comparison with bulk fertilizer products. These facets elevate the nutrients available and make it more effective for plants to take up even at reduced application rates, which might help crops grow better and become enriched with the nutrient over a much more sustainable rate than standard fertilizing strategies [2]. Nano-fertilizers can be sprayed on plants, mixed with soil or primed seeds to escape immobilization in the soil and increase plant uptake directly [4]. A series of recent experimental information demonstrates the beneficial processes of nano-ZnO based fertilizing practices on enhancing wheat production and biofortification. For instance, field studies have demonstrated that the foliar application of nano-ZnO (alone or in combinations with biofertilizers) resulted in statistically higher wheat-vegetative growth, grain yield, and grain Zn content without disturbing the soil microbial population [5-7]. These findings suggest that nano-ZnO enhances plant chlorophyll content, biomass growth and the density of grain nutrients, concerning both the agronomic performance and the nutritional quality of wheat.

Nano-Fe₂O₃ has potential for increasing Fe uptake and grain Fe concentration as well, although the field-based test of iron nanofertilizers is to date relatively scanty and requires further investigation [2]. The direct delivery of micronutrient nanoparticles to edible plant parts for an enhanced concentration of health beneficial elements such as Zn and Fe that may result in increased dietary intake of targeted micro nutrients among vulnerable groups [2, 8]. Compared with conventional agronomic biofortification, nano-based approaches can provide higher nutrient-use efficiency and lower environmental losses, addressing key challenges in modern agriculture and sustainable intensification [4]. However, the application of combined nano-ZnO and nano-Fe₂O₃ compared with conventional fertilizers has not been studied in field experiments under real agricultural conditions, particularly in semi-arid and micronutrient-deficient soils. Furthermore, comprehensive assessments that encompass variations in plant growth, yield performance, grain quality parameters, and nutrient use efficiencies, apart from biofortification traits, are limited but critically important to support wider acceptability.

This study aimed to evaluate the effects of nano-ZnO and nano-Fe₂O₃ fertilizers on wheat growth and yield, compare nano-based fertilizers with conventional zinc sulfate (ZnSO₄) and iron sulfate (FeSO₄) sources, assess their impact on grain Zn, Fe, and protein content, and determine nutrient use efficiency under semi-arid, calcium carbonate-rich soil conditions.

2. Materials and Methods

2.1. Experimental Site and Climatic Conditions

A field experiment was conducted during the 2024–2025 wheat growing season at the Shahid Agricultural Research Station in the Sharazoor Plain, Halabja Governorate, Kurdistan Region of Iraq. The experimental site was located at approximately 35°12'47" N and 45°57'33" E, with an elevation of approximately 591 m above sea level (Table 1). The total experimental area was approximately 0.22 ha, as measured using the Google Earth Pro.

The study area is characterized by a semi-arid Mediterranean-type climate with mild winters and warm summers. During the wheat growing season, the average daily temperature ranged from 6 to 22

°C, and the total precipitation was approximately 420–450 mm. These climatic conditions are typical of rain-fed wheat production systems in the Sharazoor Plain and provide suitable conditions for evaluating nano-based micronutrient fertilization strategies under semi-arid calcareous soil conditions in this region.

Table 1: Climatic characteristics of the experimental site during the 2024–2025 wheat growing season.

Climatic parameter	Value/description
Location	Halabja Technical College Farm, Sharazoor Plain, Halabja
Latitude	35°12'47" N
Longitude	45°57'33" E
Elevation	591 m above sea level
Climate type	Semi-arid Mediterranean-type
Average daily temperature during season	6–35 °C
Total seasonal precipitation	420–450 mm
Cropping condition	Mainly rain-fed wheat production

2.2. Soil Sampling and Analysis

Before sowing, composite soil samples were collected from the experimental field at a depth of 0–30 cm using an auger. Several subsamples were randomly collected from the experimental area and mixed thoroughly to obtain a representative composite sample. The samples were air-dried at room temperature, gently ground, and passed through a 2-mm sieve to remove stones, roots, and plant residues before laboratory analysis [9, 10].

Soil texture was determined using the hydrometer method [11]. Soil pH and electrical conductivity (EC) were measured in a 1:2.5 soil-to-water suspension using a calibrated pH meter and EC meter, respectively [12]. Organic matter was determined using the Walkley–Black method [13], while calcium carbonate content was measured using the calcimeter method. Total nitrogen was determined by the Kjeldahl digestion method. Available phosphorus was extracted using the Olsen method and measured spectrophotometrically, whereas available potassium was determined by flame photometry [14]. Available Zn and Fe were extracted using diethylenetriaminepentaacetic acid (DTPA) solution, and the concentrations in the extracts were quantified using atomic absorption spectrophotometry. The resulting soil physicochemical properties were used to characterize the initial fertility status of the experimental site and to guide fertilizer management during the study [15].

2.3. Plant Materials, Experimental Design, and Crop Management

Certified bread wheat (*Triticum aestivum* L.) locally known as Wafia, seeds adapted to local agro-climatic conditions were used in this study. The experiment was arranged in a randomized complete block design (RCBD) with four treatments and three replicates. Each experimental plot measured 2 m × 1 m, with a 0.5 m buffer zone between the plots to minimize treatment interference. The treatments consisted of an untreated control, conventional zinc sulfate (ZnSO₄) + iron sulfate (FeSO₄), nano-ZnO, and combined nano-ZnO + nano-Fe₂O₃.

Before sowing, the experimental field was managed according to the standard local agronomic practices for wheat cultivation in semi-arid and calcareous soils. Weed control was carried out approximately 12–15 days before planting using glyphosate at 1,500 g a.i. ha⁻¹ (grams of active ingredient per hectare) for general weed suppression, combined with carfentrazone-ethyl at 40 g a.i. ha⁻¹ for broadleaf weed control. Where grassy weeds were present, clethodim at 240 g a.i. ha⁻¹ was applied as a selective post-emergence herbicide. Herbicides were applied using a calibrated knapsack sprayer under calm weather conditions to ensure uniform coverage and to minimize spray drift.

Before sowing, the seeds were treated with the commercial formulation Standak Top® to reduce the risk of early season soil-borne fungal diseases and insect pests. The formulation contained thiram + carbendazim as fungicidal active ingredients and thiodicarb + imidacloprid as insecticidal active ingredients, applied at the recommended dose of 100 kg of seeds. Seed treatment was performed immediately before planting to ensure effective protection without interfering with the subsequent fertilizer treatments.

Sowing was carried out manually on November 10, which corresponds to the recommended wheat planting period in Halabja under rain-fed conditions. Seeds were sown at a rate of 120 kg ha⁻¹, with 17 cm spacing between rows, following local agronomic recommendations. Basal fertilization was applied at sowing based on pre-experiment soil testing and local fertilizer recommendations for each treatment. Compound nitrogen-phosphorus-potassium (NPK) fertilizer (18–18–18) was applied at 250 kg ha⁻¹, supplying 45 kg nitrogen (N) ha⁻¹, 45 kg phosphorus pentoxide ha⁻¹, and 45 kg K₂O ha⁻¹. Additional nitrogen was supplied as urea (46% nitrogen) at the tillering stage, corresponding to Zadoks growth stage. Therefore, the total seasonal nitrogen application was 120 kg N ha⁻¹.

As the initial soil test indicated boron (B) deficiency, boric acid containing 18% boron was applied uniformly to all plots as a foliar spray at a rate equivalent to 1.0 kg B ha⁻¹. This application was made to avoid boron-related variations among treatments. The crop was mainly cultivated under rain-fed conditions, with supplementary irrigation applied only when necessary to prevent severe moisture stress during critical growth stages. No severe pest or disease incidences were observed during the growing season. Wheat was harvested manually at physiological maturity on June 25, providing an approximate crop duration of 225 days from sowing to harvest. Plants from each plot were collected separately for subsequent yield, grain quality, and nutrient-use efficiency analyses. An overview of the experimental field layout, crop management practices, fertilization treatments, soil sampling strategy, and major agronomic and biofortification responses are presented in figure 1.

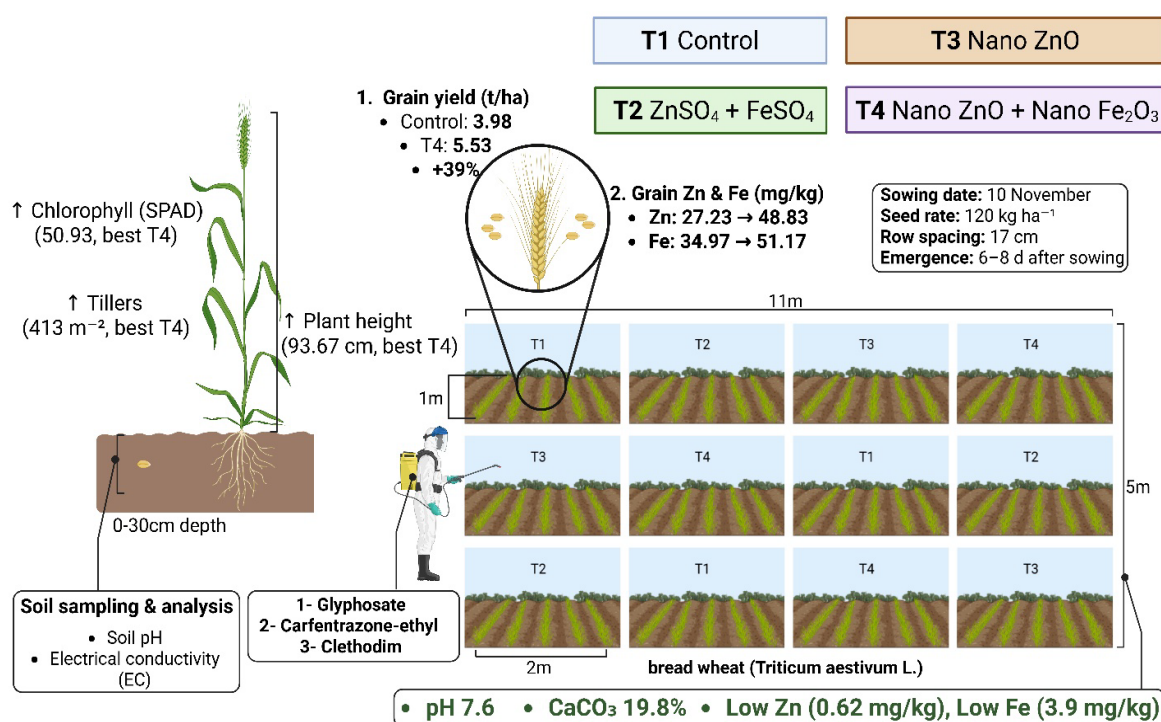


Figure 1: Experimental design, field layout, crop management practices, and major growth, yield, and biofortification responses of wheat (*Triticum aestivum* L.) under conventional and nano-based Zn and Fe fertilization in the Sharazoor Plain, Halabja, Iraq.

2.4. Nanofertilizer Preparation and Application

Commercially available ZnO and Fe₂O₃ nanoparticles with particle sizes below 100 nm were used as nanofertilizer sources in this study. Prior to application, the nanoparticles were dispersed in distilled water and subjected to ultrasonication to ensure a stable and homogeneous suspension. Nanofertilizers were applied as foliar sprays at two critical wheat growth stages, namely the tillering and booting stages, to maximize nutrient uptake efficiency. Conventional micronutrient fertilizers, ZnSO₄ and FeSO₄, were applied according to regional agronomic recommendations. To avoid nutrient deficiency and balance all plots, the same basic product with macronutrients, NPK, was applied.

2.5. Measurement of Growth Parameters

At the heading stage, growth characteristics were measured on random plants from each experimental plot. The height of the plant was calculated as the distance from the surface to the spike in centimeters [16]. The tiller number per square meter was measured using the quadrat method [17]. Leaf chlorophyll content was measured using a field-portable SPAD chlorophyll meter, and its values were represented as the SPAD chlorophyll index [18].

2.6. Yield and Yield Components

At physiological maturity, the central zone of each experiment was mechanically harvested to determine the yield and yield components. Spikes per square meter and grains per spike were counted for the whole plant. Thousand-grain weight was measured using an electronic balance [19]. The grain yield was reported on a hectare basis after being adjusted for standard grain moisture content before the regression analysis.

2.7. Grain Quality Analysis

Grain samples harvested from individual plots were oven-dried and finely powered for trait determination. The protein percentage (on a dry weight basis) was determined according to the Kjeldahl nitrogen method and estimated by multiplying the nitrogen content by 5.7 as a conversion factor [20]. The concentrations of Zn and Fe in the grain were determined through acid digestion of samples, expressed as milligrams per kilogram using atomic absorption spectrophotometry (PerkinElmer AAnalyst 800, PerkinElmer, USA) [21].

2.8. Nutrient Use Efficiency Indices

Nutrient-use efficiency indices were determined to assess the efficacy of traditional and nano-enabled micronutrient fertilization interventions on wheat productivity, nutrient acquisition, and internal nutrient utilization. Agronomic efficiency and partial factor productivity were applied for yield response to fertilizer input, recovery efficiency, and physiological efficiency for uptake utilization and biomass conversion efficiency of nutrients, respectively. The data on grain yield and nutrient uptake from the fertilized plots were compared with those from a no-fertilizer treatment to ensure that the estimates of the effects of fertilizer were accurate. Combined, the indices provide an integrated assessment of the fertilizer performance under field conditions also emphasizing a benefit of nanofertilizers in improving nutrient availability and utilization at low rates of application. The agronomic efficiency (AE) was determined by the equation [22]:

$$AE = \frac{Y_f - Y_0}{F} \quad (1)$$

Partial Factor Productivity (PFP) was calculated as [23]:

$$PFP = \frac{Y_f}{F} \quad (2)$$

Recovery Efficiency (RE) was calculated as

$$RE = \frac{(U_f - U_0)}{F} \times 100 \quad (3)$$

Physiological Efficiency (PE) was calculated as follows [24]:

$$PE = \frac{Y_f - Y_0}{U_f - U_0} \quad (4)$$

where Y_f is the grain yield of the fertilized treatment, Y_0 is the grain yield of the unfertilized control treatment, F is the amount of nutrient applied, U_f is the nutrient uptake in the fertilized treatment, and U_0 is the nutrient uptake in the unfertilized control. Grain yield, nutrient application rate, and nutrient uptake were expressed as kg ha^{-1} . AE, PFP, and PE were expressed as kg grain kg^{-1} nutrient, and RE was expressed as a percentage.

2.9. Statistical Analysis

Data were checked for normal distribution and homogeneity of variance to meet the assumptions of analysis of variance (ANOVA) before statistical analysis. The experimental data were analyzed using an RCBD model. The impact of treatments on growth traits, yield attributes, grain quality features, and nutrient-use efficiency indices was analyzed using one-way ANOVA. Where there was a significant difference among treatments, the Least Significant Difference (LSD) test at 5% probability level was used to separate means. Data are presented as mean \pm standard deviation (SD). Correlation analysis (Pearson's) was also used to estimate the relationships between grain yield, grain quality traits, and NUE indices. Statistical analyses were performed using standard statistical software (OriginPro 2026), and power and treatment effects, as well as statistical significance, were graphed.

3. Results

3.1. Soil Physicochemical Characteristics of the Experimental Site

The physicochemical characteristics of the experimental soil are presented in table 2. The soil had a clay loam texture, with sand, silt, and clay contents of 31%, 39%, and 30%, respectively. Soil pH was 7.6, indicating slightly alkaline conditions, while the electrical conductivity was 2.1 dS m⁻¹. Organic matter content was 1.15%, and calcium carbonate content was 19.8%, confirming the calcareous nature of the experimental soil. Total nitrogen and available phosphorus were 0.08% and 8.6 mg kg⁻¹, respectively, while available potassium was 245 mg kg⁻¹. Available zinc and iron concentrations were 0.62 and 3.9 mg kg⁻¹, respectively, indicating low micronutrient availability in the experimental soil. Bulk density was 1.42 g cm⁻³, while field capacity and permanent wilting point were 28.5% and 14.2%, respectively. Overall, these results show that the experimental soil was slightly alkaline, calcareous, low in organic matter, and limited in available Zn and Fe, supporting the need to evaluate Zn- and Fe-based fertilization strategies under local field conditions.

Table 2: Physicochemical characteristics of the experimental soil (0–30 cm depth) in the Sharazoor Plain, Halabja (Shahid area), Kurdistan Region of Iraq.

Soil property	Value
Sand (%)	31
Silt (%)	39
Clay (%)	30
Soil texture	Clay loam
pH (1:2.5 soil:water)	7.6
Electrical conductivity (EC, dS m ⁻¹)	2.1
Organic matter (%)	1.15
Calcium carbonate (CaCO ₃ , %)	19.8
Total nitrogen (%)	0.08
Available phosphorus (mg kg ⁻¹)	8.6
Available potassium (mg kg ⁻¹)	245
Available zinc (mg kg ⁻¹)	0.62
Available iron (mg kg ⁻¹)	3.9
Bulk density (g cm ⁻³)	1.42
Field capacity (%)	28.5
Permanent wilting point (%)	14.2

3.2. Growth Parameters

The growth parameters of wheat were significantly influenced by the various fertilization treatments ($p \leq 0.05$). Plant height, number of tillers per m² and SPAD chlorophyll index significantly increased with the enhancement in nanoparticles from control treatments to nanofertilizers (Figure 2). The plants in the combined nano-ZnO + nano-Fe₂O₃ treatment were the highest with an average plant height of 93.67 cm, which was significantly taller than those in the control and conventional

micronutrient treatments (82.33 and 87.00 cm, respectively). The same phenomenon was found for tiller density, and the order of tillering occurred as combined nano- ZnO + nano-Fe₂O₃ > nano-ZnO alone > conventional fertilizers > control. The leaf chlorophyll content, expressed as SPAD value was also enhanced significantly by the use of nanofertilizer with a maximum increase up to 50.93 ± 0.70 compared with control (40.50 ± 0.87).

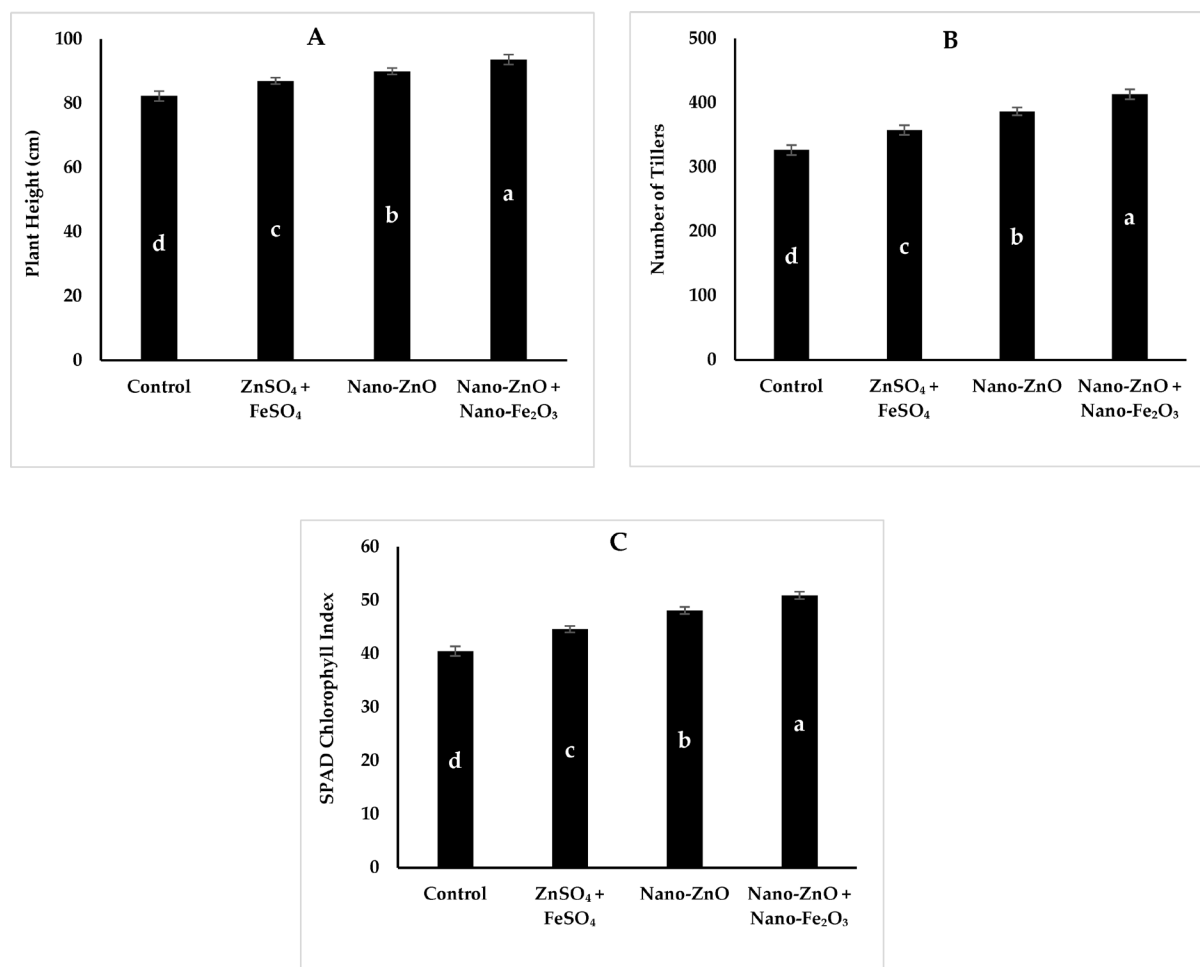


Figure 2: Effect of conventional and nano-based micronutrient fertilization on wheat growth: (A) SPAD chlorophyll index, (B) plant height, and (C) tiller number. Bars show mean ± SD (n = 3). Different letters indicate significant differences at $p \leq 0.05$ according to LSD test. LSD values were 1.35 for SPAD chlorophyll index, 2.43 cm for plant height, and 13.66 tillers m⁻² for tiller number.

3.3. Yield and Yield Components

Yield components and grain yield were significantly influenced by the fertilizer form ($p \leq 0.05$) (Table 3). The highest number of spikes per square meter was observed under the combined nano-ZnO + nano-Fe₂O₃ (495.00 spikes), followed by nano-ZnO alone (452.33 spikes), whereas the control treatment recorded the lowest value (372.33 spikes). Similarly, the number of grains per spike increased significantly under nanofertilizer application, with combined nano-ZnO + nano-Fe₂O₃ producing 55.00 grains compared with 42.00 grains in the control group. Thousand-grain weight was significantly improved by nanofertilizers, reaching 45.30 g under combined nano-ZnO + nano-Fe₂O₃, which was statistically superior to both conventional fertilization (40.90 g) and the control (38.63 g). Consequently, grain yield was maximized under combined nano-ZnO + nano-Fe₂O₃ application (5.53 Mg ha⁻¹), representing a yield increase of approximately 39% compared with the control (3.98 Mg ha⁻¹).

3.4. Grain Quality Attributes

Grain quality parameters responded significantly to the micronutrient fertilization strategies (Table 4). Grain protein content increased from 11.20% in the control to 13.30% under the combined nano treatment (nano-ZnO + nano-Fe₂O₃). Nanofertilizer application also resulted in marked improvements in grain micronutrient concentrations. The highest grain zinc concentration was recorded in the combination of nano-ZnO + nano-Fe₂O₃ (48.83 mg kg⁻¹), followed by nano-ZnO alone (43.27 mg kg⁻¹), whereas the control treatment exhibited the lowest Zn concentration (27.23 mg kg⁻¹). A similar pattern was observed for grain iron concentration, with the combination of nano-ZnO + nano-Fe₂O₃ producing the highest Fe content (51.17 mg kg⁻¹), significantly exceeding both conventional fertilization and the control. These results, as shown in table 4, indicate that nanofertilizers were effective in enhancing both the yield and nutritional quality of wheat grains.

Table 4: Conventional and nano-based micronutrient fertilization on wheat grain quality.

Treatment	Protein (%)	Grain Zn (mg kg ⁻¹)	Grain Fe (mg kg ⁻¹)
Control	11.20 ± 0.20 d	27.23 ± 0.75 d	34.97 ± 0.80 d
Conventional fertilizer (ZnSO ₄ + FeSO ₄)	11.97 ± 0.15 c	34.37 ± 0.85 c	41.63 ± 0.85 c
Nano-ZnO	12.60 ± 0.20 b	43.27 ± 1.25 b	44.10 ± 1.10 b
Combination of nano-ZnO + nano-Fe ₂ O ₃	13.30 ± 0.20 a	48.83 ± 1.35 a	51.17 ± 1.20 a
LSD value	0.38	2.16	2.16

The means connected by different letters represent significant variations according to the LSD test. Data are expressed as mean ± SD (n = 3).

3.5. Nutrient Use Efficiency Indices

The indices of nutrient use efficiencies were significantly affected by the type of fertilizer (p ≤ 0.05): generally, nanofertilizers had significantly higher assorted than conventional sources of micronutrients (Table 5). AE was highest under nano-ZnO treatment (650.00 kg kg⁻¹), followed by the combined nano-ZnO + nano-Fe₂O₃ treatment (417.12 kg kg⁻¹), while the conventional ZnSO₄ + FeSO₄ treatment showed the lowest AE (32.91 kg kg⁻¹). PFP showed the same variation trend as FWP, and nano-ZnO treatment had the highest PFP value (3139.58 kg kg⁻¹). Recovery efficiency of applied micronutrients was considerably increased with nanofertilizer application by 42.7% for nano-ZnO compared to 15.0% obtainable in conventional fertilization receiving only 18.4%. PE was also higher in the nanofertilizer treatment, which means more use of absorbed micronutrients towards grain yield.

Table 5: Effect of traditional and nano based micronutrient fertilization on nutrient use efficiency indices of wheat.

Treatment	AE (kg grain kg ⁻¹ nutrient)	PFP (kg grain kg ⁻¹ nutrient)	RE (%)	PE (kg grain kg ⁻¹ nutrient)
Conventional fertilizer (ZnSO ₄ + FeSO ₄)	32.91 ± 1.10 c	285.02 ± 5.08 c	18.4 ± 1.2 c	173.6 ± 6.4 c
Nano-ZnO	650.00 ± 6.25 a	3139.58 ± 46.91 a	42.7 ± 2.1 a	298.4 ± 9.1 a
Combination of nano-ZnO + nano-Fe ₂ O ₃	417.12 ± 10.23 b	1493.69 ± 27.21 b	35.6 ± 1.8 b	254.7 ± 8.3 b
LSD value	13.89	62.83	3.48	16.01

AE: Agronomic efficiency, PFP: Partial factor productivity, RE: Recovery efficiency, PE: Physiological efficiency. The means connected by different letters represent significant variations according to the LSD test. The data are expressed as mean ± SD (n = 3).

3.6. Correlation Analysis

There were strong and positive associations among grain yield, grain micronutrient concentration, and nutrient use efficiency indices inferred by Pearson’s correlation analysis (Table 6). Grain yield showed strong and significant positive correlations with grain Zn (r = 0.94, p ≤ 0.01) and grain Fe (r =

0.92, $p \leq 0.01$), implying that enhanced micronutrient accumulation was tightly linked to higher productivity. The highest positive correlation was recorded for recovery efficiency with agronomic efficiency ($r = 0.95$, $p \leq 0.01$), indicating that nutrient uptake efficiency is a critical factor in developing higher-yielding varieties. Protein was positively associated with yield and grain micronutrient concentration, further indicating a cooperative impact of nanofertilizers on nitrogen metabolism and micronutrient utilization.

Table 6: Pearson correlation coefficients among grain yield, grain quality, and nutrient use efficiency indices.

Parameter	Plant height	Tiller number	SPAD	Grain yield	Protein content	Grain Zn content	Grain Fe content	AE	RE	PE
Plant height	1.00									
Tiller number	0.88*	1.00								
SPAD	0.90*	0.86*	1.00							
Grain yield	0.94*	0.92*	0.91*	1.00						
Protein content	0.89*	0.84*	0.85*	0.86*	1.00					
Grain Zn content	0.91*	0.87*	0.89*	0.94*	0.87*	1.00				
Grain Fe content	0.92*	0.88*	0.90*	0.93*	0.88*	0.91*	1.00			
AE	0.87*	0.82**	0.86*	0.88*	0.79**	0.90*	0.87*	1.00		
RE	0.89*	0.84**	0.88*	0.91*	0.82**	0.93*	0.89*	0.95	1.00	
PE	0.85*	0.80**	0.83*	0.86*	0.78**	0.88*	0.85*	0.92*	0.94*	1.00

*: $p \leq 0.05$ **: $p \leq 0.01$

4. Discussion

4.1. Soil Physicochemical Characteristics of the Experimental Site

The soil properties of the experimental site helped explain the response of wheat to Zn and Fe fertilization. Although the clay loam texture was suitable for wheat cultivation under rain-fed conditions, the chemical characteristics of the soil created clear limitations on micronutrient availability. In particular, the slightly alkaline pH and calcareous nature of the soil can reduce Zn and Fe solubility through precipitation, adsorption, and fixation reactions, which may restrict their uptake by wheat roots. Similar limitations of Zn and Fe availability in alkaline calcareous soils have been reported previously, where micronutrient fixation reduces fertilizer efficiency and limits crop biofortification potential [3, 24].

Low organic matter content may further reduce nutrient mobility because organic matter can improve micronutrient availability through chelation and enhanced microbial activity. The low levels of available Zn and Fe observed in this study confirm that the experimental field was chemically constrained, even though its physical conditions were suitable for wheat growth. These findings are consistent with previous reports showing that calcareous soils commonly reduce the effectiveness of conventional Zn and Fe fertilizers due to immobilization and limited diffusion in the soil matrix [3, 25].

These soil conditions provide a strong rationale for using nano-ZnO and nano-Fe₂O₃ fertilizers. Compared with conventional ZnSO₄ and FeSO₄, nano-based fertilizers may improve micronutrient availability because of their small particle size, large surface area, better dispersion, and gradual nutrient release rates. These characteristics can enhance nutrient uptake efficiency and reduce nutrient loss under micronutrient-deficient calcareous soil conditions [4, 25]. Similar improvements in nutrient availability and fertilizer-use efficiency have been reported when nano-fertilizers were applied to calcareous soils or integrated with soil fertility management practices [26]. Therefore, the soil conditions in the present study support the evaluation of nano-enabled Zn and Fe fertilization as a practical strategy to improve wheat growth, yield, and grain biofortification in semi-arid calcareous environments.

4.2. Growth Parameters

Neither was the type of fertilization application without impact on growth responses ($p \leq 0.05$), there were clear beneficial effects for the nano-treatments in promoting morphological and physiological characteristics of wheat. The combined application of nano-ZnO + nano-Fe₂O₃ yielded the tallest plants the greatest tiller density, and the highest chlorophyll content. This is significantly higher than the untreated control and conventional fertilizers (ZnSO₄ + FeSO₄). Increased plant height and tillering is a result of greater metabolic activity and hormonal stimulation as a consequence of nanoparticle-based nutrient enrichment. It has been shown that zinc and iron nanoparticles improve the auxin metabolism and cell elongation required for better vegetative growth and increased the number of tillers [6]. Likewise, nano-ZnO has been reported to accelerate root growth and increase nutrient uptake efficiency by inducing membrane permeability and enzymatic activity under conditions of nutrient deficiency [7].

Enhancement of chlorophyll content under nano treatments is due to better availability of zinc and iron, both essential cofactors for chlorophyll biosynthesis as well as photosynthetic electron transport. Treatment with nano-ZnO has been found to increase chlorophyll a and b contents by as much as 25% in both saline and drought stressed conditions leading to photosynthetic enhancement, biomass formation [27]. Similarly, nano-Fe₂O₃ improves the generation of chlorophyll-protein complexes, so that the photosynthetic ability is maintained in alkaline soils [28]. Synergism of combined nano-ZnO + nano-Fe₂O₃ treatment is in conformity with the report of Babaei et al. [29], who reported that co-application of Zn-Fe oxide nanoparticles significantly enhanced chlorophyll stability, enzymatic antioxidant activity, and grain yield under saline stress. Again, both micronutrients function complementarily, where the role played by Zn to promote production of auxin and by Fe toward chlorophyll and energy metabolism ensure vegetative physiological vigor.

4.3. Yield and Yield Components

Wheat yield showed a significant difference for fertilizer forms. Nano based treatments significantly resulted in the highest performance among different trait of grain yield. The combined nano-ZnO + nano-Fe₂O₃ treatment produced the highest number of spikes per square meter, grains per spike, and thousand-grain weight, resulting in a remarkable grain yield, an improvement of approximately 39% over the control. These results are in line with several global studies reported synergistic effect of nano micronutrients on the productivity of wheat [30-32]. The enhancement in spike density and the number of grains per spike under nano-based fertilization may be explained by a higher nutrient utilization efficiency as well as better physiological metabolism.

The nanoparticles zinc and iron are vital for pollen formation, fertilization efficiency and spikelet differentiation leading to yield components. Prajapati et al. [31] reported that foliar application of nano-ZnO at optimum rates enhances spike density, number of grains per spike and harvest index through improved photoassimilates translocation, enzyme activities. Similarly, co-application of ZnO and Fe₂O₃ nanoparticles increased spike morphology and kernel set due to the better stabilization of chlorophyll content and antioxidant defence in reproductive stages [33]. High enhancement of 1000-grain weight under nano-based treatments indicates an efficient photosynthate partitioning and prolonged grain filling period due to the higher bioavailability of Fe, Zn. Nano-ZnO can increase the enzymes responsible for starch biosynthesis, while nano-Fe₂O₃ can improve chlorophyll and energy metabolism [34]. verall supporting the flow of assimilates to developing grains. Similar improvements have also been reported in field studies, as foliar spray of nano-ZnO applied at 900–1200 ppm resulted in >20% increase in 1000-grain weight and total grain yield compared with Zinc-ethylenediaminetetraacetic acid fertilization [32].

In addition, the co-application of nano-ZnO and nano-Fe₂O₃ played a significant role in enhancing the yield owing to the synergism of nutrient interactions. Increasing auxin biosynthesis, cell elongation was positively influenced by Zn, and the synthesis of chlorophyll and operation of redox enzymes, which are all essential for spikelet fertility and grain filling, is iron-dependent. It is reported that Zn-Fe oxide nanoparticles have a synergistic action on increasing wheat grain yield by 17–25% under saline as well as normal conditions [29]. The increased yield obtained in the present study is also in agreement

with the general opinion that nano fertilizers outperform traditional bulk formulations for a variety of reasons (control release of nutrients, higher nutrient uptake efficiency, and adaptation to abiotic stress conditions) [35]. Accordingly, the integrated use of nano-ZnO + nano-Fe₂O₃ treatment is an efficient and economical agronomic practice for improving yield and quality-related parameters in wheat grown in micronutrient-deficient calcareous soils.

4.4. Grain Quality Attributes

Micronutrient fertilization, especially nano-enabled formulations, substantially promoted wheat grain quality traits. The highest grain protein content, Zn content, and Fe concentration were observed when combination of nano-ZnO + nano-Fe₂O₃ was applied together, which were significantly superior to both the conventional micronutrient treatments and control treatment. These enhancements indicate the comparative efficiency of nanofertilizers to enhance wheat grain not only nutritionally but also physiologically. The increase of grain protein is strongly associated with improved activity of nitrogen assimilation and enzymes regulated by Zn and Fe nanoparticles. Zinc activates RNA polymerase and enzymes involved with nitrogen metabolism, whereas iron promotes chlorophyll synthesis and nitrate reductase activity which causes more accumulation of amino acids and proteins [36]. Comparable outcomes have been obtained in wheat, where foliar application of nano-ZnO increased N uptake and grain protein content by 9–15% relative to bulk ZnSO₄ [32]. Moreover, nano-Fe₂O₃ promotes electron transport and chloroplast stability which increased the efficiency of photosynthetic nitrogen utilization, meanwhile, also contributed to larger protein synthesis [7]. The pronounced increase in grain Zn and Fe concentrations found with nanofertilizer applications demonstrates the potential of these treatments to be used agronomically for biofortification.

Nano-ZnO applications improve root membrane permeability and Zn mobility within the xylem-ploem continuum, enhancing translocation to grains. Studies have also demonstrated that nano-ZnO can help in increasing the grain Zn due to wheat grains by 58.6 % with better bioavailability and no phytotoxicity [6]. Likewise, combinatorial treatment with Zn and Fe nanoparticles enhances nutrient uptake along with grain enrichment, as Fe helps in Zn transportation through controlling metal transporter genes [37]. The latest progresses in the development of nanocomposite fertilizers (e.g., mesoporous silica and graphene-based Fe–Zn nanocomposites) presented more than 50% increases in grain yield and micronutrient acquisition efficiency, affirming a synergistic effect of joint application between nano-Fe and nano-Zn [38]. Likewise, co-application of ZnNPs with plant growth-promoting bacteria has been reported to enhance Zn partitioning efficiency and grain Zn concentration by 25–40% as an environment-friendly biofortification strategy [39].

Altogether, these findings suggest that nanoparticle combination (ZnO + Fe₂O₃) not only increased yield but also significantly improved the nutritional quality of wheat grain. Higher contents of protein, Zn, and Fe are direct means to combat “hidden hunger” in cereal-dominated diets [40]. Accordingly, the nano-enabled agronomic biofortification defense is a promising sustainable and cost-effective alternative to enhance the quality of food/crop products as well as human nutrition in Zn- and Fe-deficient agroecosystems.

4.5. Nutrient Use Efficiency Indices

Fertilizer type significantly affected NUE indices, indicating that nanofertilizers were considerably better than traditional micronutrient formulations. The AE of the applied Zn varied among treatments and was highest for nano-ZnO, while mixed nano-Fe₂O₃ + nano-ZnO resulted in the second highest AE. Compared with the other treatments, conventional ZnSO₄ + FeSO₄ had the lowest AE, demonstrating better conversion ability of nutrients than nano fertilizers. The same trends were also recorded for PFP, RE, and PE, which all increased significantly under nanofertilization treatments. The higher AE and PFP in response to the nanofertilizer applications might be ascribed to nutrient-controlled release, high surface reactivity, and enhanced shoot or root absorption. On a field scale, zinc nanocomplexes increased the fertilizer use efficiency up to 10-fold with respect to the bulk ZnSO₄ at similar grain enrichments, while using one-tenth of the Zn [41].

The small size and large surface area of the nanoparticles help in better attachment to root epidermal cells and movement through the apoplast, thereby facilitating the rapid uptake of nutrients and reducing nutrient losses by leaching or volatilization [42]. For the RE, 42.7% was recorded for nano-ZnO vs. 18.4% under conventional fertilization, which indicates the targeted delivery and low nutrient loss by nano-formulations. Similarly high RE values have also been reported for bioactive zinc-coated urea (BAZU), which enhanced Zn recovery by 7-11-fold and AE by 140% compared to conventional Zn sources in rice–wheat systems [39]. Furthermore, the co-application of nano-ZnO with plant growth-promoting bacteria enhanced Zn acquisition and utilization efficiency in wheat and legumes, indicating that biologically functionalized nanoparticles also have a synergistic role. The higher PE in response to nano-fertilizers means that the nutrients absorbed were more efficiently converted to biomass and ultimately yield. This is consistent with previous research, which has shown that Fe–Zn nanocomposites enhance the formation of chlorophyll and photosynthetic efficiency and promote metabolic conversion in cereals [43]. Further, hybrid nanofertilizer formulations including Zn, Fe, and N in the hydroxyapatite matrix are reported to have more than 48% nutrient use efficiency as against 18% of conventional fertilizer [44].

In total, the high AE, RE, and PE values mentioned in this study show excellent nutrient release, retention, and utilization of the nanofertilizers. These findings provide the basis for shifting to nano-enabled nutrient management systems and suggest a sustainable means of improving fertilizer-use efficiency, minimizing input costs, and preventing environmental losses. Pearson correlations indicated strong and positive associations of these micronutrient indices with yield as well as among the nutrient-use efficiency indices. Grain yield was significantly and strongly associated with grain Zn and Fe contents, suggesting that enhanced nutrient accumulation resulted in a direct increase in productivity. RE had the strongest relationship with AE, indicating that the higher yield response was mainly contributed by increased nutrient uptake. High Zn uptake, agronomic efficiency, and yield were also reported by Nawaz et al. [45], or wheat under balanced micronutrient management, of which a strong positive correlation ($r > 0.9$) was observed between Zn uptake, agronomic efficiency, and yield. Protein content was also significantly and positively associated with grain Zn and Fe concentrations, indicating concomitant improvement in nitrogen metabolism and availability of the micronutrients, a typical attribute of physiological efficiency induced by nanofertilizers [4].

5. Conclusions

Nano-based micronutrient fertilization, particularly through the combined application of ZnO and Fe₂O₃ nanoparticles, offers a sustainable and effective approach to improving wheat growth, yield, and grain quality in calcareous, micronutrient-deficient soils. The study demonstrates that nanofertilizers enhance physiological performance, including photosynthetic efficiency and chlorophyll content, while simultaneously promoting higher nutrient uptake, better tillering, and improved spike characteristics. Compared to conventional ZnSO₄ and FeSO₄ fertilization, the nano-based approach supports efficient nutrient utilization, reduces potential environmental losses, and contributes to the biofortification of wheat grains, addressing both agricultural productivity and nutritional security, particularly in regions affected by hidden hunger. Importantly, these improvements are achieved without compromising plant health or growth, suggesting a balance between agronomic performance and ecological sustainability. The observed synergistic effects of Zn and Fe nanoparticles indicate that targeted nanonutrient strategies can optimize metabolic activity, enhance nitrogen assimilation, and stimulate key enzymatic pathways, ultimately improving protein content and micronutrient density in the grains. These findings underline the potential of nanofertilizers as a transformative tool in modern crop management. Future research should focus on long-term field assessments, soil–nanoparticle interactions, potential accumulation risks, and the scalability of these applications to diverse agroecological zones to ensure safe and sustainable implementation at the global level.

Author contributions: Aras Hama Karim Enayat: Investigation, Data curation, Resources, Validation, Farhang Hameed Awlqadr: Conceptualization, Supervision, Project administration, Funding acquisition, Writing – review & editing, Mohammed N. Saeed: Methodology, Software, Formal analysis, Validation, Writing – review & editing,

Muhammad Waqar: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft.

Data availability: Data will be available upon reasonable request by the authors.

Conflicts of interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding: The authors did not receive support from any organization for the conducting of the study.

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