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Seed-Primed and Foliar Oxozinc Nanofiber Application Increased Wheat Production and Zn Biofortification in Calcareous-Alkaline Soil

Wiqar Ahmad [1,2,3,†], Zhiyou Zou [1,*,†], Muhammad Awais [2], Fazal Munsif [4], Aziz Khan [5], Jaya Nepal [6], Masood Ahmad [7], Sultan Akbar [8], Ijaz Ahmad [9], Muhammad Shahid Khan [10], Zahid Qamar [11] and Haroon Khan [12]

1 School of Earth Sciences, Yangtze University, Jingzhou 434025, China
2 Department of Soil and Environmental Sciences, The University of Agriculture Peshawar, Amir Muhammad Khan Campus Mardan, Mardan 23200, Pakistan
3 Soil and Water Science Department, Indian River Research and Education Center, Institute of Food and Agricultural Sciences, University of Florida, 2199 South Rock Road, Fort Pierce, FL 34945, USA
4 Department of Agronomy, The University of Agriculture Peshawar, Amir Muhammad Khan Campus Mardan, Mardan 23200, Pakistan
5 Key Laboratory of Grassland Agroecosystems, Lanzhou University, Lanzhou 730000, China
6 Department of Soil, Water and Ecosystem Sciences, Indian River Research and Education Center, University of Florida, Fort Pierce, FL 34945, USA
7 Department of Horticulture, The University of Agriculture, Peshawar 25000, Pakistan
8 Department of Plant Breeding and Genetics, The University of Agriculture, Peshawar 25000, Pakistan
9 Department of PBG, The University of Agriculture Peshawar, Amir Muhammad Khan Campus Mardan, Mardan 23200, Pakistan
10 Department of Plant Protection, The University of Agriculture, Amir Muhammad Khan Campus, Peshawar 25000, Pakistan
11 Department of Applied Physics, Federal Urdu University of Science and Technology (FUUAST), Islamabad 44000, Pakistan
12 Department of Weed Science & Botany, The University of Agriculture, Peshawar 25000, Pakistan
* Correspondence: zouzhiyou1984@126.com
† These authors contributed equally to this work.

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Abstract: Low Zinc (Zn) availability in alkaline calcareous soil is one of the major causes of low cereal yield and quality. Conventional application of Zn sulfate ($ZnSO_4$) fertilizer through soil application attains minimal Zn efficiency as it is readily fixed in such soils. Oxozinc nanofiber (ZnONF) was evaluated for wheat Zn biofortification using different application methods to tackle this issue. Pots in triplicate (each with 7 kg soil) were arranged in a completely randomized design with a control treatment without Zn application. The conventional $ZnSO_4$ fertilizer recommended dose (5.5 μg Zn kg^{-1} of soil) was used for comparison and applied through soil addition, foliar spray, and seed priming, while the ZnO_{NF} was applied through foliar spray, seed coating, and seed priming (@ 0.5 kg ha^{-1}) either alone or in combination with $\frac{1}{2}ZnSO_4$ applied to the soil. The application of ZnO_{NF} significantly improved wheat plant growth as evidenced by increased plant height (14.5%), spikelets per spike (13.7%), and Zn use efficacy (611%) regardless of application methods as compared to control. The highest Zn uptake efficiency (34%) for nanofibers was obtained for theseed primed, followed by seed coating (23%) and foiar application (7%), respectively. Moreover, at the combined ZnO_{NF} and $\frac{1}{2}ZnSO_4$ application, further improvements for spike length, number of spikelets $spike^{-1}$, grain, leaf, root, and stem Zn concentrations, as well as their respective Zn contents, were noted. These results elucidated that Zn nutrition with ZnONF was either at par with or higher than the conventional $ZnSO_4$ fertilizer application despite significantly reduced ZnO_{NF} quantity, irrespective of the application method used. Additionally, the combined ZnO_{NF} and $\frac{1}{2}ZnSO_4$ (foliar spray, seed coating, or seed priming) maximized the crop Zn accumulation, wherein the $\frac{1}{2}ZnSO_4 + ZnO_{NF}$ through foliar application exceeded grain Zn biofortification. Thus, various Oxozinc nanofibers application modes may be recommended for wheat biofortification either separately or in combination with $ZnSO_4$ in Zn deficient calcareous soils for improved Zn nourishment.

Keywords: nano-fertilizer; Zn biofortification; alkaline soil; calcareous soil; wheat

1. Introduction

Alkaline calcareous soils with continuous cereals cultivation have decreased crop productivity and quality due to declining soil fertility in developing countries [1]. Among essential plant nutrients, Zn is one widely deficient micronutrient in cereal-based cropping system areas [2]. Low soil availability is a major hurdle to achieving high crop production [3]. Zn deficiency is most common in alkaline calcareous soils having low phyto-available Zn concentration [4,5], comprising around 50% of the agricultural land in the world [3]. Resultantly, low Zn crops produced on such soils do not meet human bodily and functional requirements, which results in various health issues [6]. Nutritional deficiency reduces production and adversely affects the crop quality required for proper human nutrition; therefore, improving grain Zn content would reduce the intensity of Zn deficiency-associated health problems in humans. The World Health Organization's recommended daily intake of Zn for an adult human is 15 mg day^{-1}; however, around 25% of the world's population, mostly in developing countries, is less than that amount. However, Zn deficiency could be reduced in plants by following various practices, i.e., supplementary application, using diverse diets, and food and crop plants bio-fortification [6]. Conventionally, mineral $ZnSO_4$ fertilizers are most commonly used because of their high solubility and low price [7]. However, fixation reactions reduce zinc bioavailability in soils with low organic matter, high carbonate content, and high pH, causing calcite adsorption or $Zn(OH)_2$ or $ZnCO_3$ precipitation, making conventional fertilizers ineffective for crop zinc uptake [5,8,9]. Wheat is one of the major staple foods for approximately 40% of the world's population living in developing countries [10]. Zn biofortification of wheat grain is a technique involving improving its inherent Zn status through the external application of Zn in the form of solid or liquid fertilizers to the crop at the proper growth stage. With the global population expected to reach 9.8 billion by 2050 [11], a major challenge for scientists is not only to increase food production by multiple folds but also to enhance the nutritional qualities of the food produced to feed more people, particularly in developing regions [12]. This would require not only improving the current technology but also the identification of new areas of research in this field. One such promising technology is nanotechnology, i.e., the use of engineered nanomaterials that are a billionth of the size of a meter and offer unique properties owing to their minute dimensions.

Recently, nanotechnology has been getting attention for mitigating nutritional stress and securing sustainable crop management required for potential production. Given their small size, large specific surface area, and high reactivity, nanoparticles (NPs) have great application potential in agriculture [13]. Nanoparticles of essential elements may serve as essential plant nutrients [14–17]. Supplementing Zn via nano Zn alternatives offer a potential in wheat Zn biofortification, especially in alkaline calcareous soils where Zn is easily fixed in soils. Zn oxide (ZnO) nanoparticles have been widely tested [14] for crop growth, showing effectiveness at low ($\leq$100 mg kg^{-1}) concentrations. In contrast, at a high concentration (1000 mg kg^{-1}), ZnO nanoparticles enhanced cucumber crop Zn accumulation but not crop growth [18]. Nanoscale powders of different elements can be used as fertilizers and pesticides [19] with efficient and controlled release of pesticides, herbicides, and fertilizers and the detection of soil moisture and nutrients. Nano-fertilizers are readily absorbable by plants and possess the potential to increase growth and yield [20]. Nano ZnO particles with a size range of 25 nm at 1000 ppm concentration demonstrated improved germination, seedling vigor, root and stem growth, chlorophyll content, and pod yield by 34% in peanuts [21]. ZnO nanoparticle colloidal solution is used as fertilizer. It serves as a plant's nutrient source and prevents the use of synthetic fertilizers, thus reviving the soil to an organic state [20]. Nano-fertilizers are used in minimal quantity compared to conventional fertilizers yet have been proven to increase wheat yield by 20–25% [22].

ZnO nanoparticles for Zn deficiency mitigation and crop biofortification have been widely reported. However, the application of ZnO nanofibers for crops' Zn biofortification has rarely been studied. Through the well-known laboratory procedure of electrospinning, nanofibers can be prepared from zinc nanoparticles to enhance their qualities. Nanofibers of desired size and shapes can be produced from polymeric solutions using electrospinning techniques under controlled laboratory conditions [23]. The Zn nanoparticles can be fabricated into specific nanofibers to achieve high surface area, porosity, and reactivity, which can potentially improve crop uptake.

The main objective of this study was to evaluate the effectiveness of novel Oxozinc (ZnO) nanofibers as a Zn nano-fertilizer applied through priming or foliar in comparison with conventionally used $ZnSO_4$ under alkaline calcareous silty loam soil conditions. We hypothesized that because of the higher surface area and reactivity of Zn nanofibers compared to conventional fertilizers, Zn use efficiency and wheat crop quality would be improved at a lower application amount.

2. Materials and Methods

2.1. Preparation and Characterization of ZnO Nanofiber

Oxozinc nanofiber (ZnO_{NF}) was prepared locally in the laboratory using the electrospinning method following the protocols described by [24]. In brief, ZnO nanoparticles were first prepared using the chemical precipitation method from Zn acetate ($C_4H_6O_4Zn$) and sodium hydroxide (NaOH) reaction, as described by [25]. The Zn nanoparticle obtained was then mixed with polyvinyl alcohol (PVA) at a ratio of 1:2, and the solution was mounted on the electrospinning injector with a needle hole diameter of 1 mm. The injector was set at 0.5 mL/h outflows in front of an aluminum foil at a 20 cm distance, receiving the solution jet with nanofibers. The prepared ZnO nanofibers were characterized using a scanning electron microscope (SEM; Model: JSM-5910; Make: JEOL, Japan; Energy: 30 KV Magnification) (Figure 1) and X-Ray Diffractometer (XRD; Model: JDX-3532; Make: JEOL, Japan; Voltage: 20–40 kV; Current: 2.5–30 mA; X-Rays: CuKa (Wavelength = 1.5418 Å); 2Theta-Range: 0 to 160°) (Figure 2) for the material identification. It was observed using SEM (Figure 2) that nanoparticles that were subjected to electrospinning were round, irregular, and hexagonal in shape and individual as well as compounded. The pure nanofibers selected for the experiment were chosen from the batch, as observed in Figure 2c,d. The XRD spectra (Figure 2) confirmed the formation of nanofiber and revealed that peaks existed at 2 θ between 30 and 40 [26].

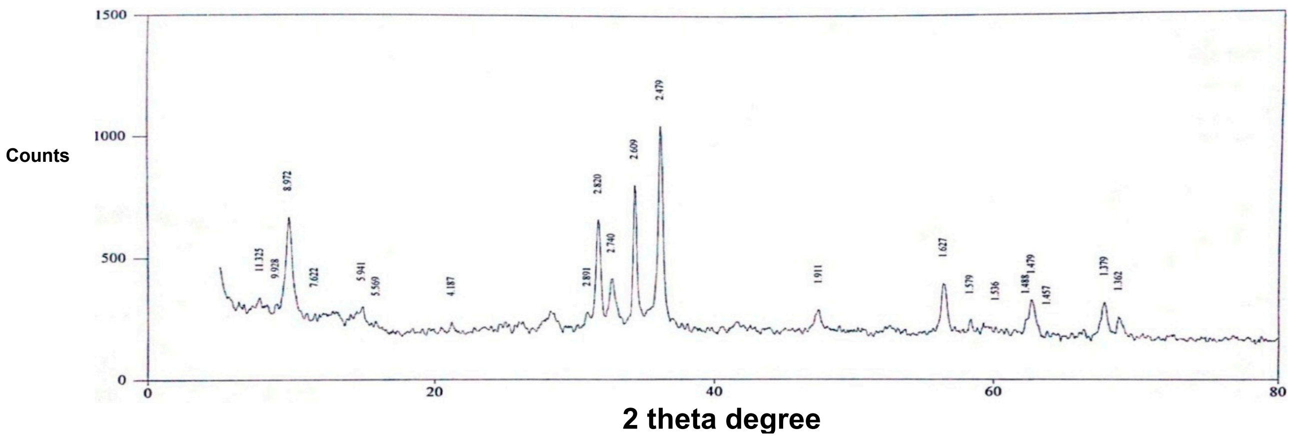

Figure 1. XRD spectra of ZnO nanoparticles used in the experiment. For convenient comparison, the abscissa and ordinate of XRD patterns are displayed at the same row under the same condition and are scaled to the same range.

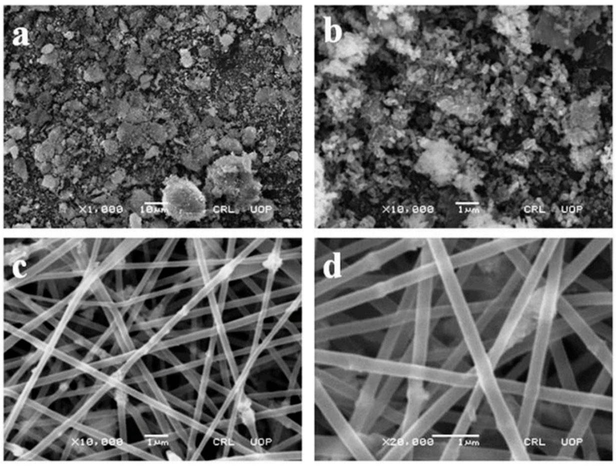

Figure 2. SEM images of the ZnO (**a**) nanoparticles at 10 μm, (**b**) nanoparticles at 1 μm, (**c**) nanofibers at 10 μm, and (**d**) nanofibers at 1 μm.

2.2. Application of ZnO Nanofibers to Wheat Crop in Pots

The study was conducted in pots (8″ top, 6″ bottom, and 12″ height accommodating 7 kg soil) with 10 treatments and 3 replications arranged in a completely randomized design. The experimental treatments include Control, $ZnSO_4$(f), $ZnSO_4$(s), $ZnSO_4$(p), ZnO_{NF}(f), ZnO_{NF}(c), ZnO_{NF}(p), $\frac{1}{2}ZnSO_4$(s) + ZnO_{NF}(f), $\frac{1}{2}ZnSO_4$(s) + ZnO_{NF}(c), $\frac{1}{2}ZnSO_4$(s) + ZnO_{NF}(p) where the suffix f, s, p, and c means application through foliar, soil addition, seed priming, and seed coating, respectively. Soil for pots was collected from a cultivated field (Table 1). The concentration of $ZnSO_4$ was 0.2% for foliar application and seed priming and 5.5 μg kg^{-1} of soil for soil application as per local recommendations. The concentration of ZnO nanofiber was 0.016% for foliar and 0.05% for seed priming application and seed coating using polyvinyl alcohol (PVA) as a coating agent and an electrospinning machine as a coating instrument. The lower application rate for Zn oxide nanofiber was selected based on seed germination tests and preliminary trials in the laboratory. For seed coating, healthy seeds were selected and placed in aluminum foil to receive the nanofiber solution spray on the seeds. Seeds inside the aluminum foil were shuffled at a constant interval for uniform coating results [27]. Wheat (*Triticum aestivum* L.) seed, variety Wadan-2017, purchased from the Cereal Crop Research Institute Pir Sabak, Nowshehra, Khyber Pakhtunkhwa, Pakistan, was sown at the rate of 9 seeds pot^{-1}. Plants in pots were then thinned to 4 pot^{-1} until maturity. The crop was harvested 160 days after sowing (DAS). A filtrate collected from a 1:5 soil:water suspension was sent to an EC meter (DDS-11A, Nanjing, China) for electrical conductivity measurement [28], and a pH meter (HM-12P, Japan) (1:5 H_2O) was used to determine soil pH. A hydrometer procedure was used to determine soil texture [29]. Soil organic matter was determined using the chromic acid wet oxidation method [30]. Soil-available Zn, P, and K were extracted from 1:2 soil:ammonium bicarbonate di-ethylene tri-amine penta-acetic acid (AB-DTPA) with shaking for 30 min and filtration with Whatman 42 filter paper [31]. Soil-available Zn was determined using an atomic absorption spectrophotometer (Perkin Elmer Model 2380, Champaign, IL, USA) directly in the filtrate. Available P was determined through color development with ascorbic acid and reading with a spectrophotometer (U-3900H, Hitachi-Hitech) at 880 nm. Available K was determined with a flame photometer (Jenway-PFP7). The pictorial view of the experiment at different stages is given in Figure 3.

Table 1. Pre-sowing characteristics of the soil used in the experiment.

Parameters	Unit	Value
Texture	-	Silty loam
pH (1:5 H_2O)	-	7.7
EC (1:5)	(dS m^{-1})	0.467
OM	(%)	1.13
Lime ($CaCO_3$)	(%)	9.4
Total soil N	(%)	0.22
AB-DTPA Ext. Zn	mg kg^{-1}	0.81
AB-DTPA Ext. P	mg kg^{-1}	5.77
AB-DTPA Ext. K	mg kg^{-1}	102.6

Figure 3. Pictorial view of the experiment at different stages (Source: Authors) (**a**) (20DAS), (**b**) (75DAS), (**c**) (130DAS), and (**d**) (155DAS) representing the periodic growth stages of wheat in pots.

2.3. Data Collection

In each pot, the plant's height was measured with measuring tape stretched from the bottom to the top of the plant. Spike length was also measured with a measuring tape, and the number of spikes spike^{-1} was counted manually in each pot. At maturity, spikes in each pot were counted and threshed to calculate the number of grains spike^{-1}.

2.4. Plant Sample Analysis

Zn concentration in straw, grain, leaves, and root samples was determined through wet acid digestion ($HNO_3/HClO_4$ digestion) [32] and subsequently analyzed for Zn concentration on an atomic absorption spectrophotometer. Zn uptake (µg pot^{-1}) was calculated as

Zn uptake = (Straw yield × Zn concentration (mg kg^{-1}) + (Grain yield × Zn concentration) + (leaves dry weight × Zn concentration) + (root dry weight × Zn concentration)

Zn uptake efficiency (*ZUE*) was determined as

$$ZUE\ (\%) = \frac{Zn\ content_{treatment} - Zn\ content_{control}}{Zn\ applied} \times 100$$

2.5. Statistical Analysis

ZnO nanofiber and $ZnSO_4$ application and replications were treated as fixed and random effects, respectively. Normality and homoscedasticity assumptions of the parametric test of all studied traits were checked prior to statistical analysis using the Shapiro–Wilks and Levene's test, and it was found that collected data were normally distributed. After verifying the normality of the data, they were analyzed and presented using analysis of variance (ANOVA). The experimental data on the investigated parameters were subjected to a variance analysis suitable for CRD design using StatistiX 8.1 computer software. For parameters with significant F-values ($p \leq 0.05$), a post-hoc analysis using the LSD test was performed to assess significant differences between the means. At a significance threshold of $p \leq 0.05$, the Least Significant Difference (LSD) test was employed. [33].

3. Results

3.1. Growth Parameters

The effects of different treatments on wheat growth and yield parameters (plant height, spike length, number of spikelets per spike, and grains per spike) are presented in Table 2. Use of $ZnSO_4$ fertilizers and ZnO nanofibers (ZnO_{NF}) through either mode of application to the wheat crop showed significant ($p \leq 0.05$) improvement in plant height and non-significant improvement (up to 1.6 cm) in spike length (Table 2). Soil application, foliar spray, and seed priming of Zn through $ZnSO_4$ fertilizer at the recommended dose (5.5 µg kg^{-1} of soil) recorded 92, 86, and 88 cm plants with 18, 11, and 13% increase in plant height over the Zn control, respectively. However, the ZnO nanofiber seed priming (ZnO_{NF}(p)), seed coating (ZnO_{NF}(c)), and foliar application (ZnO_{NF}(f)) showed 94, 92, and 82 cm plants with 20, 18, and 6% increase in plant height over the Zn control, respectively. Combined $\frac{1}{2}ZnSO_4$(s) (half of the recommended $ZnSO_4$ applied to soil) in combination with either of the ZnO_{NF} seed coating ($\frac{1}{2}ZnSO_4$(s) + ZnO_{NF}(c)), seed priming ($\frac{1}{2}ZnSO_4$(s) + ZnO_{NF}(p)), and foliar application ($\frac{1}{2}ZnSO_4$(s) + ZnO_{NF}(f)) also showed significantly ($p \leq 0.05$) improved plant height (92, 92, and 85 cm, respectively) with 18, 18, and 9% increase over the Zn control, respectively. Spike length with $ZnSO_4$ priming, foliar, and soil application was 11.7, 11.3, and 11.2 cm, respectively. Using ZnO nanofiber applications as seed coating, priming, and foliar, the spike length was 12.0, 11.8, and 11.0 cm, respectively, while these modes of ZnO_{NF} when combined with $\frac{1}{2}ZnSO_4$ applied to soil, spike length was 12.1, 11.2, and 11.3 cm, respectively compared to spike length in the control (10.5 cm).

Table 2. Plant height, spike length, spikelets per spike, and grains per spike of wheat as affected by different modes and sources of Zn nutrition.

Treatments	Plant Height	Spike Length	Spikelets $Spike^{-1}$	Grain $Spike^{-1}$
Control	78^{b}	10.5	17.7^{c}	39.3^{d}
$ZnSO_4$(f)	86^{ab}	11.3	18.7^{bc}	47.0^{b}
$ZnSO_4$(s)	92^{a}	11.2	19.3^{abc}	51.0^{ab}
$ZnSO_4$(p)	88^{ab}	11.7	19.3^{abc}	44.3^{c}
ZnO_{NF}(f)	82^{ab}	11.0	19.0^{abc}	43.7^{c}
ZnO_{NF}(c)	92^{a}	12.0	20.7^{a}	52.3^{a}
ZnO_{NF}(p)	94^{a}	11.8	20.7^{a}	52.0^{a}
$\frac{1}{2}$ $ZnSO_4$(s) + ZnO_{NF}(f)	85^{ab}	11.3	20.7^{a}	49.0^{b}
$\frac{1}{2}$ $ZnSO_4$(s) + ZnO_{NF}(c)	92^{a}	12.1	20.0^{ab}	42.0^{c}
$\frac{1}{2}$ $ZnSO_4$(s) + ZnO_{NF}(p)	92^{a}	11.2	18.7^{bc}	50.3^{ab}
LSD $_{(p \leq 0.05)}$	12	ns	1.8	2.6

Suffix f, s, p, and c means application through foliar, soil addition, seed priming, and seed coating, respectively. NF: nanofiber, means with different letters are statistically significant at $p \leq 0.05$.

3.2. Yield Parameters

Application of $ZnSO_4$ fertilizers or ZnO_{NF} by either method to wheat crop significantly ($p \leq 0.05$) enhanced the spikelets and grain count spike^{-1} (Table 2). The spikelets count spike^{-1} and grains count spike^{-1} with $ZnSO_4$ applied to soil ($ZnSO_4$(s)) at 5.5 µg kg^{-1} of soil were 19.3 and 51. For $ZnSO_4$ treated as a foliar spray ($ZnSO_4$(f)), the spikelets count spike^{-1} and grain count spike^{-1} were 18.7 and 47, while for $ZnSO_4$ seed priming ($ZnSO_4$(p)), these were 19.3 and 44.3, respectively. The increase in spikelet count spike^{-1} with $ZnSO_4$ application as soil, foliar, and seed priming was 9, 6, and 9%, and grain count spike^{-1} was 30, 20, and 11% over the control, respectively (Table 2). The ZnO_{NF} coating, priming, and foliar application recorded spikelets count spike^{-1} of 20.7, 20.7, and 19, showing an edge of 17, 17, and 7%, and grain count spike^{-1} was 52.3, 52.0, and 43.7 with 33, 32, and 11% increase over the control, respectively. A combination of $\frac{1}{2}ZnSO_4$ applied as soil with ZnO_{NF} each as foliar spray (ZnO_{NF}(f)), seed coating (ZnO_{NF}(f)), and seed priming (ZnO_{NF}(f)) registered the spikelets count spike^{-1} of 20.7, 20, and 18.7, with a 17, 13, and 6% increase over the control (17.7), respectively, and grains count spike^{-1} of 49, 42, 50.3 with a 25, 7, 28% increase over the control (39.3), respectively.

3.3. Zn Concentration in Plant Tissues

$ZnSO_4$ fertilizers and ZnO_{NF} treatments through different modes of application to wheat crops significantly affected the Zn concentration in grain, leaves, stem, and roots ($p \leq 0.05$; Table 3). Wheat grain exhibited maximum Zn concentration (29 µg g^{-1}) with $ZnSO_4$ foliar spray at the recommended dose (5.5 µg kg^{-1} of soil); however, when $ZnSO_4$ was treated as seed priming or applied to the soil at the recommended dose, Zn concentration in grain was 26 and 25.6 µg g^{-1}, respectively. While all these values were statistically similar, their respective increase in grain Zn concentration over the control was 39, 25, and 23%. When ZnO_{NF} as foliar spray, seed coating, and seed priming was applied, the grain Zn concentration was 29.3, 26.1, and 25.7 µg g^{-1}, achieving an increase in grain Zn concentration by 41, 25, and 24%, respectively, over the control. The combined application of $\frac{1}{2}ZnSO_4$ as soil and ZnO_{NF} as foliar spray and seed priming resulted in significantly ($p \leq 0.05$) higher grain Zn concentration (32.9 and 31.1 µg g^{-1}, respectively) over all the other treatments. Combined $\frac{1}{2}ZnSO_4$ as soil and ZnO_{NF} applied as seed coating recorded significantly ($p \leq 0.05$) lower grain Zn concentration (25.7 µg g^{-1}) as compared to other Zn treatments with a similar mode of application (foliar spray and seed priming), whereas each one registered an increase of 58, 49, and 23%, respectively, over the control.

Table 3. Zn concentration in leaf, stem, root, and grain of wheat as influenced by different modes and sources of Zn nutrition.

Treatments	Zn Concentration (µg g^{-1})			
	Grain	**Leaf**	**Root**	**Stem**
Control	20.8 ^{d}	17.8 ^{d}	21.5 ^{f}	11.1 ^{d}
$ZnSO_4$(f)	29.0 ^{ab}	21.2 ^{abc}	53.5 ^{a}	17.5 ^{b}
$ZnSO_4$(s)	25.6 ^{bc}	20.8 ^{abc}	26.3 ^{ef}	15.0 ^{c}
$ZnSO_4$(p)	26.0 ^{bc}	18.8 ^{cd}	36.0 ^{bc}	16.6 ^{bc}
ZnO_{NF}(f)	29.3 ^{ab}	18.9 ^{cd}	34.1 ^{cd}	11.7 ^{d}
ZnO_{NF}(c)	26.1 ^{bc}	20.0 ^{bcd}	37.4 ^{bc}	16.6 ^{bc}
ZnO_{NF}(p)	25.7 ^{bc}	19.1 ^{cd}	29.3 ^{de}	20.4 ^{a}
$\frac{1}{2}$ $ZnSO_4$(s) + ZnO_{NF}(f)	32.9 ^{a}	18.9 ^{cd}	38.4 ^{bc}	12.9 ^{d}
$\frac{1}{2}$ $ZnSO_4$(s) + ZnO_{NF}(c)	25.7 ^{bc}	22.4 ^{ab}	28.0 ^{e}	16.5 ^{bc}
$\frac{1}{2}$ $ZnSO_4$(s) + ZnO_{NF}(p)	31.1 ^{a}	23.3 ^{a}	40.8 ^{b}	20.2 ^{a}
LSD $_{(p \leq 0.05)}$	3.9	2.7	5.4	2.1

Suffix f, s, p, and c means application through foliar, soil addition, seed priming, and seed coating, respectively. NF: nanofiber, means with different letters are statistically significant at $p \leq 0.05$.

In leaves, Zn concentration (21.2 µg g^{-1}) was the maximum for the recommended dose (5.5 µg kg^{-1} of soil) of $ZnSO_4$ through foliar application, while for soil application and seed priming, it was 20.8 and 18.8 µg g^{-1}, respectively, where each one achieved an increase of 19, 17, and 6% over the control (17.8 µg g^{-1}), respectively. For ZnO_{NF}, the maximum leaf Zn concentration was noted with ZnO_{NF} seed coating (20 µg g^{-1}), followed by ZnO_{NF} seed priming (19.1 µg g^{-1}) and ZnO_{NF} foliar spray (18.1 µg g^{-1}), where each one registered an increase in Zn content in leaf by 13, 7, and 6%, respectively, over the control. In the combined $\frac{1}{2}ZnSO_4$ applied as soil and ZnO_{NF} applied each as seed priming, seed coating, and foliar spray, the leaf Zn concentration was 23.3, 22.4, and 18.9 µg g^{-1}, where each one registered an increase of 31, 26, and 6% over the control, respectively.

The root Zn concentrations for $ZnSO_4$ as foliar spray, seed priming, and soil addition at the recommended dose (5.5 µg kg^{-1} of soil) were 53.5, 36, and 26.3 µg g^{-1}, and recorded an increase of 149, 68, and 23%, over the control (21.47 µg g^{-1}), respectively. For ZnO_{NF} applied through seed coating, foliar spry, and seed priming, the root Zn concentrations were 37.4, 34.1, and 29.3 µg g^{-1}, each one accruing an increase of 74, 59, and 36%, respectively, over the Zn control. In the case of $\frac{1}{2}ZnSO_4$ applied as soil combined with ZnO_{NF} applied as either seed priming, foliar spray, or seed coating, the root Zn concentrations were 40.8, 38.4 µg g^{-1}, and 28 µg g^{-1}, where each one registered an increase of 90, 79, and 30%, respectively, over the control. With regards to stem Zn concentration, application of $ZnSO_4$ foliar spray, seed priming, and soil addition at the recommended dose (5.5 µg kg^{-1} of soil) recorded 17.5, 16.6, and 15 µg g^{-1} stem Zn concentration while having a 58, 49, and 35% increase over the control (11.1 µg g^{-1}), respectively (Table 3). For ZnO_{NF} seed priming, seed coating, and foliar spray, the stem Zn concentrations were 20.4, 16.6, and 11.7 µg g^{-1}, showing 84, 50, and 5% higher stem Zn concentrations over the control, respectively. However, the $\frac{1}{2}$ $ZnSO_4$ applied as soil combined with ZnO_{NF} either as seed priming, seed coating, and foliar spray recorded 20.2, 16.5, and 12.9 µg g^{-1} stem Zn concentration, each one registered an increase of 82, 48, and 16%, respectively, over the control.

Table 3 shows that Zn fortification through seed priming of the wheat crop with ZnO_{NF} resulted in the maximum and significantly higher Zn uptake efficiency (ZUE, 34%) than the rest of the treatments. Application of ZnO_{NF} to the wheat crop as seed coating with a ZUE value of 23% followed the ZnO_{NF} priming, while the difference between the two higher ZUE values was significant. Application of the sole $ZnSO_4$ to the soil through seed priming or foliar spray at the recommended level or its application in half of the recommended dose to soil plus ZnO_{NF} with either method (foliar, seed coating, or priming) did not reflect an increase or improvement in ZUE in wheat crop, while all of them were statistically similar. The ZUE for $ZnSO_4$ applied to the soil at the recommended dose was 3%, while for foliar spray and seed priming, the ZUE was 2% each. When $\frac{1}{2}ZnSO_4$ applied to soil was combined with ZnO_{NF} as foliar spray, seed coating, and seed priming, the ZUE improved to 5, 4, and 6%, respectively; however, the improvement was still non-significant statistically and remained at par with $ZnSO_4$ applied at the recommended dose to soil.

3.4. Zn Uptake

Application of $ZnSO_4$ fertilizers and ZnO_{NF} through different methods to wheat crops significantly ($p \leq 0.05$) affected the Zn accumulation in grain, leaf, root, stem, and total Zn uptake (Table 4). The grain total Zn content with $ZnSO_4$ application as foliar spray, soil addition, and seed priming was 242, 233, and 211 µg pot^{-1}, each one accruing an increase of 63, 58, and 43%, respectively, over the control (148 µg pot^{-1}). The order of grain Zn content with the application of ZnO_{NF} through different methods was: seed priming (283 µg pot^{-1}) > seed coating (232 µg pot^{-1}) > foliar spray (215 µg pot^{-1}) while each one accrued 92, 57, and 45% increase over the control, respectively. When $\frac{1}{2}ZnSO_4$ soil addition was combined with ZnO_{NF} application as foliar spray, seed priming, and seed coating, their respective grain Zn content (380, 334, and 252 µg pot^{-1}) increased by 157, 126, and 71%, respectively, over the control.

Table 4. Zn content in different parts of the crop and total Zn uptake as affected by different modes and sources of Zn nutrition.

Treatments	Zn Uptake (µg pot^{-1})					ZUE (%)
	Grain	Leaf	Root	Stem	Total Uptake	
Control	148 ^{d}	84 ^{b}	75 ^{d}	119 ^{d}	427 ^{d}	
$ZnSO_4$(f)	242 ^{bcd}	127 ^{ab}	188 ^{a}	220 ^{bc}	776 ^{ab}	3 ^{c}
$ZnSO_4$(s)	233 ^{bcd}	114 ^{ab}	76 ^{d}	211 ^{c}	633 ^{bcd}	2 ^{c}
$ZnSO_4$(p)	211 ^{cd}	105 ^{ab}	103 ^{cd}	224 ^{bc}	642 ^{bcd}	2 ^{c}
ZnO_{NF}(f)	215 ^{cd}	84 ^{b}	91 ^{cd}	126 ^{d}	516 ^{cd}	7 ^{c}
ZnO_{NF}(c)	232 ^{bcd}	123 ^{ab}	132 ^{abcd}	235 ^{bc}	722 ^{abc}	23 ^{b}
ZnO_{NF}(p)	283 ^{abc}	111 ^{ab}	113 ^{bcd}	340 ^{a}	847 ^{ab}	34 ^{a}
$\frac{1}{2}$ $ZnSO_4$(s) + ZnO_{NF}(f)	380 ^{a}	129 ^{ab}	139 ^{abc}	190 ^{cd}	838 ^{ab}	5 ^{c}
$\frac{1}{2}$ $ZnSO_4$(s) + ZnO_{NF}(c)	252 ^{bcd}	145 ^{a}	87 ^{cd}	247 ^{bc}	731 ^{abc}	4 ^{c}
$\frac{1}{2}$ $ZnSO_4$(s) + ZnO_{NF}(p)	334 ^{ab}	139 ^{a}	162 ^{ab}	291 ^{ab}	926 ^{a}	6 ^{c}
LSD $_{(p \leq 0.05)}$	106	52	58	75	259	8.0

Suffix f, s, p, and c means application through foliar, soil addition, seed priming, and seed coating, respectively. NF: nanofiber, $\frac{1}{2}$ $ZnSO_4$ soil: half of the recommended $ZnSO_4$ applied to soil. Means with different letters are statistically significant at $p \leq 0.05$.

The leaf total Zn content for $ZnSO_4$ foliar spray was 127 µg pot^{-1}, while for soil addition and seed priming, leaf Zn content was 114 and 105 µg pot^{-1}, showing an increase of 51, 35, and 25% over the leaf Zn content in the control treatment (84 µg pot^{-1}), respectively. With the application of ZnO_{NF} through seed coating, the leaf Zn content was 123 µg pot^{-1}; with ZnO_{NF} application as seed priming, the leaf Zn content was 111 µg pot^{-1}, while for its foliar spray, the leaf Zn content was 84 µg pot^{-1}. Variation in leaf Zn content with ZnO_{NF} application as seed coating and seed priming showed an increase of 47% and 33%, respectively, over the Zn control, while ZnO_{NF} foliar spray recorded no change in leaf Zn content as compared with the control treatment. With $\frac{1}{2}ZnSO_4$ as soil addition in combination with ZnO_{NF} application either as seed coating, seed priming, or foliar spray, the leaf Zn contents recorded were 145, 139, and 129 µg pot^{-1} and the accrued increase in leaf Zn content by each treatment was 73, 66, and 53% over the Zn content in leaf recorded for the control treatment (84 µg pot^{-1}).

In roots, Zn content with $ZnSO_4$ treatment as foliar spray, seed priming, or soil addition at the recommended dose (5.5 µg kg^{-1} of soil) was 188, 103, and 76 µg pot^{-1}, showing an increase of 149%, 36%, and 1% over the root Zn content in the control (75 µg pot^{-1}) treatment, respectively (Table 4). Significant ($p \leq 0.05$) variation in root Zn content was also observed with ZnO_{NF} application with seed coating, having root Zn content of 132 µg pot^{-1}, seed priming with 113 µg pot^{-1}, and foliar spray with 91 µg pot^{-1}, where the increase over the control for each ZnO_{NF} application method was 75%, 49%, and 21%, respectively. With the application of $\frac{1}{2}ZnSO_4$ as soil addition in combination with ZnO_{NF} as seed priming, the root Zn content was 162 µg pot^{-1}, followed by $\frac{1}{2}ZnSO_4$ as soil addition in combination with ZnO_{NF} foliar spray with root Zn content of 139 µg pot^{-1}, and $\frac{1}{2}ZnSO_4$ as soil addition in combination with ZnO_{NF} seed coating with root Zn content of 87.0 µg pot^{-1}, respectively, accruing an increase of 115%, 85%, and 15%, over the root Zn content in the control (75.4 µg pot^{-1}), respectively.

Stem Zn content with the application of $ZnSO_4$ as seed priming was 224 µg pot^{-1}; with the application of $ZnSO_4$ as foliar spray or soil addition, the stem Zn content was 220 and 211 µg pot^{-1}, respectively, whereas each of the above application methods recorded an increase of 87%, 85%, and 77%, over the stem Zn content in the control pots (119 µg pot^{-1}), respectively. Significant ($p \leq 0.05$) variation in stem Zn content was observed for ZnO_{NF} application; as seed priming, the stem Zn content was 340 µg pot^{-1}, followed by seed coating with stem Zn content of 235 µg pot^{-1}, and then the foliar spray with stem Zn content of 126 µg pot^{-1}, whereas the increase in stem Zn content for each ZnO_{NF} application method was 185%, 97%, and 5%, respectively, over the stem Zn content in the control

(Table 4). With $\frac{1}{2}ZnSO_4$ applied as soil combined with ZnO_{NF} as seed priming, the stem Zn content was 291 µg pot^{-1}, followed by $\frac{1}{2}ZnSO_4$ applied as soil combined with ZnO_{NF} as seed coating (stem Zn content 247 µg pot^{-1}), and $\frac{1}{2}ZnSO_4$ applied as soil combined with ZnO_{NF} as foliar spray (stem Zn content 190 µg pot^{-1}), and each one showed an increase in stem Zn content by 143%, 107%, and 59%, respectively, over the control (119 µg pot^{-1}).

Total Zn uptake by wheat crop significantly ($p \leq 0.05$) varied with different modes of $ZnSO_4$ application at the recommended dose (5.5 µg kg^{-1} of soil) (Table 4); the total Zn uptake for $ZnSO_4$ foliar spray was 776 µg pot^{-1}, for seed priming and soil addition, the Zn total uptake was 642 and 633 µg pot^{-1}, respectively. These quantities of Zn total uptake by wheat crop accrued through different modes of $ZnSO_4$ application recorded an increase of 82%, 50%, and 48% over the Zn total uptake in the control (427 µg pot^{-1}), respectively. The Zn total uptake with ZnO_{NF} seed priming was 847 µg pot^{-1}, followed by Zn total uptake, with ZnO_{NF} seed coating (722 µg pot^{-1}) and foliar spray (516 µg pot^{-1}), each accruing 98%, 69%, and 21%, respectively, over the Zn control. However, $\frac{1}{2}ZnSO_4$ applied as soil combined with ZnO_{NF} seed priming recorded the maximum Zn total uptake of 926 µg pot^{-1}. The $\frac{1}{2}ZnSO_4$ applied as soil plus ZnO_{NF} as foliar, and $\frac{1}{2}ZnSO_4$ applied as soil plus ZnO_{NF} as seed coating resulted in 838 and 731 µg pot^{-1} total Zn uptake by wheat crop while showing a 117%, 96%, and 71% increase over the Zn total uptake in the control, respectively.

4. Discussion

4.1. Effect of Zn on Wheat Growth and Yield Parameters

Plant height was statistically similar among the Zn source and methods of application, but all the Zn treatments were significantly ($p \leq 0.05$) higher over the Zn control regardless of application method (Table 2). This means that ZnO nanofiber (ZnO_{NF}) was an effective alternative Zn source despite its application at a highly reduced rate (0.5 kg Zn ha^{-1}) as compared to conventional $ZnSO_4$ fertilizers. The soil was not only Zn deficient but also alkaline in nature and low in soil organic matter content (Table 1), which could further cause inhibition in Zn^{+2} absorption by the plants. Soil pH is the primary soil factor affecting Zn distribution in soil, wherein it is more readily released when the soil pH is acidic and more readily adsorbed on soil matrix at higher pH, especially in cases when the soil OM content is low [34]. However, any improvement in growth and yield under such Zn deficiency and alkaline conditions may be related to external Zn application [35]. The results also elucidated that both the seed coating and seed priming of ZnO_{NF} edged over the soil application of $ZnSO_4$ because of their reduced chances of Zn fixation from ZnO_{NF} and improved chances of Zn absorption by the plants [36]. Foliar applied Zn from either $ZnSO_4$ or ZnO_{NF}, or their combination rendered improvement in plant height and was 7–9% lower; the number of spikelet $spike^{-1}$ and the number of grains $spike^{-1}$ were 3 and 10% lower for foliar $ZnSO_4$ and 10–22% for foliar ZnO_{NF} than their other counterpart methods of application viz soil application, seed coating, seed priming, or their combinations (Table 2). Although previous authors showed a more prominent effect of foliar application of ZnO NP on plant growth than any other Zn source [37], here, the low performance in plant growth using foliar application could be attributed to its application at mid or latter growth stages rather than other modes of application where Zn availability is increased at the start of the crop growth. Additionally, the shape and size of nanomaterial are key in affecting the absorption through the leaves more readily [38], and thus, nanofibers may not be as readily available through foliar application compared to other Zn oxide forms. Keeping the Zn sources constant, timely availability of Zn from either source and by any mode improves the plant growth through enhancing the growth hormone Indole Acetic Acid [39], chlorophyll content [40], photosynthetic activity [41], and enzymes acid and alkaline phosphatase, phytase, and dehydrogenase activities [41] resulting in improved plant height, spike length, spikelets $spike^{-1}$, and grains $spike^{-1}$.

Contrary to foliar application, $ZnSO_4$ seed priming was 3% higher in spikelets spike^{-1} but 9% lower in grain spike^{-1} (Table 2), indicating a maximum of seed primed with Zn exhaustion until grain development. Soil application of $ZnSO_4$ and seed coating and priming of ZnO_{NF} were the highest in spikelets and grain counts spike^{-1} (Table 2), perhaps due to Zn availability throughout the growth period. Previous workers also reported higher grain production with foliar ZnO NP application [37,40]. However, this study revealed ZnO_{NF} and $ZnSO_4$ foliar application as synonymous with its improved grain yield rather than vegetative growth, revealing its translocation to grain development better than any other mode of application. The pitfall of lower vegetative growth in the case of foliar application was masked by its combined application with $\frac{1}{2}ZnSO_4$ as soil addition by recording 4–11% more spikelets spike^{-1} and 18% more grain spike^{-1} than the other modes of application (Table 2). In this case, the soil-applied $ZnSO_4$ supports the initial crop growth, and foliar application of ZnO_{NF} supports the grain development along with crop growth. Studies by [42] support Zn application for higher grain and biomass yield, and [43] observed the highest grain yield and grain NP and Zn uptake from the mixture of $ZnSO_4$ and foliar ZnO_{NP} compared to sole $ZnSO_4$ fertilizer, while [41] reported a significant increase in shoot and root lengths, root area, chlorophyll, leaf protein, and biomass yield as a result of increased dehydrogenase activities by ZnO nano-fertilizers, which indicated improved microbial activities in the rhizosphere and the resultant nutrient mobilization for plant uptake. Pandey [44] also reported improved plant growth as a result of nano ZnO application. We can deduce that zinc nanofiber can offer the potential to mitigate the inherent fixation issue associated with alkaline calcareous soil and improve wheat growth, irrespective of application methods.

4.2. Comparative Effects on Zn Uptake and Use Efficiency

Higher Zn concentration was observed with $ZnSO_4$ foliar in grain and leaf (by 14–16% and 2–13%, respectively) than with $ZnSO_4$ seed priming or soil application. However, the ZnO_{NF} foliar spray was 15–17% higher in leaf Zn concentration than the seed coating and approximately equal to seed priming (Table 3). These differences, although statistically similar, indicate a more facilitated translocation of Zn from foliar-applied ZnO_{NF} to grain compared to $ZnSO_4$. This might be ascribed to the nano size of the applied ZnO_{NF}. The combination of $\frac{1}{2}ZnSO_4$ + ZnO_{NF} foliar showed 11% higher ($p \leq 0.05$) grain but 25% less leaf Zn concentration than its counterpart treatments (Table 2). The effectiveness of foliar Zn application alone or in combination with $ZnSO_4$ for grain Zn content was evident from our results compared to other application modes. Elshayb et al. [43] supported our results and reported significantly ($p \leq 0.05$) higher Zn uptake with a mixture of ZnO_{NP} and $ZnSO_4$. The Zn controlling factors, such as carbonate content and high soil pH (Table 1) or the generally prevalent nutrient exhaustion in cereal-growing soils, could support a higher crop response to the combination of ZnO_{NF} and $ZnSO_4$. Furthermore, Zn foliar spray near the heading stage could result in maximal absorption and utilization for translocation to grain for grain development. Grain Zn concentration with $ZnSO_4$ soil addition and seed priming and ZnO_{NF} seed coating and priming were comparable (Table 2), suggesting ZnO_{NF} is a useful substitute for conventional $ZnSO_4$ fertilizers. Moreover, the combined application of ZnO_{NF} foliar spray or seed priming with $\frac{1}{2}ZnSO_4$ soil addition surpassed all other modes of application in terms of Zn use in wheat grains and could be adapted for successful wheat grain Zn biofortification. Previous works such as [45] recommended ZnO nanoparticle seed priming for higher growth, photosynthesis, and yield parameters than control. Saleem et al. [46] reported a significant increase in wheat grain Zn content and yield by applying Zn fertilizers. Since Zn transfers from vegetative parts to developing grains, Zn presents transport through the phloem; therefore, in addition to soil factors such as high pH and calcareousness, the availability of water from soil could have affected the Zn content in grain from soil-applied $ZnSO_4$ or seed coated and primed ZnO_{NF}, while this problem can have little effect in the case of foliar application since, being accompanied with water, the foliar applied Zn has considerably swift movement in wheat [35].

Higher Zn concentration in roots and stem for $ZnSO_4$ foliar (Table 3) shows ready mobility of Zn from source (leaves) to sink (stem and onward to roots). The root and stem Zn concentration in the case of ZnO_{NF} also confirmed that Zn travels from foliar spray towards the root and from seed priming towards the upper parts since root Zn concentration in the case of seed priming of ZnO_{NF} is significantly ($p \leq 0.05$) lower than the other two application modes. Irrespective of the Zn source, this trend of Zn movement indicates a source–sink relationship, and the travel is always from source (foliar application) to sink (grain and roots) and vice versa for seed priming, soil application, and seed coating. Previous research [47] also revealed that Zn influx into the plant is concentration-dependent, suggesting it is carrier-mediated and metabolism-dependent, and its uptake from the soil into the root and translocation to shoots indicate its movement across root cells' plasma membranes. This might also be true for seed priming, seed coating, and combined $\frac{1}{2}ZnSO_4$ + ZnO_{NF} foliar spray, which might have enhanced Zn translocation from roots to shoots [48].

Results for grain Zn content indicated the maximum and significantly higher grain Zn content (by 157%) with combined $\frac{1}{2}ZnSO_4$ and ZnO_{NF} (foliar spray), followed by sole ZnO_{NF} foliar spray (by 92%), in leaf Zn content for combined $\frac{1}{2}ZnSO_4$ and ZnO_{NF} (seed coating) (73%), in root Zn content (149%) for sole $ZnSO_4$ (foliar spray and in stem Zn content for sole ZnO_{NF} (seed priming) (185%) (Table 4). While all these methods variably affected Zn accumulation in different parts of the crop, foliar application of ZnO_{NF} in combination with $\frac{1}{2}$ $ZnSO_4$ as soil addition surpassed the rest of the methods for Zn fortification of wheat grain. The maximum and significant increase in total Zn uptake with $\frac{1}{2}ZnSO_4$ + ZnO_{NF} seed priming (117%) might be due to more biological yield and, therefore, does not stand as a suitable marker for wheat Zn biofortification. However, improved Zn uptake efficiency (ZUE) through ZnO_{NF} seed priming (34%) followed by its seed coating (23%) also shows that these treatments are suitable for application to wheat crops. Application of the sole $ZnSO_4$ (2–3%) in either method or its half-dose addition to soil combined with ZnO_{NF} (4–6%) in either method did not improve ZUE in wheat crops.

The lowest Zn uptake from $ZnSO_4$ (Table 4) may explain the lowest Zn accumulation in grain. In the case of the combination of ZnO_{NF} seed priming and coating along with $\frac{1}{2}ZnSO_4$ as soil, a lower concentration of root Zn might have resulted in low Zn uptake from soil and upward translocation and, resultantly, lower grain Zn fortification than the foliar application. Our results were consistent with [45], suggesting ZnO nano-fertilizer with higher Zn uptake and accumulation in various plant parts; however, the effect of ZnO nanofiber and its most suitable application method on Zn concentration and content in various plant parts was never reported. Our results in this regard found that the application of ZnO nanofiber relative to a conventional ($ZnSO_4$) Zn source significantly improved crop Zn nutrition and grain biofortification in wheat crops. The results are in agreement with the findings of [49], while according to [50], the application of Zn-EDTA and $ZnSO_4.7H_2O$ significantly enhanced the Zn use efficiency of rice over $ZnCl_2$, $Zn_3(PO_4)_2$ and oxide. However, the Zn use efficiency with Zn EDTA was found to be significantly superior to $ZnSO_4.7H_2O$. Further studies on understanding the mechanisms of Zn nanofiber uptake by crops in different soils with different application methods would provide comprehensive information to optimize this potential efficiently (Figure 4).

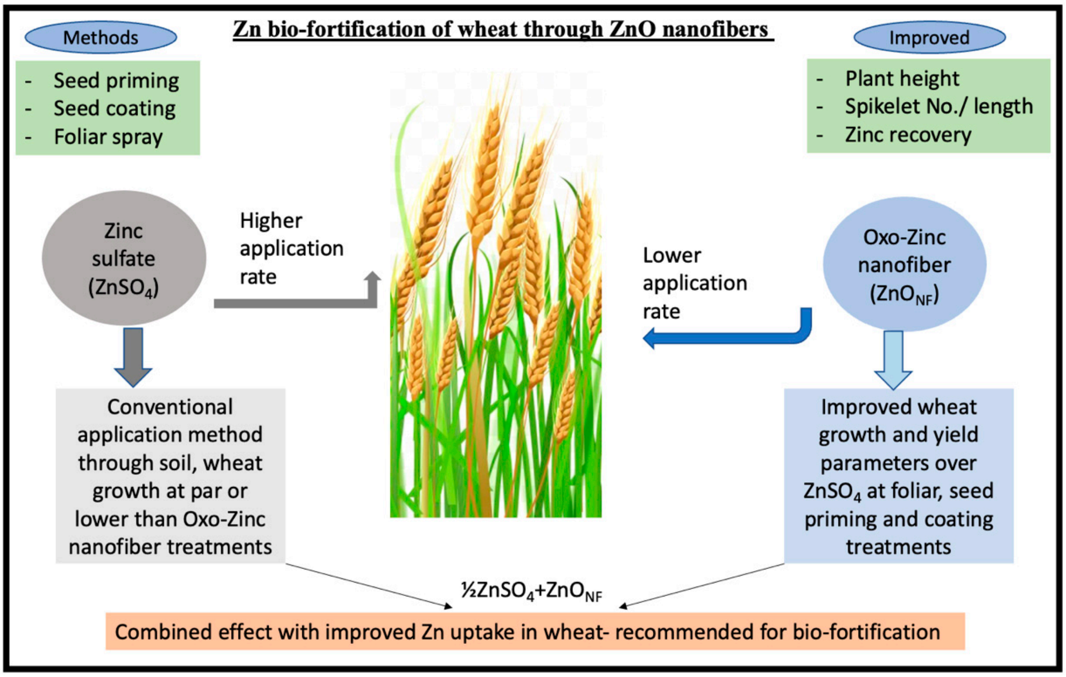

Figure 4. Graphical presentation of increased wheat production and Zn biofortification in wheat.

5. Conclusions

Wheat growth, yield, nutritional, and Zn uptake traits improved as compared to control with Zn nutrition applied. As compared to conventional Zn source ($ZnSO_4$), ZnO nanofiber (ZnO_{NF}) was required in significantly lower quantity (0.5 kg ZnO_{NF}-Zn ha^{-1} vs. 5.5 µg kg^{-1} of soil $ZnSO_4$-Zn ha^{-1}) but improved wheat growth Zn uptake and quality with various application methods. Specifically, ZnO_{NF} applied as seed priming and foliar spray produced more yield than its application as seed coating. Furthermore, the combined ZnO_{NF} (foliar spray, seed coating, and seed priming) and $\frac{1}{2}ZnSO_4$ maximized the Zn nutrient accumulation in different parts of the wheat. In particular, the $\frac{1}{2}$ $ZnSO_4$ + ZnO_{NF} through foliar application attained the highest Zn uptake in wheat grain. Future research must focus on the safety, bioavailability, and toxicity of various NFs and NPs utilized for improving crops. Moreover, ZnO nanofiber (ZnO_{NF}) application may be tested under abiotic stresses such as drought, salinity, etc., for inducing tolerance in crops and enhancing crop yields under stress conditions.

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