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Article

Exogenous Application of Zinc Sulphate at Heading Stage of Wheat Improves the Yield and Grain Zinc Biofortification

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Abstract: Wheat is the leading staple food in the world, particularly in developing countries, which lacks a mechanism of zinc absorption; when compared to pulses, more attention is consequently important to be given to the wheat crop. Micronutrient deficiencies and especially zinc deficiency influences one-third of the world population. In addition to this, it is also essential for the growth and development of plants and animals. A pot and field experiment was conducted to check the effect of foliar application of zinc sulphate on three different wheat varieties at the same time. Treatment consisted of three zinc levels (control, 4%, 6%) in the form of zinc sulphate (21% Zn) applications were applied on various wheat varieties (Zincol, Fakher-e-Bhakkar, Faisalabad-2008) at different growth stages (tillering, booting and heading). Different zinc levels showed different results on wheat varieties in both experiments. Results revealed that var. Fakher-e-Bhakkar was best at 6% zinc application for more plant height, the number of spikelets, spike length, 100-grain weight, biological and grain yield per plant as compared to other varieties and treatments. Antioxidants and nutritional quality (protein, gluten, starch and zinc contents) showed variable behavior both on wheat varieties and zinc application. It is concluded that Fakher-e-Bhakkar was found to be the most responsive cultivar at 6% zinc application for improvement in growth, yield-related traits and nutritional quality. So it is recommended for achieving maximum yield and yield components and grain zinc contents of wheat under agro-climatic conditions of Layyah, Punjab-Pakistan.

Keywords: wheat varieties; zinc biofortification; seed yield; antioxidants; nutritional quality traits

1. Introduction

The recorded growth of the agriculture sector by 2.67% is significantly higher than the last year, which was 0.58. According to the economic survey of Pakistan during 2019–2020, wheat is a major staple food and is grown on an area of 9.1 million hectares. The per capita wheat consumption in Pakistan is higher than other countries of the world.

Wheat (*Triticum aestivum* L.) is the main strategic cereal crop for almost all of the world's populations. It is the major staple food around the world for about two billion people (36%) population. Globally, wheat grain provides 55% of the carbohydrates and 20% of the energy in meals. Among the countries of the world, Pakistan rank 8th in wheat production, and it contributes about 3.17% to world wheat production. In Pakistan, it is the leading food grain crop having the main position in the economy. Micronutrients play a vital role in plant physiological processes. The plant's requirement for micronutrients is low but also has importance equal to macronutrients [1]. In Pakistan, most soils are calcareous in nature, in which nutrients become unavailable because of high pH, low organic matter, salt stress, and an imbalanced application of NPK fertilizers are also responsible for nutrient deficiency in soil [2].

Rice and wheat cultivated soil are deficient in some of the major micronutrients such as zinc, boron, manganese, iron, copper and molybdenum. In the area of southern Punjab, 70% of wheat cultivated soils are deficient in zinc [3]. Zinc plays an important role in the health, the functioning of the immune system, the signaling of the intestine and growth [4]. This is the reason zinc is related to many health issues, such as an increase in infection rate, impaired learning, and abnormal immune system function [4]. Malnutrition is a global problem as almost one-quarter of the world population is affected by one or extra micronutrient malnutrition disorders [5]. In Pakistan, 12 million children are stunted, and 22.1% of women and 18.6% of children (under the age of five) have a deficiency of Zn. In the case of Zn deficiency in women, Punjab has the maximum share (24.1%), observed via way of means of Baluchistan (23.4%), and Sindh (21.4%), while Khyber Pakhtunkhwa has the lowest prevalence (15.9%) [6].

However, the Zn nutritional disorder can be improved by bio-fortification, which is an effective and economical method [7,8]. The agronomic and genetic approaches include appropriate plant and soil fertilizer application for zinc biofortification [9]. Agronomic biofortification is not only important for the development but also in the utilization potential and mobilization of micronutrients [9–12]. Bouis and Saltzman [13] assert that the crops produced by biofortification are consumed by 20 million people around the world. The deficiencies of this micronutrient can be overcome by introducing these elements by seed, leaf, and soil treatment.

Soils that are based on the cultivation of cereals have a deficiency of Zn, and it is also accepted that it is the most deficient micronutrient. Because of this unavailability, severe economic losses occur, and it also decreases the plant's development along with its yield. Another option to meet the deficiency of nutrients is the foliar application of them [14]. When the roots are not able to provide nutrients, then the foliar application is helpful [15].

Application of Zn at various growth phases of wheat significantly improved its accumulation in its grain. In recent studies, it was concluded that maximum zinc was accumulated when it was applied at the grain filling stage of the crop, and it has more concentration to accumulate later at a stage known as the milky stage during the grain development. Grain zinc concentration becomes higher at phases such as the dough and the milky stage than initial growth stages of wheat as a result of foliar spray of zinc [16].

The preferred zinc application seems to improve the general overall performance of the plants in the field. Biofortification of wheat is needed to deal with the trouble of micronutrient deficiency in humans [17]. Zinc in fertilizers can substantially enhance the quality and yield of crops.

So, the main objectives behind our research were to identify the most appropriate concentration of zinc for wheat crops and identify the potential of zinc to improve the productivity, yield, and nutritional quality of grain and increase the zinc contents of bread wheat under a semi-arid climate.

2. Materials and Methods

2.1. Experimental Plot

The current studies were held (1) pot study and (2) field experiment at Agronomy Research Area, College of Agriculture, BZU Bahadur Campus Layyah, Punjab-Pakistan during winter season 2019–2020. The climatic condition of this region is semi-arid to the sub-tropical with hot and dry summer and cool winter (Figure 1). The soil was sandy loam having a pH of (8.3) with a soil organic matter of 0.58%, EC (0.59 dSm^{-1}), available phosphorus and extractable potassium of 5.1 and 53 (mg kg^{-1}), respectively.

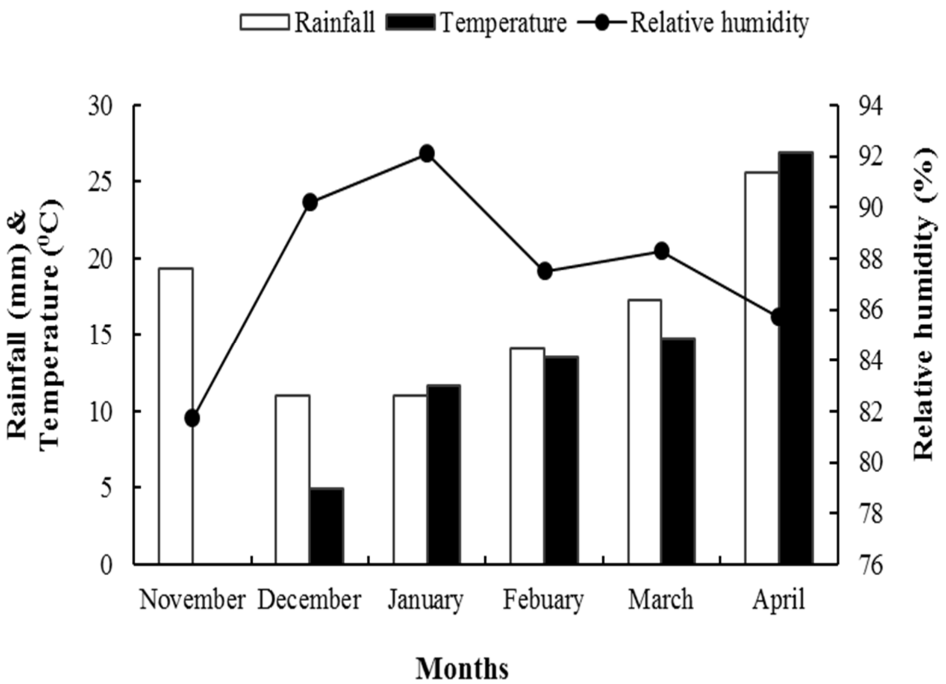

Figure 1. Meteorological data of the experimental site.

2.2. Experimental Design and Treatments

The treatment consisted of three zinc levels (control, 4%, 6%) in the form of zinc sulphate (21% Zn) applications which were applied at different growth stages (tillering, booting, and heading) on various wheat varieties (Zincol, Fakher-e-Bhakkar, Faisalabad-2008). All the treatment mixtures have been randomly allocated to experimental plots using a whole randomized design (CRD) with three replications and 27 general pots.

2.3. Collection of Materials

Wheat seeds of Zincol and Faisalabad-2008 were obtained from Ayyub Agriculture Research Institute (AARI), Faisalabad-Pakistan, while Fakher-e-Bhakkar was taken from Arid Zone Research Institute (AZRI), Bhakkar-Pakistan. The seeds that were healthy, equal in size and had good strength were selected for sowing of the crop. Zinc was obtained from the source zinc sulphate having (21% *w*/*w* Zn) and was collected from the Sigma-Aldrich Company Pvt. Islamabad, Pakistan. The soil was taken from Hafizabad Research Farm College of Agriculture, BZU, Bahadur campus Layyah, Punjab-Pakistan. The soil was dried, made porous and then mixed with farmyard manure. Earthen pots were purchased from Green Land Nursery, Layyah, Punjab-Pakistan of appropriate size (Height = 0.5 m, Diameter = 0.45 m) were filled with 15 kg of dry soil.

2.4. Crop Husbandry

In the pot study, the crop was sown on 20 November 2019 at a depth of 3 cm. Ten seeds were sown in each pot, then thinning was performed, and five plants from each pot were maintained. In the field experiment, the crop was sown on 26 November 2019 with a hand seed drill. The row to row distance was 22 cm. Four subsequent irrigations were applied during the whole crop period after seedling emergence. All other agronomic practices were carried out as recommended by the wheat crop. Recommended nitrogen, phosphorus and potassium (100, 50 and 50 kg ha^{-1}) applications were made in the form of urea, potash and DAP. Harvesting of the crop was undertaken on the 20–21 April 2020 in pot and field experiments, respectively.

2.5. Data Recording

In the pot study, tags on the plants were used to measure the various morphological traits (plant height, number of productive tillers, root length, number of spikes per plant, spike length, spikelets per spike, and grains per spike). The 100-seed weight was counted with the help of an automatic seed counter. The tagged plants were harvested from each of the pots and then sun-dried for two weeks. This dried plant was weighed on an electric balance to measure the biological and seed yield per plant. The straw yield was obtained by subtracting the grain yield from the biological yield. In the field experiment, ten plants were selected randomly from each plot to calculate the morphological traits (plant height, number of productive tillers and spikes per plant, spike length and spikelets per spike). The 1000-seed weight was counted with the help of an automatic seed counter. An area of 1 m^2 was harvested from each plot and then sun-dried for two weeks. The dried plant was weighed on an electric balance to measure the biological and seed yield. The straw yield was obtained by subtracting the grain yield from the biological yield.

Different quality traits, i.e., superoxide dismutase (μg/g protein), peroxidase (μmol/g FW), catalase (μg/g protein) and MDA (μmol/g FW) were measured spectrophotometrically. For the foliar application treatment, superoxide dismutase (μg/g protein), peroxidase (μmol/g FW), catalase (μg/g protein) and MDA (μmol/g FW) in the leaves were measured; while for the soil Zn treatment, they were measured in the roots. Fresh plant tissues were milled into small pieces in liquid nitrogen. Na_3PO_4 buffer (0.05 M, pH $\frac{1}{4}$ 7.2) was used to standardize the solutions and centrifuged at 4 °C for 15 min in superoxide dismutase (SOD) and peroxidase (POD), the activities were measured based on the protocol of Chen et al. [18].

Protein content was determined by the Kjeldahl method (Instruction manual VELP Scientific Model: HM-IV) manufactured by Bio base Industry (Jinan, China) Co., Ltd. Two grams' sample was taken, and then added a tablet of digestion mixture and 10 mL sulphuric acid. The digested sample was diluted. The proteins are 'digested' (wet oxidized) in sulfuric acid with a catalyst (mercury and selenium tablets now succeed by the much safer potassium and copper sulfate tablets). After the distillation, the sample was titrated against sodium hydroxide. Protein was determined after multiplying the correction factor with nitrogen percentage. Starch was measured by NIR instrument (instruction Manual Omeg Analyzer G). The wheat sample was taken in a hopper which used an 18 mm sample spacer for the reading of the starch content value [19]. Gluten content was determined by the glutomatic apparatus used in an ISO-17025 certified CT Lab [20]. A 10 g sample of flour was weighed and placed into the glutomatic washing chamber on top of the polyester screen. The sample was mixed and washed with a 2% salt solution for 5 min. The wet gluten was removed from the washing chamber, placed in the centrifuge holder, and centrifuged. The Zn concentration in grains was measured according to Rashid [21], who revealed that wet ashing of grains harvested at the final stage was finished. Samples were ground through a grinder and then weighed after drying in an oven at 70 °C for 24 h. Digestion was conducted on a digestion plate (Heidolph, Chicago, IL, USA model, R3003) in a di-acid ($HClO_4$:HNO_3 at 3:10 v/v ratio). Zn concentration in grains and shoots was measured by using the atomic absorption spectrophotometer (Shimadzu, UV-1201, Kyoto, Japan).

2.6. Statistical Analysis

The collected data on the parameters were statistically analyzed by the application software, "Statistix 8.1". The least significant difference (LSD) test at a 5% probability level was applied to compare the treatment means [22].

3. Results

3.1. Morphological Traits

In the pot experiment, the different wheat morphological traits, i.e., plant height, spikelets per spike, spike length, grain per spike and root length, were significantly ($p < 0.05$) affected by the wheat varieties (Tables 1 and 2) but under the field investigation these remained non-significant (Table 1). Among the wheat varieties, maximum plant height, spikelets per spike, grain per spike, root length and spike length were recorded in the Fakher-e-Bhakkar as compared to other varieties. Improvement in morphological traits was observed with increasing the Zn level. The highest level of foliar applied zinc (6%) produced the maximum spikelets per spike, grain per spike and spike length, followed by control treatment as compared to the Zn levels of 4%.

Table 1. Plant height, number of productive tillers, number of spikes, number of spikelet per spikes, and spike length as affected by different foliar applied zinc and wheat varieties grown under controlled environment.

Sources	Plant Height (cm)		Number of Productive Tillers		Spikelets Spike^{-1}		Spike Length (cm)	
	Pots	Field	Pots	Field	Pots	Field	Pots	Field
Wheat varieties (Var)								
Zincol	88.1A	78.7	13	10	44B	17	9.8B	9.9
Fakher-e-Bhakkar	91.5A	81.2	11	10	53A	18	12A	10.7
Faisalabad 2008	72.0B	79.7	12	9	33C	18	9.5B	10.8
LSD ($p \leq 0.05$)	3.86	ns	ns	ns	1.53	ns	1.26	ns
Zinc levels (Zn)								
Control	84.2	81.4A	12	9B	43AB	18AB	10.7	10.8A
4%	83.5	76.9B	12	10B	42B	19A	9.7	10.7AB
6%	83.8	81.2A	12	11A	44A	17A	10.8	9.9B
LSD ($p \leq 0.05$)	ns	2.08	ns	0.71	1.53	1.05	ns	0.81
Significance								
Zinc levels	ns	*	ns	*	*	*	ns	*
Varieties	*	ns	ns	ns	*	ns	*	ns
Var × Zn	ns	ns	ns	*	ns	*	ns	*

Means sharing the same case letter do not differ significantly at $p \leq 0.05$; * = significant at $p \leq 0.05$; ns = non-significant.

3.2. Yield Related Traits

Different wheat yield traits, i.e., biological/grain yield and straw yield, were significantly ($p < 0.05$) influenced by the wheat varieties and application of Zn fertilization (Table 3). Among the wheat varieties, maximum biological/grain yield and straw yield were recorded in Fakher-e-Bhakkar under pot study conditions, while in the field experiment, the maximum yield attributes were in Fasialabd-2008 as compared to other varieties. Improvement was observed with increasing the Zn level. The highest level of foliar applied zinc (6%) produced maximum biological/grain yield and straw yield compared to the control plot.

Table 2. Grain per spike and root length as affected by different foliar applied zinc and wheat varieties grown under controlled environment.

Sources	Grains Spike^{-1}	Root Length (cm)
Zincol	39B	13.7B
Fakher-e-Bhakkar	55A	15.9A
Faisalabad 2008	31B	12.8B
LSD ($p \leq 0.05$)	7.67	1.27
Control	45	14.5A
4%	40	13.1B
6%	40	14.7A
LSD ($p \leq 0.05$)	ns	1.27
Significance		
Zinc levels (Zn)	ns	*
Varieties	*	*
Var × Zn	ns	ns

Means sharing the same case letter do not differ significantly at $p \leq 0.05$; * = significant at $p \leq 0.05$; ns = non-significant.

Table 3. Yield and yield related traits as affected by different foliar applied zinc and wheat varieties grown under controlled environment.

Sources	1000-Seed Weight (g)		Biological Yield		Grain Yield		Straw Yield	
	Pots	Field	Pots (g plant^{-1})	Field (Kg ha^{-1})	Pots (g plant^{-1})	Field (Kg ha^{-1})	Pots (g plant^{-1})	Field (Kg ha^{-1})
Wheat Varieties (var)								
Zincol	50.9	74.1	58.7B	5307.5AB	26.7B	1695.2A	32.9AB	3612.3B
Fakher-e-Bhakkar	70.2	79.2	78.4A	4987.1B	43.5A	1101.4C	39.4A	3885.7AB
Faisalabad 2008	70.1	77.2	47.1C	5551.0A	27.8B	1492.0B	26.7B	4058.0A
LSD ($p \leq 0.05$)	ns	ns	2.60	356.3	1.98	93.3	7.40	294.87
Zinc levels (Zn)								
Control	70.0	77.9	57.7C	4615.3B	31.9B	1416.1	33.4	3199.2B
4%	60.7	77.3	60.6B	5561.2A	31.4B	1368.0	32.6	4193.2A
6%	60.6	75.3	66.0A	5669.1A	34.7A	1505.6	33	4163.5A
LSD ($p \leq 0.05$)	ns	ns	2.60	232.8	1.98	ns	ns	220.36
Significance								
Zinc levels (Zn)	ns	ns	**	*	**	ns	ns	*
Varieties	ns	ns	**	*	**	*	*	*
Var × Zn	ns	ns	**	*	**	ns	ns	*

Means sharing the same case letter do not differ significantly at $p \leq 0.05$; * = significant at $p \leq 0.05$; ** = highly significant at $p \leq 0.05$ and $p \leq 0.01$; ns = non-significant.

3.3. Antioxidants Traits

Different antioxidants traits, i.e., superoxide dismutase SOD (µg/g protein), peroxidase POD (µmol/g FW), catalase CAT (µg/g protein), and malondialdehyde MDA (µmol/g FW) were significantly ($p < 0.05$) influenced by the varieties and application of Zn (Figure 2).

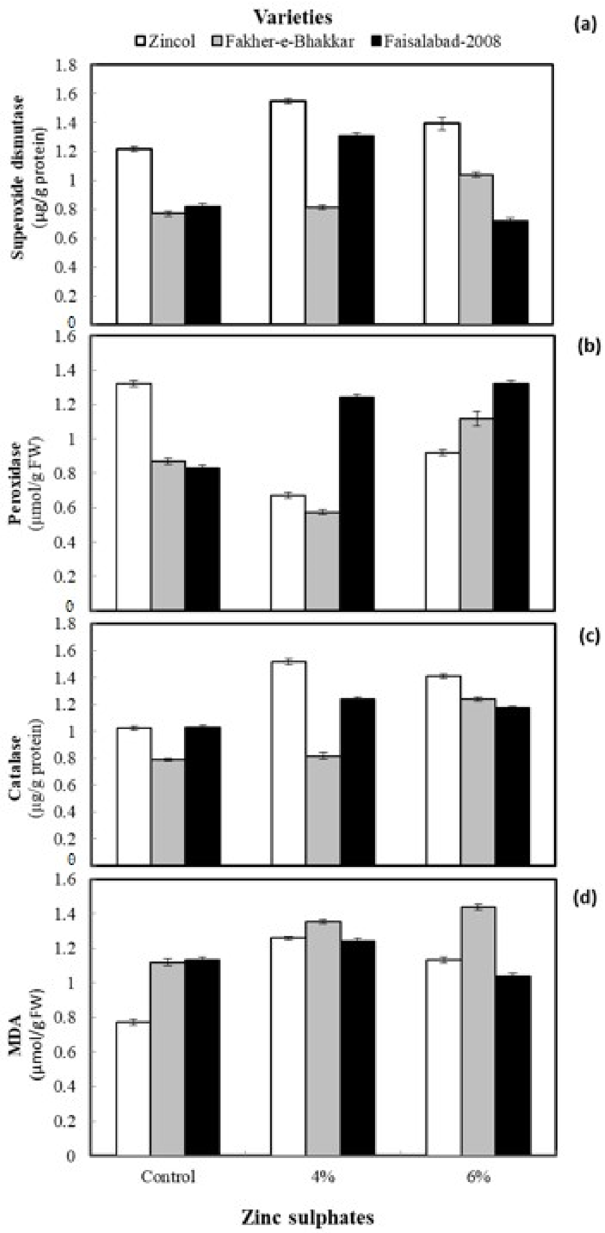

Figure 2. Interactive effect of varieties × zinc sulphates on the antioxidants of bread wheat grains grown under a semi-arid environment. (**a**)superoxide dismutase; (**b**) peroxidase; (**c**) catalase and (**d**) MDA. Error bars represent the standard error (n = 5).

Among the zinc levels, the maximum amount of superoxide dismutase (µg/g protein) was recorded in the Fakher-e-Bhakkar wheat variety as compared to other varieties. Regarding the zinc levels, the highest superoxide dismutase (µg/g protein) were produced under (4%) zinc application as compared to the control and zinc (6%). The highest peroxidase (µmol/g FW) was noted under the zincol wheat cultivar. While among zinc levels, the maximum for peroxidase (µmol/g FW) was produced under controlled conditions. Among the wheat cultivars, zincol produced maximum catalase (µg/g protein); however, under the zinc application, the highest of the catalase (µg/g protein) was recorded under (4%) foliar-applied zinc. Figure 2 shows that the highest of MDA (µmol/g FW) was produced by the wheat cultivar Fakher-e-Bhakkar as compared to other varieties. While regarding the foliar-applied zinc maximum of MDA (µmol/g FW) was recorded under highest level for foliar-applied zinc (6%).

3.4. Quality Variables

Analysis of variance shows that among the wheat cultivars, the maximum protein percentage was recorded in zincol, while regarding the zinc level when (4%) zinc was applied through foliar application, the highest protein was calculated. It is reported that the maximum starch percentage was recorded in the wheat cultivar Fakher-e-Bhakka under (4%) zinc application. Wheat gluten fulfills an essential biological role in the major grain storage protein fraction. Among the wheat cultivars, the maximum gluten percentage was noted in Faisalabad-2008 when (4%) zinc was applied (Figure 3). Results of this revealed that maximum grain zinc contents were observed in the zincol cultivar where 6% zinc sulphate was foliarly applied while minimum gain zinc contents were recorded in Faislabad-2008 where no zinc was applied (Figure 4).

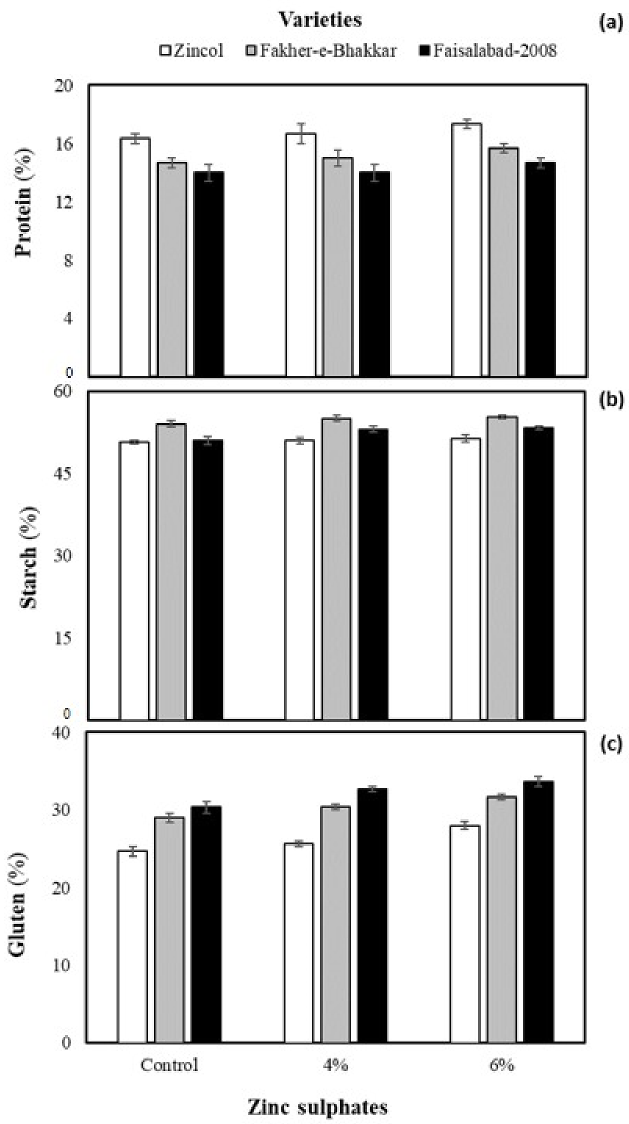

Figure 3. Interactive effect of varieties × zinc sulphates on the nutritional quality of bread wheat grains grown under a semi-arid environment. (**a**), protein; (**b**), starch; (**c**), gluten. Error bars represent the standard error (n = 5).

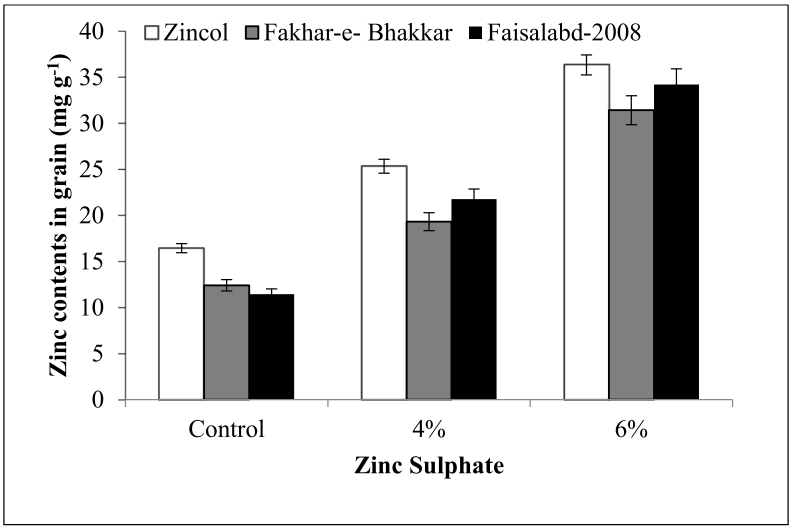

Figure 4. Interactive effect of varieties × zinc sulphates on the grain zinc contents of bread wheat grains grown under a semi-arid environment. Error bars represent the standard error (n = 5).

4. Discussion

Wheat growth is an important parameter, but the final and improved wheat yield is determined by different factors if they are not affected by any stress or negative environmental influence. So, the growth parameters of wheat might have been influenced by different zinc applications, either foliar- or soil-applied or both. Esfandiari et al. [23] reported that timing of foliar zinc application to wheat is a highly responsible factor for increasing zinc content in wheat crop, most important in the endosperm part that is the predominant grain fraction used in many countries and a large pool of Zn in vegetative tissues of plants the during grain filling stage (e.g., via foliar Zn spray) is an important practice to increase grain Zn and contribute to human nutritional health.

Wheat is reported to be a poor source of zinc, having less than 20 mg kg^{-1} in most of the cultivars, which should be more than 50 mg kg^{-1} in the dry weight of wheat grains [24]. The levels of Zn in plants usually range between 10 and 100 mg kg^{-1} of DW, and toxicity symptoms usually become visible in crop species at Zn > 300 mg kg^{-1} leaf DW. Zinc is not only responsible for wheat growth and yield components, but it is also very useful in increasing crop water-use efficiency [18]. At higher temperatures, grain development is reported to be reduced during dry matter accumulation and the duration of reproductive growth and the grain filling stage [25]. Zinc application might also be in contest with this yield-limiting stress with the increased thermo-tolerance of the photosynthetic apparatus of wheat during high temperatures during the ripening stage and maturation of wheat crop [26].

Oxidative stress appears to also have essential poisonous results on wheat [18], as antioxidative structures play critical roles in plant responses to outside stressors. To lessen oxidative strain, wheat flowers undertake antioxidant shielding mechanisms that contain enzymatic and non-enzymatic antioxidant structures [27]. From the results of this study, it was revealed that antioxidant enzyme activities such as SOD, POD, CAT and MDA were improved with zinc application. This improvement is due to Zn application that alleviated the ROS induced damage in plants. These findings corroborate with the recent results observed in different plants where the Zn lowered the levels of MDA and H_2O_2 in plants under situations of adverse conditions [28,29]. One of the main processes underlying stress to plants is increased formation of ROS but produce oxidative bursts by interfering with the antioxidant defense system [30] that promotes the MDA contents due to lipid peroxidation [31]. During the evolution, the plants have established well-organized defense mechanisms to detoxify the ROS [32]. For example, plants possess antioxidant enzymes that eliminate ROS accumulation. Our results revealed enhanced activity of CAT and SOD in the tissues of foliage under Zn treated plants, and these results clearly demonstrated that Zn treatment maximized the activities of SOD and CAT. SOD converts the O_2—to less toxic H_2O_2, and it forms the first line of defense in the antioxidant plant system; to this end, CAT scavenges the H_2O_2 [33,34]. Zn mediated the antioxidant enzyme activities enhancement in the wheat leaves under Cd stress [29]. Foliar application of Zn had a significant positive effect on wheat grain yield and its components, as well as quality of grains [23,35]. El-Habbasha et al. [35] reported a significant impact of foliar-applied Zn on wheat protein content and plays an important role in protein synthesis and protein functions.

In the present study, foliar application of zinc helps in improving many of the vegetative as well as yield components of the wheat. This could be related to the improved physiology of plants as seen in photosynthesis, enhanced nutrient uptake, auxin activity, thermo-tolerance, and water use efficiency. So, by increased zinc application, wheat grain zinc content could accumulate in high amounts. Liu et al. [36] also recommended that foliar application of zinc was more preferable as it could increase yield attributes and grain zinc content up to 80%. Ram et al. [37] reported a simultaneous increase in wheat yield and grain zinc content in wheat.

Zinc foliar application at the early milk stage of grain filling is reported to significantly increase zinc concentration in wheat grain [38]. Velu et al. [39] also emphasized that frequent application of zinc at the early milk stage (up to 10 or every third day) increased grain yield. In our experiments using pots and a field, the effect of zinc levels on wheat varieties were observed; the calculated results showed significant results for morphological as well as for yield traits such as plant height, the number of tillers, spikelets per spike, spike length, root length, biological yield, grain yield and hundred or thousand seed weight, all these parameters were calculated with maximum values in wheat variety Fakher-e-Bhakkar as compared with the other two varieties; similarly, the highest values were recorded for 6% if the zinc levels that were applied on the wheat crop compared to the control and 4% [15]. Zinc content increased considerably along with a progressive increase in seed size and weight. Foliar application of zinc at the milk and dough stage has also been reported to accumulate more zinc in grains than its application at earlier stages such as the stem elongation and booting stage [16].

So different results are found for wheat varieties and zinc application levels from pot and field experiments. This might be due to the fact that different varieties have a different genetic make-up and hence respond differently to different zinc application methods [24]. Furthermore, it is reported that zinc application methods increased the grains yield of wheat significantly.

5. Conclusions

It is concluded from the above findings that different zinc application levels have different impacts on different yield parameters of wheat in different study environments, i.e., field and pot experiments. Amongst wheat varieties, the Fakher-e-Bhakkar variety produced more plant height, number of spikes per plant, spike length, root length, biological and grain yield and nutritional quality as compared to other varieties and treatments. It is concluded that Fakher-e-Bhakkar was most beneficial at 6% zinc application for improvement in growth, yield-related traits and nutritional quality of the wheat crop. Therefore, it is recommended for achieving maximum yield and yield components of wheat under agro-climatic conditions of Layyah, Punjab-Pakistan.

Author Contributions: A.S. (Ahmad Sher) conceived the idea. B.S. conducted the experiment. A.S. (Abdul Sattar) and M.I. collected the literature review. S.U.-A., M.T.H., A.M., A.Z., J.I. and A.Q. provided technical expertise to strengthen the basic idea. B.H.E., A.E.A., A.F.G., K.A.I., A.S. (Abdul Sattar) and A.Q. helped in statistical analysis. A.S. (Ahmad Sher) and A.Q. proofread and provided intellectual guidance. All authors have read and agreed to the published version of the manuscript.

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