Enhancing wheat production and quality in alkaline soil: A study on the effectiveness of foliar and soil applied zinc

Farhat Ullah Khan [1] , **Adnan Anwar Khan** [2] , **Qu Yuanyuan** [3] , **Qi Zhang** [1] , **Muhammad Adnan** [4] , **Shah Fahad** [Corresp., 5] , **Fatima Gul** [6] , **Muhammad Ismail** [1] , **Shah Saud** [7] , **Shah Hassan** [8] , **Xu Xuexuan** [Corresp. 1]

[1] Institute of Soil and Water Conservation, Northwest A&F University, Yangling, China
[2] College of Natural Resources and Environment, Northwest A&F University, Yangling, China
[3] College of Grassland Agriculture, Northwest A&F University, Yangling, China
[4] Department of Agriculture, University of Swabi, Swabi, Pakistan
[5] Department of Agronomy, Abdul Wali Khan University, Mardan, Pakistan
[6] Department of Horticulture, University of Agriculture Peshawar, Peshawar, Pakistan
[7] College of Life Science, Linyi University, Linyi, China
[8] Department of Agricultural Extension Education and Communication, The University of Agriculture, Peshawar, Peshawar, Pakistan

Corresponding Authors: Shah Fahad, Xu Xuexuan
Email address: shah_fahad80@yahoo.com, xuxuexuan@nwsuaf.edu.cn

Cultivations of high yield varieties and unbalanced fertilization have induced micronutrient deficiency in crops especially in wheat across the globe.Zinc (Zn) deficiency is most common in alkaline calcareous soils. Therefore, this study aimed to evaluate the effect of Zinc, applied either alone or in combination with foliar or soil application, on the quality and production of wheat grown in alkaline soil. Zn was applied in the form of zinc sulfate ($ZnSo_4$), applied both to the soil and as a foliar spray during the sowing and tillering stage, respectively. Results showed that Zn fertilization to wheat irrespective of modes of its application significantly increased grain and biological yield, grain per spike and 1000 grain weight over control, however, its effect was more noticeable when applied as 7.50 kg ha^{-1} of soil Zn in combination with foliar Zn at 2.5 kg ha^{-1}. The Zn application significantly increased the grain protein content, from 9.40 % in control to a maximum 11.83 % with an application rate of 10 kg Zn ha^{-1}. Similarly, Zinc addition promoted wheat Zn, Nitrogren (N), Phosphoreus (P) and Potassium (K) uptake. Moreover, Zinc application had a significant impact on the P content in the grains. Therefore, zinc supplementation is very crucial for improving crop quality and wheat yield.

Enhancing wheat production and quality in alkaline soil: A study on the effectiveness of foliar and soil applied zinc

Farhat Ullah Khan[1a], Adnan Anwar Khan[2a], Qu Yuanyuan[3], Qi Zhang [1], Muhammad Adnan[4], Shah Fahad[5*], Fatima Gul[6], Muhammad Ismail[1], Shah Saud[7], Shah Hassan[8], Xu Xuexuan[1*]

[1] **Institute of Soil and Water Conservation, Northwest A&F University, Yangling, Shaanxi 712100, China (farhatk304@gmail.com; 1047800045@qq.com; ismailrafiq344@gmail.com)**

[2] **College of Natural Resources and Environment, Northwest A&F University, Yangling, Shaanxi 712100, China. (adnan_aup@yahoo.com)**

[3] **College of Grassland Agriculture, Northwest A&F University, Yangling, Shaanxi 712100, China (quyuanyuan@nwafu.edu.cn)**

[4] **Department of Agriculture, University of Swabi, Pakistan (**madnan@uoswabi.edu.pk**)**

[5] **Department of Agronomy, Abdul Wali Khan University Mardan, Khyber Pakhtunkhwa 23200, Pakistan (Shah_fahad80@yahoo.com)**

[6] **Department of Horticulture, University of Agriculture Peshawar, 25130, Khyber Pakhtunkhwa, Pakistan; (afnankhan123@gmail.com)**

[7] **College of Life Science, Linyi University, Linyi, Shandong 276000, China;(**saudhort@gmail.com (S.A)

8 **Department of Agricultural Extension Education and Communication, The University of Agriculture, Peshawar, 25130, Khyber Pakhtunkhwa, Pakistan;** 184108@hainanu.edu.cn

[*] **Corresponding author:** Shah Fahad (shah_fahad80@yahoo.com); Xu Xuexuan, xuxuexuan@nwsuaf.edu.cn

[a] These authors contributed equally to this work.

Abstract: Cultivations of high yield varieties and unbalanced fertilization have induced micronutrient deficiency in crops especially in wheat across the globe. Zinc (Zn) deficiency is most common in alkaline calcareous soils. Therefore, this study aimed to evaluate the effect of Zinc, applied either alone or in combination with foliar or soil application, on the quality and production of wheat grown in alkaline soil. Zn was applied in the form of zinc sulfate ($ZnSo_4$), applied both to the soil and as a foliar spray during the sowing and tillering stage, respectively. Results showed that Zn fertilization to wheat irrespective of modes of its application significantly increased grain and biological yield, grain per spike and 1000 grain weight over control, however, its effect was more noticeable when applied as 7.50 kg ha^{-1} of soil Zn in combination with foliar Zn at 2.5 kg ha^{-1}. The Zn application significantly increased the grain protein content, from 9.40 % in control to a maximum 11.83 % with an application rate of 10 kg Zn ha^{-1}. Similarly, Zinc addition promoted wheat Zn, Nitrogren (N), Phosphorous (P) and Potassium (K) uptake. Moreover, Zinc application had a significant impact on the P content in the grains. Therefore, zinc supplementation is very crucial for improving crop quality and wheat yield.

Keywords: Zinc, foliar spray, wheat yield, grain quality, alkaline soil

1. Introduction

Wheat (~~*TriticumestivumL.,*~~) belongs to ~~a~~ family Poaceae and is the key cereal crop for the vast majority of people on globe. For almost 36% of the global population (2 billion people) ~~W~~heat is the most favored staple food. Wheat accounts for nearly 55% of all carbohydrates and 20% of all food calories consumed worldwide (Azeem et al. 2019; Jat et al. 2018). Pakistan is one of the top wheat-producing countries in the world, ranking 8th and accounting for approximately 3.17% of the total global wheat production (Sher et al. 2022). Wheat's substantial production contribution makes it a crucial food grain crop for the country's economy. Wheat grain yield is affected by several biotic and abiotic factors. Among abiotic factors, one of the primary causes of low yield is nutritional imbalance and lack of essential macro and micro nutrients (Azeem et al. 2019). Though to some extent wheat yield has been improved due to ~~inclusion~~ of nitrogen and phosphorous ~~application~~ to the crop by many farmers but yet it potential yield has not been achieved (Khan et al. 2017). By 2050, the world's population is expected to reach 9.8 billion, presenting a major challenge for scientists to not only increase food production ~~exponentially~~ but also improve the nutritional quality of the food produced, especially in developing regions (Ahmad et al. 2023).

Alkaline calcareous soils in developing countries, due to declining soil fertility, have resulted in decreased crop productivity and quality (Bhatt et al. 2020). Zn deficiency is most common in alkaline calcareous soils. Micronutrients, like zinc (Zn) is neglected which is as much essential as macronutrients. The nature of most soils in Pakistan is calcareous, which can cause nutrients to become unavailable due to factors such as high pH, low organic matter, salt stress, and imbalanced application of synthetic fertilizers (Hafeez et al. 2013). These issues can collectively contribute to nutrient deficiencies in the soil. It is now an established fact that Pakistan's soil is deficient in zinc, which can cause a ~~further~~ decrease in the yield of certain cereal crops, such as wheat. ~~Therefore,~~ those who consume a diet high in cereals may experience health issues linked to ~~M~~icronutrient deficiencies (zinc), such as impaired brain and immune function, stunted growth in children, harm to physical development, weakened resistance to disease, unfavorable pregnancy outcomes in women, and increased morbidity and mortality (Cakmak 2008). In Pakistan, approximately 12 million children suffer from stunted growth, whereas zinc deficiency affects 22.1% of women and 18.6% of children under the age of five (Sher et al. 2022).

In Pakistan ~~(~~Yoshida & Tanaka 1969)~~,~~ first recognized zinc problem in soils and later studies confirmed the widespread Zn deficit in all rice-growing regions. In alkaline soil of Pakistan ~~(~~Khattak et al. 2015), observed that ~~W~~heat responded well when Zn applied @ 5 kg ha^{-1} and 15 kg ha^{-1}, increased the yield as 18% and 41% over control, respectively. They also observed that application of Zn through foliar spray and soil increased the ~~W~~heat grain protein content by 29.5%. In another study, ~~(~~Walaszek et al. 2018) reported that Zn has a strong affinity for clay adsorption and also tends to accumulate unusable Zn hydroxide in alkaline pH.. Reducing Zn deficiency in plants can be achieved through a variety of practices such as supplementary applications, diverse diets, and bio-fortification (Peter et al. 2017). Mineral Zn fertilizers are conventionally the most commonly used due to their low cost and high solubility (Roohani et al. 2013). However, fixation reactions can reduce the bioavailability of zinc in soils with low organic matter, high carbonate content, and high pH. This can lead to the adsorption ~~of~~ calcite or precipitation of $Zn(OH)_2$ or

$ZnCO_3$, making conventional fertilizers ineffective for crop zinc uptake (Recena et al. 2021; Rehman et al. 2020). Zinc foliar application is a technique used to enhance the zinc status of wheat by externally applying solid or liquid zinc fertilizers to the crop leaves at the appropriate growth stage.

There have been numerous attempts to address Zn deficiency. However, increasing zinc content in food crops using agronomic methods is an excellent strategy to address its deficit in developing world. The size of the soil's accessible Zn pools has a significant impact on zinc levels. The majority of the cereal-growing regions, there are some chemical and physical obstacles that considerably decrease access of Zn to plant roots. Ultimately the newly developed cultivars (biofortified) would not take up the adequate amount of zinc from soil and translocate it in the grain. Therefore, it necessitates have a short term strategy to enhance Zn in cereal grains. In this connection agronomic biofortification is a fast idea to above problems and presents useful and sustainable solution to the existing breeding programmers (Cakmak 2008). Keeping the above facts in view, the current study aimed to address the problem of limited Zinc availability in alkaline soils. This was achieved by evaluating the following set of objectives (i) to evaluate the effect of Zinc application, either alone or in combination with foliar or soil application, on the quality and production of wheat grown in alkaline soil (ii) to determine the optimal rate of Zinc application for maximum wheat yield and quality (iii) to investigate the effect of Zinc application on the protein content of wheat grains in alkaline soil.

2. MATERIALS AND MATHODS

2.1 Experimental Site

In the alkaline soil of the Kohat region (72°N, 33°50'E and 72°30'N, 33°25'E), a field experiment was carried out to assess the impact of soil and foliar applied zinc on the growth, yield, and quality of wheat crop during Rabi 2020-2021. The study site has a humid and subtropical climate with a yearly average rainfall of 638 mm. During the summer months of May to September, the mean maximum and minimum temperatures are 39 and 25 °C, respectively. According to the International Union of Soil Sciences (IUSS) working Group WRB, the pH and EC in the soil were 7.6 and 0.32 dSm^{-1}. The soil texture was clay loam with 22, 38, 40% silt, sand, clay respectively. Furthermore, soil organic matter (0.69%), AB-DTPA extractable P (1.5 mg kg^{-1}), Potassium (55.00 $mgkg^{-1}$), and Zn was (0.58 $mgkg^{-1}$).

2.2 Experimental Procedure

The Randomized Complete Block Design (RCBD) was used in this study, with three replications. The area of each plot was 9 m^2 (3 m x 3), and there were a total of 33 plots. Wheat was sown at 120 kg ha^{-1} on 22nd November, 2020, and harvested on 5th May, 2021. There were 11treatments including; control, soil Zn 5 kg ha^{-1}+0 kg ha^{-1} foliar Zn (T1), soil Zn 3.75+1.25foliar Zn (T2), Soil Zn 2.5+foliar Zn 2.5 (T3), Soil Zn 1.25+foliar Zn3.75 (T4), Soil Zn 0+foliar Zn 5 (T5), Soil Zn 10+foiliar Zn 0 (T6), Soil Zn 7.25+foliar Zn2.50 (T7), Soil Zn 5.00+foliar Zn 5.00 (T8), Soil Zn 2.50+foliar Zn 7.50 (T9) and Soil Zn 0+foliar Zn 10 (T10). Soil application of Zn was done at the time of sowing and foliar spray was done at tillering stage. Each treatment received a base dose of N P K @ 120:90:60 that were applied as Urea, DAP, and SOP, respectively, at the time of sowing.

2.3 Agronomic observation

For data concerning tillers per plant, 5 plants were randomly taken from each treatment, counted after that averaged. 1000 grains were collected randomly from each treatment via grain counter machine and weighed. For grain and Biological yield central four rows of each treatment were threshed by mini wheat thresher, and then weighed to convert into kg ha^{-1}.

2.4 Soil and plant analysis

Appropriate techniques were used to determine the pH of the soil by (McLean 1983) and electrical conductivity (EC) by (Rhoades 1996). The (Bouyoucos 1962) and (Nelson & Sommers 1996) methods were used to determine the texture and organic matter content, respectively. The AB-DTPA extractable P, K and Zn were determined by the method of (Soltanpour & Schwab 1977). Total Nitrogen in samples was determined by Kjeldhal method (Bremner 1996) . 0.5 g of grain flour samples was obtained in triplicate to determine the protein content of the grain. After adding 0.25 mL of protein buffer, centrifuging the mixture for 15 minutes at 13 K rpm after combining it with a vortex mixer. By using an ultraviolet spectrophotometer, for spectroscopic analysis, the supernatant was taken out at E-280 nm in mg/mL, or nearly 1 optical density (OD). % protein was estimated as follows by multiplying the absorbance by the dilution factor:

$$\%\, protein = absorbance\, reading \times dilution \times \frac{100}{1000}$$

Applying sodium dodecyl sulphate-polyacrylamide gel electrophoresis, the impact of Zn on the protein composition of wheat grain was investigated (Sironi et al. 2005).

2.5 Statistical Analysis

Using SPSS version 20.0 software, the data were statistically examined by variance analysis (ANOVA) and compared using the Duncan test ($P < 0.05$).

3. RESULT

3.1 Agronomic attributes

The results indicate that certain rates of zinc fertilizer application, whether applied as soil or foliar, can have a positive impact on both the biological and grain yield of wheat (Table 1). Regardless of the method, Zn application had a considerable positive impact on grain yield. The plots with the maximum grain yield (3639 kg ha^{-1}) received soil + foliar Zn at rates of 7.50 kg ha^{-1} and 2.50 kg ha^{-1}, respectively; followed by plots received soil Zn 5 kg ha^{-1} + 5 kg ha^{-1} foliar Zn with an average yield (3608 kg ha^{-1}). The lowest grain yield (2712 kg ha^{-1}) was found in the control.The yield was higher in plots that received zinc both as soil and foliar spray. Similarly, the highest dry matter in wheat was found in the plots whereZn was given as soil and foliar @ 7.50 kgha^{-1} and 2.50 kg ha^{-1}, respectively, which produced 8802 kg ha^{-1} biological yield,followed by plots that received 5 kg ha^{-1} of zinc fertilizers in both soil and foliar, which had an average dry matter of 7994 kg ha^{-1}.The lowest biological yield was found in under control plot.

The outcome of our experiment predicts that tiller per plant were statistically significant (Table 1). The maximum number of tillers (7.56) were found in the plots received Zn fertilizer at the rate 10 kg Zn ha^{-1} as foliar, followed by the plot which were administered with zinc through soil + foliar (5:5 kg ha^{-1} and 2.5:7.5 kg ha^{-1}) respectively, producing 7.50 and 7.45 tiller per plant. Most of the treatments were significantly different from the control. The numbers of tiller per plant were more in plots where foliar ratio of Zn was kept higher than soil.

The results showed that Zn addition to wheat crop had increased 1000 grain weight but not significantly with exception of T_1 and T_2. Data on 1000 grain weight ranges from 40.36 -48.37g. The maximum 1000 grain weight was recorded in T_8 (48.373g) and T_7 (47.470 g) followed by T_6 (47.380 g) while the minimum (40.357 g) was recorded in T_1 (control).

3.2 Grain Zn, P and K nutrition

The findings regarding grain Zn, K and P as affected by Zinc addition at various levels through soil and foliar are presented in Table 2.These results indicated that zinc application synergies P up boot stage and translocate parallel to each other but after depletion they hinder each other translocation. The lowest concentration (0.12%) of grain's P was found in the control. Maximum Zn concentration (0.51%) was recorded in control plot followed by (0.49%) in soil application Zn @ 5 kg ha^{-1}. The minimum Zn in Wheat grain was recorded in plots receiving Zn both as soil and foliar application. Zn level has a considerable impact on wheat grains' potassium content. Potassium concentration in grain increased 6.24% over the control with foliar Zn spray @ 5 kg ha^{-1}. The plot that received Zn as soil @ 10 kg ha^{-1} had the minimum Wheat grain's K content.

3.3 Grain protein content

Zinc fertilization, whether applied as foliar spray or to the soil, significantly increased the protein content of the wheat grains (Table 3). There are significant differences among the means of some treatments while some have shown simple increase over control. From above T_6 all are significantly different from rest of the treatments. These treatments are at par with each other statistically showing 14.54% to 25.88 % increase over control. As a whole all the 2-10 treatments showed increase in protein content from 2.48% to 25.88% over control. The data also showed that soil Zn increasing level in combination with decreasing level of foliar amount enhanced protein % in grain gradually.

3.4 Correlation analysis

Zn in grain was negatively correlated with the others (Table 4). Phosphorous in grain was positively correlated with the others except potassium in grain, and the positive correlation between phosphorous in grain and grain weight was not significant. The protein content of Wheat grains and grain's potassium were significantly linked. Grain weight was positively correlated with till per plant, grain yield, biological yield. There are positive correlations among protein content, biological yield, grain yield and tiller per plant, except protein content and tiller per plant.

4. Discussion

4.1 Wheat ~~Y~~yield and yield components

Zn was applied as soil and foliar @ 7.50 kg ha^{-1} and 2.50 kg ha^{-1}, respectively, which greatly improved wheat grain production. These results highlight the significance of zinc availability in reducing the wheat yield gap (the difference amid realized and expected production). According to ~~(~~Khattak et al. 2006), in alkaline soils of Pakistan, adding Zn at a rate of 5 kg ha^{-1} or twice as a foliar spray increased maize grain yield by 29 % and 14%, respectively. With soil + foliar treated plots, better photosynthetic activity and biomass accumulation resulted in better yields, this could be explained by a sufficient Zn supply up until harvesting and an increase in several enzymatic activity, which in turn led to greater yields (Hussain & Yasin 2004; Paramesh et al. 2014). Zn increases photosynthesis, carbohydrate transformation, and seed development, according to the study of ~~(~~Alloway 2008). Our findings are consistent with results of ~~(~~Imran & Rehim 2017), who revealed that in comparison to soil application alone, combine application of soil + foliar would be more advantageous for enhancing grain yield and plant development. Other authors have also ~~been~~ shown benefits of using Zn fertilization to wheat yield and yield components (Cakmak et al. 2010; Nawaz et al. 2015).

A significantly greater ~~production of~~ biological yield was observed when both foliar and soil Zinc supply were used in combination. It might be because of the wheat crop's successful response to foliar Zn spraying. The fact that zinc is known to crucial for different enzymes as a metal component or as a functional, structural, or regulatory co-factor may be supportive of the increase in biological output caused by zinc nutrition (Singh et al. 2018), essential ingredient in the production and breakdown of proteins, nucleic acids, and carbohydrates (Cakmak 2008), as a result, it plays a crucial role in biomass synthesis as a co-factor of auxins and lipids (Chattha et al. 2017; Hassan et al. 2019). Furthermore, ~~(~~Mosanna & Behrozyar 2015) shown that soil Zn with foliar treatment had greater pigment content and biological outcome production of maize crop (75 and 54 %, respectively). According to a prior study, maintaining protein synthesis in wheat meristems necessitates a high Zn concentration (Mat Hassan et al. 2012). Our results are supported by ~~(~~Paramesh et al. 2020), who recorded highest wheat straw yield (6.92 t ha^{-1}) by both soil Zn application + foliar spray.

The numbers of tiller plant were more in plots where foliar ratio of Zn was kept higher than soil. Our findings are in accordance with the result of ~~(~~Soleimani 2006), they reported that foliar zinc spray improved the number of plant tillers.. Zn is thought to influence carbohydrate metabolism through photosynthesis and sugar conversions, and Zn deficiency can cause net photosynthesis to drop by 50–70%, causing drastic changes in yield components (Alloway 2004). The current experiment's findings are also corroborated by ~~(~~Gul et al. 2011; Ramzan et al. 2020), they came up with the conclusion that Zn treatment raised the proportion of productive tillers; this could be due to additional nutrients being available to growing tillers. Our results showed that Zn addition to wheat crop had increased 1000 grain weight but not significantly with exception of the treatment (Soil Zn7.50+Foliar Zn2.50). Zinc is required for growth hormone synthesis, starch production, and maturation, all of which contribute to seed weight gain (Sarkar et al. 2007). Zinc external fertilization increased 1000-grain weight, possibly reason could be zinc's strong mobility within the plant from leaves to stems, roots, and maturing grains (Rengel 2001). Another study found that when zinc is applied to wheat, the weight of 1000 grains increases substantially (Torun et al. 2001).

4.2 Plant nutrient concentration in wheat grain

Data regarding grain Zn, K and P as affected by Zinc addition at various levels through soil and foliar indicated that grain P decreased while Zn concentration increased with Zn fertilizer either soil or foliar application, this might be caused by the negative interaction between Zn and P. These results also indicates that zinc is friendly with P up to boot stage and translocate parallel to each other but after depletion there they hinder each other translocation to given part if the one increases the other decreases and vice versa. (Ryan et al. 2008) mentioned that because of the dilution effect, increasing P fertilizer application causes Zn deficiency in plants. Almost identical results in alkaline soils were achieved by (Khattak et al. 2006), who shown that P retention in leaf and grain increased by zinc increasing level, yet it behaved differently in parts of plant. It showed much concentration in grain with zinc increasing level by either method of its application. After attaining maximum value of phosphorous with 10 kg ha^{-1} addition one foliar application of zinc presented higher concentration of P in leaves while two foliar application indicated a decrease in this content (-13.5%). They attributed these phenomena to Zn and P antagonistic effect on each other. Our results are consistent with those of (Paramesh et al. 2014), who observed soil + foliar Zn spraying produced the maximum P concentration in wheat grain. Plants absorbed more P from the soil after receiving zinc as a foliar spray (Bukvić et al. 2003).

Zn levels have a significant on the K content of wheat grains. It was evident that potassium concentration in grain increased considerably over the control with soil Zn + foliar spray. The increase in K content of wheat grain with soil Zn + foliar spray might be due to zinc synergistic relationship with potassium (Razzaq et al. 2013). Zn application through foliar spray have been shown to improve the macro-nutrient components of plants (Sayed et al. 2004). (Szakal 1989) observed that Zn treatment in maize grain increased the Mg and K content.

4.3 Grain protein content

Wheat grain's protein content has increased as a result of soil or foliar applications of zinc fertilizer. Zn was one of the main nutrient in improving the protein and amino acid content of grains, according to the current research findings. Zinc is a stimulating component that enhances indoleacetic acid synthesis, increasing the protein and amino acids content in grains. In another study conducted in alkaline soils, (Khattak et al. 2015) found that Zn treatment increased grain protein content, with the most effective treatment beingZn at 5 kg ha^{-1} applied to the soil, with one foliar spray. The findings of this study were likewise consistent with those of (Paramesh et al. 2014), who shown that application of Zn 25 kg ha^{-1} into the soil resulted in an 11.8 % increase in grain's protein compared to the control. Zn helps to increase wheat β-Carotene content, which improves protein and other quality characteristics (Kharub & Gupta 2003). (Ozturk et al. 2006) shown that during the seed formation, maximum zinc uptake and protein synthesis happen at the same time. According to (Barunawati et al. 2013), Zn increased dry matter building and N uptake in the soil and on the leaves application, which in turn raised the protein content of grains. Zn shortage is linked to N metabolism; when Zn absorption in plants is reduced, the amount of protein in the plant reduces dramatically. A thinkable reason for improvement in protein content is that zinc shortage impairs both RNA and ribosome production and structural integrity, resulting in increased RNase activity. As a result, protein synthesis necessitates appropriate Zn supplementation (Patel et al. 2007). The zinc nutrition could change the protein composition of wheat grain (Peck et al. 2008). Wheat grain's protein and grain zinc concentration were found to be positively correlated (Gao et al. 2012).

5. CONCLUSIONS.

The application of Zinc, whether through foliar spray or soil application, was found to benefit both the yield and quality of Wheat. The treatment effect was more noticeable when applied as 7.50 kg ha^{-1} of soil Zn in combination with foliar Zn at 2.5 kg ha^{-1}. Potassium and phosphorous grain concentrations were enhanced through Zn addition to the crop, regardless of application method. The levels of Zn were increased by increasing the Zn application until the boot stage, but its translocation to the seed part appeared to be weaker. As a result, the retention of Zn by the grain did not increase, and it may have even slightly decreased, perhaps due to an antagonistic effect. That's why, Zn application is recommended in order to increase wheat grain production and quality. Further research could be conducted to optimize the timing, dosage, and method of Zinc application to crops in order to maximize its efficiency and minimize any potential negative effects. Additionally, the use of Zinc in agricultural practices could be expanded to increase the productivity and quality of other crops beyond wheat, particularly in regions with Zinc-deficient soils.

AUTHOR CONTRIBUTIONS

Farhat Ullah Khan and Adnan Anwar Khan conducted the research**; Qu Yuanyuan and Qi Zhang** performed lab analysis and provided the resources; **Muhammad Adnan, Shah Saud, Shah Hassan and Shah Fahad** reviewed and edited the manuscript; **Fatima Gul and Muhammad Ismail** analyzed the data. **Xu Xuexuan** supervised the experiment.

CONFLICT OF INTEREST

This article's publication does not include any conflicts of interest.

REFRENCES

Ahmad W, Zou Z, Awais M, Munsif F, Khan A, Nepal J, Ahmad M, Akbar S, Ahmad I, Khan MS, Qamar Z, and Khan H. 2023. Seed-Primed and Foliar Oxozinc Nanofiber Application Increased Wheat Production and Zn Biofortification in Calcareous-Alkaline Soil. *Agronomy* 13:400.

Alloway B. 2004. Zinc in soils and crop nutrition, vol. 130. *International Zinc Association, Brussels, Belgium*.

Alloway BJ. 2008. Zinc in soils and crop nutrition. https://doi.org/10.1094/CCHEM-87-1-0010

Azeem I, Khan AA, Bibi H, Azeem K, Naz F, . I, Khan K, Shah SAA, and Shah K. 2019. 22. Nitrogen sources and Bioaab inclined yield and nutrients uptake specifically in Triticum aestivum. *Pure and Applied Biology (PAB)*:2311-2325V 2318. http://dx.doi.org/10.19045/bspab.2019.80178

Barunawati N, Hettwer Giehl RF, Bauer B, and Von Wirén N. 2013. The influence of inorganic nitrogen fertilizer forms on micronutrient retranslocation and accumulation in grains of winter wheat. *Frontiers in Plant Science* 4:320. https://doi.org/10.3389/fpls.2013.00320

Bhatt R, Hossain A, and Sharma P. 2020. Zinc biofortification as an innovative technology to alleviate the zinc deficiency in human health: a review. *Open Agriculture* 5:176-187. doi:10.1515/opag-2020-0018

Bouyoucos GJ. 1962. Hydrometer method improved for making particle size analyses of soils 1. *Agronomy journal* 54:464-465. https://doi.org/10.2134/agronj1962.00021962005400050028x

Bremner JM. 1996. Nitrogen ‐ total. *Methods of soil analysis: Part 3 Chemical methods* 5:1085-1121. https://doi.org/10.2136/sssabookser5.3.c37

Bukvić G, Antunović M, Popović S, and Rastija M. 2003. Effect of P and Zn fertilisation on biomass yield and its uptake by maize lines (Zea mays L.). *Plant soil environ* 49:505-510.

Cakmak I. 2008. Enrichment of cereal grains with zinc: agronomic or genetic biofortification? *Plant and soil* 302:1-17. https://doi.org/10.1007/s11104-007-9466-3

Cakmak I, Pfeiffer WH, and McClafferty B. 2010. Biofortification of durum wheat with zinc and iron. *Cereal chemistry* 87:10-20. https://doi.org/10.1094/CCHEM-87-1-0010

Chattha MU, Hassan MU, Khan I, Chattha MB, Mahmood A, Chattha MU, Nawaz M, Subhani MN, Kharal M, and Khan S. 2017. Biofortification of wheat cultivars to combat zinc deficiency. *Frontiers in plant science* 8:281. https://doi.org/10.3389/fpls.2017.00281

Gao X, Lukow OM, and Grant CA. 2012. Grain concentrations of protein, iron and zinc and bread making quality in spring wheat as affected by seeding date and nitrogen fertilizer management. *Journal of Geochemical Exploration* 121:36-44. https://doi.org/10.1016/j.gexplo.2012.02.005

Gul H, Said A, Saeed B, Mohammad F, and Ahmad I. 2011. Effect of foliar application of nitrogen, potassium and zinc on wheat growth. *ARPN J Agric Biol Sci* 6:56-59.

Hafeez B, Khanif Y, and Saleem M. 2013. Role of zinc in plant nutrition-a review. *American Journal of Experimental Agriculture* 3:374-391.

Hassan MU, Chattha MU, Ullah A, Khan I, Qadeer A, Aamer M, Khan AU, Nadeem F, and Khan TA. 2019. Agronomic biofortification to improve productivity and grain Zn concentration of bread wheat. *Int J Agric Biol* 21:615-620.

Hussain F, and Yasin M. 2004. Soil fertility monitoring and management in rice-wheat system. *Annual Report Islamabad, Pakistan: LRRP, National Agricultural Research Centre*:1-33.

Imran M, and Rehim A. 2017. Zinc fertilization approaches for agronomic biofortification and estimated human bioavailability of zinc in maize grain. *Archives of Agronomy and Soil Science* 63:106-116. https://doi.org/10.1080/03650340.2016.1185660

Jat ML, Stirling CM, Jat HS, Tetarwal JP, Jat RK, Singh R, Lopez-Ridaura S, and Shirsath PB. 2018. Soil processes and wheat cropping under emerging climate change scenarios in South Asia. *Advances in agronomy* 148:111-171. https://doi.org/10.1016/bs.agron.2017.11.006

Khan K, Sharif M, Azeem I, Khan AA, Ali S, Khan I, and Khan A. 2017. Phosphorus solubility from rock phosphate mixed compost with sulphur application and its effect on yield and phosphorus uptake of wheat crop. *Open Journal of Soil Science* 7:401-429. https://doi.org/10.4236/ojss.2017.712028

Kharub A, and Gupta S. 2003. Quality traits in durum and aestnum wheat genotypes as influenced by Zn application. *Indian Journal of Agricultural Research* 37:48-51.

Khattak SG, Dominy PJ, and Ahmad W. 2015. Assessment of ZN interaction with nutrient cations in alkaline soil and its effect on plant growth. *Journal of Plant Nutrition* 38:1110-1120. https://doi.org/10.1080/01904167.2015.1009093

Khattak SG, Malik A, Parveen Q, and Ibrar M. 2006. Assessing maize yield and quality as affected by Zn as soil or foliar application. *Sarhad Journal of Agriculture* 22:465.

Mat Hassan H, Marschner P, McNeill A, and Tang C. 2012. Growth, P uptake in grain legumes and changes in rhizosphere soil P pools. *Biology and Fertility of Soils* 48:151-159. 10.1007/s00374-011-0612-y

McLean E. 1983. Soil pH and lime requirement. *Methods of soil analysis: Part 2 Chemical and microbiological properties* 9:199-224.

Mosanna R, and Behrozyar EK. 2015. Effect of zinc nano-chelate foliar and soil application and different growth stages on physiological performance of maize (Zea mays L.). Biological Forum: Research Trend. p 1327.

Nawaz H, Hussain N, Yasmeen A, Arif M, Hussain M, Rehmani MIA, Chattha MB, and Ahmad A. 2015. Soil applied zinc ensures high production and net returns of divergent wheat cultivars. *J Environ Agric Sci* 2:2313-8629.

Nelson DW, and Sommers LE. 1996. Total Carbon, Organic Carbon, and Organic Matter. *Methods of Soil Analysis*, 961-1010.

Ozturk L, Yazici MA, Yucel C, Torun A, Cekic C, Bagci A, Ozkan H, Braun HJ, Sayers Z, and Cakmak I. 2006. Concentration and localization of zinc during seed development and germination in wheat. *Physiologia plantarum* 128:144-152. https://doi.org/10.1111/j.1399-3054.2006.00737.x

Paramesh V, Dhar S, Dass A, Kumar B, Kumar A, El-Ansary DO, and Elansary HO. 2020. Role of integrated nutrient management and agronomic fortification of zinc on yield, nutrient uptake and quality of wheat. *Sustainability* 12:3513. https://doi.org/10.3390/su12093513

Paramesh V, Dhar S, Vyas A, and Dass A. 2014. Studies on impact of phosphoenriched compost, chemical fertilizer and method of zinc application on yield, uptake and quality of maize (Zea mays). *Indian Journal of Agronomy* 59:613-618.

Patel K, Kumar A, and Durani S. 2007. Analysis of the structural consensus of the zinc coordination centers of metalloprotein structures. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics* 1774:1247-1253. https://doi.org/10.1016/j.bbapap.2007.07.010

Peck A, McDonald G, and Graham R. 2008. Zinc nutrition influences the protein composition of flour in bread wheat (Triticum aestivum L.). *Journal of Cereal Science* 47:266-274. https://doi.org/10.1016/j.jcs.2007.04.006

Peter JG, Ammar W, Joseph A-G, Maria H, Roman G, Edward JMJ, and Martin RB. 2017. Approaches to reduce zinc and iron deficits in food systems. *Global Food Security* 15:1-10. https://doi.org/10.1016/j.gfs.2017.03.003

Ramzan Y, Hafeez MB, Khan S, Nadeem M, Batool S, and Ahmad J. 2020. Biofortification with zinc and iron improves the grain quality and yield of wheat crop. *International Journal of Plant Production* 14:501-510. https://doi.org/10.1007/s42106-020-00100-w

Razzaq K, Khan A, Malik A, Shahid M, and Ullah S. 2013. Foliar application of zinc influences the leaf mineral status, vegetative and reproductive growth, yield and fruit quality of 'Kinnow'mandarin. *Journal of Plant Nutrition* 36:1479-1495. https://doi.org/10.1080/01904167.2013.785567

Recena R, García-López AM, and Delgado A. 2021. Zinc Uptake by Plants as Affected by Fertilization with Zn Sulfate, Phosphorus Availability, and Soil Properties. *Agronomy* 11:390.

Rehman A, Farooq M, Ullah A, Nadeem F, Im SY, Park SK, and Lee D-J. 2020. Agronomic Biofortification of Zinc in Pakistan: Status, Benefits, and Constraints. *Frontiers in Sustainable Food Systems* 4. 10.3389/fsufs.2020.591722

Rengel Z. 2001. Genotypic differences in micronutrient use efficiency in crops. *Communications in Soil Science and Plant Analysis* 32:1163-1186. https://doi.org/10.1081/CSS-100104107

Rhoades J. 1996. Salinity: Electrical conductivity and total dissolved solids. *Methods of soil analysis: Part 3 Chemical methods* 5:417-435. https://doi.org/10.2136/sssabookser5.3.c14

Roohani N, Hurrell R, Kelishadi R, and Schulin R. 2013. Zinc and its importance for human health: An integrative review. *J Res Med Sci* 18:144-157.

Ryan MH, McInerney JK, Record IR, and Angus JF. 2008. Zinc bioavailability in wheat grain in relation to phosphorus fertiliser, crop sequence and mycorrhizal fungi. *Journal of the Science of Food and Agriculture* 88:1208-1216. https://doi.org/10.1002/jsfa.3200

Sarkar D, Mandal B, and Kundu MC. 2007. Increasing use efficiency of boron fertilisers by rescheduling the time and methods of application for crops in India. *Plant and soil* 301:77-85. https://doi.org/10.1007/s11104-007-9423-1

Sayed R, Solaiman B, and Abo-El Komsan E. 2004. Effect of foliar sprays of some 3 mineral nutrients, GA3 and or bio-stimulant on yield and fruit quality of Valencia orange trees grown in sandy soil. *Egypt J Appl Sci* 19:222-238.

Sher A, Sarwar B, Sattar A, Ijaz M, Ul-Allah S, Hayat MT, Manaf A, Qayyum A, Zaheer A, Iqbal J, Askary AE, Gharib AF, Ismail KA, and Elesawy BH. 2022. Exogenous Application of Zinc Sulphate at Heading Stage of Wheat Improves the Yield and Grain Zinc Biofortification. *Agronomy* 12:734.

Singh A, Singh Ná, Afzal S, Singh T, and Hussain I. 2018. Zinc oxide nanoparticles: a review of their biological synthesis, antimicrobial activity, uptake, translocation and biotransformation in plants. *Journal of materials science* 53:185-201. https://doi.org/10.1007/s10853-017-1544-1

Sironi E, Sessa F, and Duranti M. 2005. A simple procedure of lupin seed protein fractionation for selective food applications. *European Food Research and Technology* 221:145-150. https://doi.org/10.1007/s00217-005-1151-2

Soleimani R. 2006. The effects of integrated application of micronutrient on wheat in low organic carbon conditions of alkaline soils of western Iran. 18th world congress of soil science.

Soltanpour P, and Schwab A. 1977. A new soil test for simultaneous extraction of macro‐ and micro‐ nutrients in alkaline soils. *Communications in soil science and plant analysis* 8:195-207. https://doi.org/10.1080/00103627709366714

Szakal P. 1989. Effect of zinc and copper amine complexes on the yield and quality of maize. *XIX–HUNGAROCHEM Konferencia Murtagyazas Novenyvedelem*:43-47.

Torun A, Gültekin I, Kalayci M, Yilmaz A, Eker S, and Cakmak I. 2001. Effects of zinc fertilization on grain yield and shoot concentrations of zinc, boron, and phosphorus of 25 wheat cultivars grown on a zinc-deficient and boron-toxic soil. *Journal of plant nutrition* 24:1817-1829. https://doi.org/10.1081/PLN-100107314

Walaszek M, Del Nero M, Bois P, Ribstein L, Courson O, Wanko A, and Laurent J. 2018. Sorption behavior of copper, lead and zinc by a constructed wetland treating urban stormwater. *Applied Geochemistry* 97:167-180. https://doi.org/10.1016/j.apgeochem.2018.08.019

Yoshida S, and Tanaka A. 1969. Zinc deficiency of the rice plant in calcareous soils. *Soil Science and Plant Nutrition* 15:75-80. https://doi.org/10.1080/00380768.1969.10432783

Table 1 (on next page)

Table 1. Wheat grain, biological yield, tiller per plant, and thousand grain weight as affected by different levels of soil and foliar applied Zn

Table 1. Wheat grain, biological yield, tiller per plant, and thousand grain weight as affected by different levels of soil and foliar applied Zn

Zn (kg ha^{-1})		Grain yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Tiller per plant	Thousand grain weight (g)
Soil	Foliar				
0	0	2712.67 ^{f}	6033.33^{g}	6.59 ^{d}	40.36 ^{c}
5	0	2928.00 ^{e}	6422.33 ^{f}	7.22^{abc}	43.57^{bc}
3.75	1.25	3130.00 ^{d}	6921.67 ^{e}	7.07^{abcd}	45.11^{ab}
2.5	2.5	3237.00 ^{d}	7034.67 ^{e}	6.69 ^{d}	46.08^{ab}
1.25	3.75	3252.33 ^{d}	7246.67 ^{d}	6.87^{cd}	46.68^{ab}
0	5	3378.00 ^{c}	7387.67 ^{d}	6.89^{cd}	47.38^{ab}
10	0	3454.67 ^{c}	7631.00 ^{c}	7.02^{bcd}	47.47^{ab}
7.5	2.5	3639.67 ^{a}	8002.33 ^{a}	7.44^{ab}	48.37 ^{a}
5	5	3608.67^{ab}	7994.00 ^{a}	7.50^{ab}	47.15^{ab}
2.5	7.5	3588.00^{ab}	7890.33^{ab}	7.44^{ab}	45.38^{ab}
0	10	3504.00^{bc}	7786.33^{bc}	7.56 ^{a}	47.01^{ab}

*values carrying different letter (s) in columns are significantly different at $P \leq 0.05$

Table 2(on next page)

Table 2. Grain Zn, K and P as affected by different levels of Zn as soil and foliar

Table 2. Grain Zn, K and P as affected by different levels of Zn as soil and foliar

Zn (kg ha^{-1})		Zn	K	P
Soil	**Foliar**	_____ **Concentration (%)** _____		
0	0	0.51^{a}	0.50^{bcd}	0.12^{c}
5	0	0.49^{a}	0.51^{ab}	0.13^{bc}
3.75	1.25	0.45^{b}	0.49^{de}	0.13^{abc}
2.5	2.5	0.44^{b}	0.48^{ef}	0.14^{abc}
1.25	3.75	0.46^{b}	0.47^{f}	0.14^{ab}
0	5	0.44^{b}	0.52^{a}	$014^{ab.}$
10	0	0.45^{b}	0.45^{b}	0.14^{ab}
7.5	2.5	0.44^{b}	0.50^{bc}	0.14^{ab}
5	5	0.39^{c}	0.50^{bcd}	0.15^{a}
2.5	7.5	0.39^{c}	0.49^{cde}	0.15^{a}
0	10	0.39^{c}	0.50^{cde}	0.15^{a}

*values carrying different letter (s) in columns are significantly different at P≤ 0.053.3

Table 3(on next page)

Table 3. Protein in grain as affected by different levels of Zn as soil and foliar application

Table 3. Protein in grain as affected by different levels of Zn as soil and foliar application

Zn (kg ha^{-1})		Protein in Grain	Increase over control
Soil	Foliar	(Mean Value)	(%)
0	0	9.40 a	
5	0	9.63 de	2.48
3.75	1.25	9.83 cde	4.6
2.5	2.5	10.13 bcde	5.32
1.25	3.75	10.20 bcde	8.51
0	5	10.53 abcde	12.05
10	0	11.83 a	25.88
7.5	2.5	11.46 ab	21.99
5	5	11.23 abc	19.5
2.5	7.5	10.96 abcd	16.67
0	10	10.76 abcde	14.54

*values carrying different letter (s) in columns are significantly different at $P \leq 0.05$

Table 4(on next page)

Table 4. Pearson's correlation coefficients

Table 4. Pearson's correlation coefficients

	Grain Weight	Tiller Per Plant	Grain yield	Biological yield	Protein content	K in Grain	P in Grain	Zn in Grain
Grain Weight	1	0.349*	0.649**	0.645**	0.459**	-0.008	0.333	-0.474**
Tiller Per Plant		1	0.604**	0.597**	0.324	0.247	0.438*	-0.608**
Grain yield			1	0.971**	0.631**	0.039	0.678**	-0.832**
Biological yield				1	0.652**	0.068	0.739**	-0.838**
Protein content					1	0.348*	0.472**	-0.466**
K in Grain						1	-0.21	0.048
P in Grain							1	-0.685**
Zn in Grain								1

** indicate significance at 95% level.