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Comprehensive Study on Foliar Application of Zn (Fortification) on Growth, Yield and Zinc Content in Wheat

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Abstract: This experiment was conducted at Latif Agriculture Farm, Tandojam, District Hyderabad during Rabi season 2020-2021 to assess the effects of zinc applied in the form of foliar on wheat yield and growth characteristics, employing a randomized complete block design (RCBD). Zinc was sprayed in various concentrations (0, 0.10%, 0.30%, and 0.50%) on leaves at different growth stages. Results revealed that the foliar zinc application along with NPK recommended doses significantly raised the plant height (73.46 and 85.63) at 90 days and harvest time on T_4 relative to the control (59.32 and 73.24), respectively. The number of grains per spike showed a consistent rise with increasing zinc application, with T_4 and T_3 displaying significantly higher grain numbers (26.89) than the control. The thousand grain weight significantly increased and the highest was observed at T_4 compared to the control and other treatments. Moreover, grain yield significantly increased in T_4, recording the highest yield compared to the control and other treatments. Zinc concentration in grains displayed a significant rise with increasing zinc application, notably higher in T_4 than other treatments. Zinc application also increased zinc content in wheat straw, with T_4 exhibiting the highest (29.55 µg g^{-1}) zinc content compared to the control and other treatments. Overall, the findings demonstrate the positive impact of foliar application of zinc on various growth stages of wheat, emphasizing its potential in enhancing grain yield and quality. This study advocates for the implementation of foliar application of zinc strategies to fortify wheat crops, thus contributing to agricultural practices aimed at increasing productivity and nutrient enrichment.

Keywords: Zn fortification, foliar spray, zinc content, wheat genotype.

Introduction

Wheat holds significant value as a cereal crop in Pakistan and serves as a fundamental dietary staple. The country's demand for wheat continues to rise owing to population growth and comparatively low productivity per unit area (Nazeer *et al.*, 2020). On a global scale, wheat grains constitute 55% of the carbohydrates and 20% of the energy in diets (Sher *et al.*, 2022) Pakistan holds the 8th position in worldwide wheat production, contributing approximately 3.17% to the total global output. Wheat stands as the primary food grain crop, occupying a pivotal role in the country's economy (Sher *et al.*, 2022), and being a significant cereal crop, serves as the primary provider of calories, protein, and crucial micronutrients like zinc (Zn) and iron (Fe) for the majority of individuals in developing nations (Saltzman *et al.*, 2017; Velu *et al.*, 2017). Zinc, an essential micronutrient has been identified as deficient in Pakistani soil (Bhatti *et al.*, 2021). The deficiency of zinc was initially recognized in 1967 as the primary cause of "hadda disease" in rice, marking the first instance of a recognized micronutrient disorder in the country (Bhatti *et al.*, 2021; Yoshida and Tanaka, 1969). It was documented that around 70% soils of Pakistan suffer from zinc deficiency (Imtiaz *et al.*, 2010). In addition, it is also observed that 70% soils are zinc deficient where wheat is cultivated (Sher *et al.*, 2022; Maqsood *et al.*, 2009).

The acknowledged scarcity of zinc in Pakistan's soils emphasizes the crucial need for its application in crop cultivation practices (Bhatti *et al.*, 2021). This issue is prevalent in southeast region of the Asia continent-India, Pakistan, Iran, and Turkey (Cakmak, 2008). The increasing global occurrence of zinc deficiency in soils is becoming a critical issue due to its adverse effects on human health. These effects include stunted growth, delayed sexual development, cognitive impairments, weakened immune functions, and abnormalities in the central nervous system (Singh *et al.*, 2005). To alleviate zinc deficiency among impoverished individuals in developing nations, the utilization of zinc-enriched food sources is essential. Alternatively, a sustained supply of zinc-rich foods is needed to address the growing zinc deficiency among the growing population of developing countries.

This process, known as agricultural biofortification, is important for increasing zinc levels in plant-based food sources (Nestel *et al.*, 2006; Cakmak, 2008). Zinc malnutrition in humans is more frequent in the regions where cereals are eaten as staple foods (Bughio *et al.*, 2021). Conventional methods involving soil-based zinc fertilization have historically been utilized to tackle this concern. Nevertheless, contemporary studies emphasize the benefits of foliar application as a swift and effective approach in providing zinc to plants (Bouis and Saltzman, 2017; Zou *et al.*, 2012). Spraying zinc on the leaves helped the crops grow better, leading to higher yields. This happened because it involves to regulate the stomata openings in the leaves, making photosynthesis more efficient, boosting chlorophyll production, and increasing the size of the leaves (Ma *et al.*, 2017; Sultana *et al.*, 2016). Applying nutrients through the leaves (foliar application) shows promise as a smart farming technique. It allows nutrients to be directly absorbed and transported from the leaf surface to the plant's phloem, needs much smaller effective quantities in situations, where the absorption of micronutrients is disrupted due to low soil temperature and moisture levels (Rehman *et al.*, 2018). Foliar application of zinc on leaves observed and moved rapidly with the plants (6 to 12 hours) after spray (Doolette *et al.*, 2018). Increased grain yield and higher zinc levels in grains have been documented through the application of zinc. This observation aligns with prior research wherein other investigators have noted enhancements in grain yield through both zinc fertilization and zinc bio fortification (Ma *et al.*, 2017; Rehman *et al.*, 2018; Faran *et al.*, 2019; Asif *et al.*, 2019). The application of Zn increased physiological traits of wheat (Bhutto *et al.*, 2016). We hypothesized that the spray of zinc in the form of liquid will boost the growth, yield and zinc content in wheat. However, there is very limited research found on zinc biofortification. Considering the above fact, this study was conducted on spray of Zinc at different growth phases with different zinc concentrations to enhance the yield and zinc content in grain and straw.

Materials and Methods

The study was conducted at the Latif Agriculture Farm located in Tandojam, District Hyderabad during Rabi season 2020-2021. Before the current study, the field had been used for growing cotton crop. In that specific area, the average rainfall measured 260 mm, while the temperature averaged at 45°C. The field trial was structured on complete randomized block design (RCBD) to reduce spatial variability. The wheat variety TD-1 which is short duration variety, was employed in plots measuring 48 m^2, with each plot spanning approximately 4 m^2. As a precautionary measure against soil-borne diseases, fungicides were applied to treat the wheat seeds. The prescribed and recommended dose of fertilizers was practical, applying NPK at rates of 120, 60, and 40 kg per hectare. The full doses of phosphorus (P) and potassium (K) were applied as single super phosphate (SSP) and sulfate of potash (SOP), respectively. Half of nitrogen (N) was given at the time of sowing, while the remaining half of N in two equal parts at 1st irrigation after 15 days followed by 2nd with the interval of 30 days respectively. The experiment included spraying zinc on leaves using four treatments, each repeated three times. The treatments were as follows: T_1: control (0), where only distilled water was used; T_2: (0.10%); T_3: (0.30%); and T_4: (0.50%). Zinc, in the form of zinc sulfate ($ZnSO_4$), was sprayed at different growth stages: during tillering, booting, and the milky stage. Subsequent irrigations were given at intervals of 15 days. During the harvest, three plants were randomly chosen from each replication to note specific observations, comprising the plant's height, the quantity of grains in each spike, the weight of 1000 grains (g), and the grain yield (measured in kilograms per acre), zinc content in the grains, and in the wheat straw. Plant height was observed four times, and for further observations, the plants were manually cut and threshed. In the end, the yield was obtained by harvesting and threshing each individual plot with a tiny thresher. A digital balance was used to measure the grain weight of each treatment in kilograms, which was then calculated to tons per hectare.

Zinc Concentration in Grain and Straw

The process for determining Zn concentration involved several steps. Firstly, harvested grain and straw were separately dried in an oven at 68° C for 48 hours. Once dry, both the grain and straw were pulverized separately using a Grinder Machine (ANEX, Germany). The samples were then digested using a method described by Estefan *et al.*, (2013). Specifically, one gram of the oven-dried grain and straw were separately using a mixture of nitric and perchloric acids (HNO_3-$HClO_4$) in a 2:1 ratio on a hot plate. After digestion, the samples were chilled and diluted with 25 milliliters of distilled water. The produced samples were then subjected to analysis with an atomic absorption spectrometer Estefan *et al.* (2013).

Soil analysis samples of soil from depths of 0-15 cm and 15-30 cm were collected and dispatched to soil and plant analysis laboratory to assess various physical and hemical properties. Bouyoucos hydrometer method (Bouyoucos, 1962) was ascertained to analyze the soil texture. To measure soil pH and electrical conductivity, the soil was mixed with distilled water for extract at 1:2.5 ratio. The pH meter was used to analyze the soil pH and electrical conductivity of 2:1 soil water extract was determined by EC meter. The Soil Organic Matter (SOM) was calculated using the Walkley-Black method (Gelman *et al.*, 2011). The soil samples' zinc content was analyzed using the AB-DTPA method described by Estefan *et al.* (2013) and measured by the Atomic

Absorption Spectrophotometer.

Statistical Analysis

The collected data was analyzed using statistical software (SPSS 22.0) to detect variations between the variable replications and treatments. One way of Analysis (ANOVA) was conducted using Minitab 17 software from Minitab Ltd. USA. To compare the treatments, **Noubles** test was applied at a significance level of 0.05 P value (Bhatti et al., 2021).

Results and Discussion

Before wheat sowing, the physical and chemical characteristics of the soil were assessed at two depths (0–15 cm and 15–30 cm). The soil was found to be alkaline in nature with pH levels measuring 8.84 ± 0.08 and 8.64 ± 0.05 at the respective depths. The soil possessed a sandy loam texture, low organic matter content (0.14% to 0.26%) and non-saline (0.003 to 0.022 dS m^{-1}) behavior. The extractable zinc content was noted to be marginal at 1.35 µg g^{-1} at the 0-15 cm depth and low at 1.12 µg g^{-1} at the 15-30 cm depth.

Impact of Foliar Zinc Spray on Plant Height of Wheat

The results from Table 1 summarize the plant height observations of the crop. Initially, the plant height at the start of the crop's growth did not show any significant impact at the time of foliar application of zinc. However, as the crop progressed, the plant height significantly was affected by the application of spray of zinc. At 60 days, there was a notable effect, and at 90 days and during the crop's harvest, this effect was highly significant. The treatment T_4, which involved the application of recommended doses of NPK along with foliar Zn application (0.50%) at 25, 50, and 75 days after sowing, exhibited the tallest plant (73.46±1.72 and 85.63±1.30 cm) compared to the control (59.32±0.90 and 73.24±3.00 cm) at 90 days and at harvest, respectively. Similar findings were reported by Ankush *et al.* (2022) indicating that foliar application of Zn at 0.50% concentration during 30, 45, and 60 days, in conjunction with 100% recommended doses of NPK fertilizers, resulted in a maximum plant height of 97.07 cm compared to the other seven treatments. Gul *et al.* (2011) observed that the maximum height of wheat plants reached 100 cm when a spray containing N (0.50%) + K (0.50%) + Zn (0.50%) was applied twice, contrasting with the control group which measured 75 cm with no spray application. Bhutto *et al.* (2016) found that applying a 2% foliar spray resulted in the highest recorded wheat plant height of 66.1 cm. Mohsin *et al.* (2014) reported a notable increase in maize plant height ranging from 20 to 25% following foliar application of Zn. The mean of the data is four. The means denoted by distinct letters (a, b, and C) exhibit a significant difference from one another at $p<0.05$. The values pertain to the standard error ± (n = 3).

Application of Foliar Zinc on Number Grain per Spike of Wheat

The count of grains was conducted on three selected plants from randomized plots in each replication. The application of foliar zinc exhibited a highly significant effect on the number of grains per spike (Fig. 1).

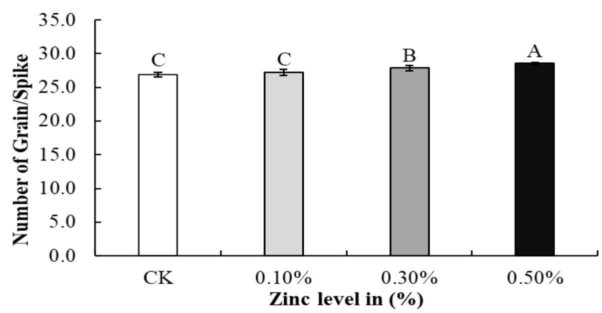

Fig. 1 The quantity of grain or spike following zinc application. The letters A, B, and C denote statistically significant distinction among the treatments according to the slightest significant distinction test at the 5% significance rank.

Table 1. Foliar application of Zn on plant height of wheat crop Plant Height (cm)

Treatments	30 Days	60 Days	90 Days	At Harvest
T1= Control (0)	16.246^{a}±1.01	32.813^{a}±1.82	59.321^{a}±0.90	73.236^{a}±3.00
T2= $ZnSO_4$ 0.10%	17.193^{a}±0.86	34.913^{ab}±1.62	62.391^{b}±2.18	77.026^{ab}±2.32
T3= $ZnSO_4$0.30%	15.876^{a}±0.77	36.555^{ab}±2.37	66.534b^{c}±2.35	81.281^{bc}±2.16
T4= $ZnSO_4$ 0.50%	16.016^{a}±0.63	39.406^{b}±1.34	73.462^{c}±1.72	85.633^{c}±1.30

As the amount of Zn applied increased, a consistent rise in the grains per spike noted. The highest (28.56±0.19) grains per spike was recorded at T_4 treated with zinc (0.50%), followed by T_3 (27.89±0.38), which received zinc (0.30%) at 25, 50, and 75 days after sowing, whereas, the grains per spike were observed non-significant at T_1 and T_2 respectively. Noreen *et al*. (2019) similarly observed that the foliar application of 4mM zinc resulted in the highest grain count per spike, reaching 73.20 grains. Likewise, Bhutto *et al.* (2016) observed an increased in grains number in one spikelet, as the concentration of zinc increases.

Foliar Zinc Impact on Thousand Grain Weight of Wheat

The data regarding the thousand grain weight showed a notable increase in thousand-grain weight due to the foliar application of zinc (Fig. 2). Specifically, the foliar Zn treatment (T_4) of 0.50% (45.55 ±1.09 g), applied at 25, 50, and 75 days after sowing, led to substantially greater grain weight compared to the control treatment (T_1) involving distilled water spray (40.16 ±1.76 g) as well as the other treatments. Similarly, T_2 and T_3 received 0.1% and 0.30% respectively observed non-significant along with control. Additionally, Bhutto *et al.* (2016) observed zinc applied at 2% in the form of spray found higher seed index value of (51 g) compared to 1.5% zinc (47.3 g). Moreover, Khattak *et al.* (2016) recorded the highest 1000 grain weight of 50.6 g in the treatment that received 15 kg $ZnSO_4$ in the soil and a 0.50% $ZnSO_4$ solution as a foliar spray. Mohsin *et al.* (2014) found notable results demonstrating rise in 1000 grain weight through the application of zinc as a foliar spray on maize.

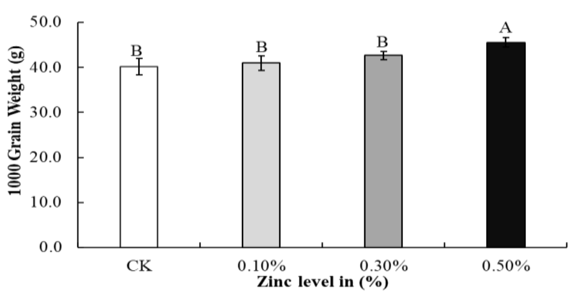

Fig. 2 Weight in thousands of grains following zinc application. Based on the least significant distinction test at the 5% significance rank, different letters (A, B, and C) denote significant distinction between the treatments.

Impact of Foliar Zinc on Yield kg/acre of Wheat

The data regarding yield of grain showed a highly significant enhancement was recorded over the control (Fig. 3). After application of foliar zinc, the grain yield of wheat significantly increased compared with the control. Whereas the highest (1979±14.39 kg/acre) grain yield was obtained in T_4, where zinc (0.50%) was applied at 25, 50, and 75 days after sowing. Similarly, as the zinc concentration decreased, grain yield was found low at 0.1% and 0.3%, and the lowest grain yield was detected at control (1705±16.67 kg/acre).

The increase in physiological traits of wheat can be linked to the role of zinc in numerous physicochemical and biological processes within the plant. Zinc plays a crucial part in enzymatic activities, photosynthesis, fruit development and maturation, stimulation of plant hormones, and the formation of starch (Bhatti *et al.*, 2021). The findings align with various studies in the field. For instance Zoz *et al.* (2012) observed that around 14% rise was noted in number of grains/yield compared to the control when applying 216 g ha^{-1} Zn through leaf spraying during tillering and booting stages. Bhutto et al. (2016) reported that the foliar application of Zn at rates of 2.0%, 1.5%, and 1.0% resulted in respective grain yields of 5540.7 kg ha^{-1}, 4840.7 kg ha^{-1}, and 4517.7 kg ha^{-1}. Ankush *et al.* (2022) found significantly higher grain yield (61.70 q/ha) in T_7 (RDF+ Zinc Spray @ 15 + 30 + 60 DAS) compared to other treatments, showing the positive impact of zinc foliar spray on grain yield. Similarly, Noreen *et al.* (2019) reported an enhanced grain yield in maize crops ranging from 20% to 22% due to zinc foliar application. Sher *et al.* (2022) indicated that the highest foliar zinc level (6%) led to the maximum grain yield. Furthermore, Nazeer *et al.* (2020) highlighted a 13.32% enhancement in total biological yield due to exclusive foliar application of zinc. These studies collectively emphasize the positive influence of zinc foliar spray on grain yield across different crops and application rates.

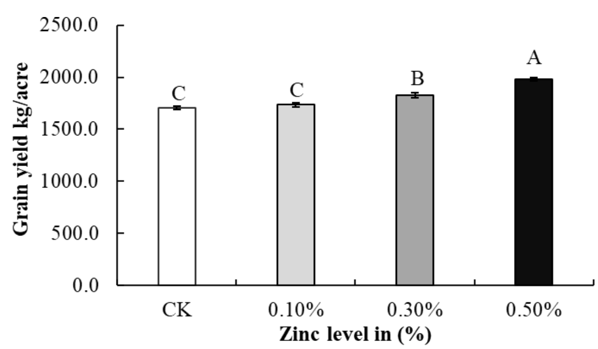

Fig. 3 Wheat grain yield following zinc application. According to the least significant distinction test at the 5% significance rank, special letters (A, B, and C) denote significant distinctions between the treatments.

Impact of Foliar Spray on Zinc Content in Grain

Foliar application of zinc significantly affected the zinc concentration in wheat grain (Fig 4). As the application of zinc increased, there was a consistent rise observed in the zinc content within the wheat grain. Comparing with control the highest (75.51 ± 0.80 µg g^{-1}) zinc content in grains were observed at T_4 treated with 0.50% zinc followed by T_3 treated with (0.30% zinc) were received the (63.83 ± 1.51 µg g^{-1}) zinc content in grain. However, the T_2 treated with (0.10% zinc) was observed (52.85 ± 0.95 µg g^{-1}) significantly higher compared to control T_1 (46.06 ± 1.09 µg g^{-1}). Comparatively, the foliar application of zinc resulted in an increase in zinc content within the wheat grains, when utilizing zinc applications from 0.10% to 0.50%. This method of zinc application appears to be notably effective, resembling zinc fortification. Numerous scientific studies have demonstrated similar outcomes. For example, El-Dahshouri *et al.* (2017) highlighted that Zn content was recorded higher after the foliar spray on wheat grains compared to the control group that received no zinc application, across two growing seasons.

Jiang *et al.* (2022) recorded rise in zinc concentration in wheat grain within a range of 12.6 mg kg^{-1} (from 46.7 to 59.3 mg kg^{-1}) due to foliar application of zinc. Rahim *et al.* (2022) achieved the highest zinc concentration in wheat grain at 66.36% with a Zn application of 7.5 mg/kg compared to the control. Sher *et al.* (2022) graphically demonstrated an increase in zinc concentration correlating with higher zinc application rates, even up to 6% Zn. Similarly, Noreen *et al.* (2019) reported zinc content increased up to 0.023 mg g^{-1} with a 4 mM zinc application. Moreover, Jiang *et al.* (2008) observed a threefold enhancement in the zinc content of grains from 18.7 to 50 mg kg^{-1} compared to the control, which represented an 83.5% increase in grain zinc content. These studies collectively reinforce the beneficial effect of foliar zinc application on enhancing zinc content within wheat grains across various experimental settings and application rates.

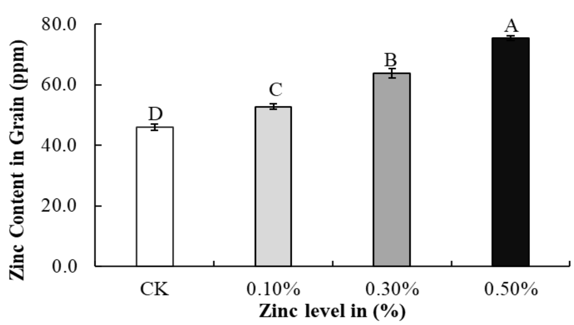

Fig. 4 Zinc content of grain following zinc application. According to the least significant distinction test at the 5% significance rank, special letters (A, B, and C) indicate significant distinctions between the treatments.

Effect of Zinc Foliar Spray on Zinc Content in Straw

The effect of applying zinc through foliar methods remained notably significant concerning the zinc concentration found in wheat straw ($P < 0.05$; Fig 5). As the application of zinc increased, a consistent rise in zinc concentration within the wheat straw was observed. Among all treatments, the highest (29.55 ± 1.94 µg g^{-1}) zinc content in wheat straw was found at T_4 treated with (0.50% zinc), followed by T_3 with 0.30% zinc (25.80 ± 0.86 µg g^{-1}) and T_2 with 0.10% zinc (21.50 ± 1.42 µg g^{-1}). These values were significantly higher compared to the plants treated solely D-Ionized water (17.68 ± 0.71 µg g^{-1}). Moreover, in contrast to the control group, the foliar application of zinc resulted in an increase in zinc concentration within the wheat straw, when applying zinc concentrations from 0.10% to 0.50%. Similar findings were reported by Maqsood *et al.* (2009), indicating an increase in zinc concentration in wheat straw as the application of zinc increased, ranging from 29.80 to 51.22 µg g^{-1}. Likewise, Jiang *et al.* (2008) noted an enhancement of zinc concentration within the shoot of rice as the zinc application level increased.

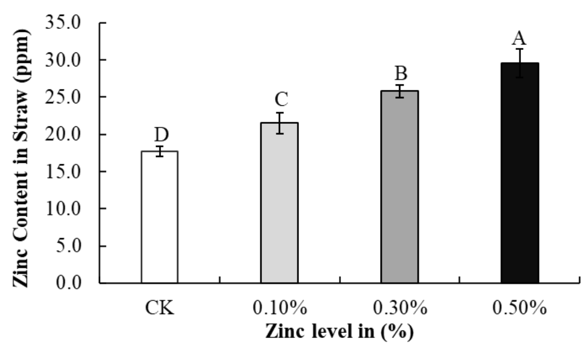

Fig. 5 Zinc content of wheat straw following zinc application. According to the least significant distinction test at the 5% significance rank, special letters (A, B, and C) indicate significant distinctions between the treatments.

Conclusion

It was concluded that foliar zinc application at 0.50% concentration significantly enhanced wheat growth and yield parameters, including plant height, grains per spike, 1000-grain weight, and grain yield. The study emphasizes the potential of this approach to address zinc deficiency in crops, promoting sustainable agriculture and contributing to improved food security. The positive impact on zinc content in both grains and straw was observed. We, recommended that the viability of foliar zinc application along with recommended NPK doses increases the yield and also completes the nutritional diet for humans. Research focusing zinc application is needed for the future with implications for global agricultural practices and human nutrition.

Conflicts of interest**:** The authors declare that there is no conflict of interest.

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