

Kashf Journal of Multidisciplinary Research Vol: 02 - Issue 2 (2025) P-ISSN: 3007-1992 E-ISSN: 3007-200X

https://kjmr.com.pk

ENHANCING MAIZE PRODUCTION THROUGH TRICHODERMA APPLICATION AND ZINC SUPPLEMENTATION

Muhammad Abdullah Khan Mansoor Department of Agronomy University of Agriculture Peshawar ,Pakistan. Shahen Shah Department of Agronomy University of Agriculture Peshawar ,Pakistan. Romaisa Amin* University of agriculture Faisalabad.

Abstract

*Corresponding author: Romaisa Amin (<u>romaisa75142@gmail.com</u>) DOI: <u>https://doi.org/10.71146/kjmr290</u>

Article Info





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Trichoderma harzianum is known for enhancing crop productivity, and zinc is an essential nutrient for plant growth. Zinc deficiency can hinder plant growth and reduce yields, which highlights the importance of adequate zinc availability for optimal crop development. Therefore, a trial was conducted during the summer of 2023 at The University of Agriculture, Peshawar, to investigate the effects of Trichoderma harzianum and varying zinc levels on maize. Trichoderma harzianum treatment with a control plot was assigned to the main plots, whereas various levels of zinc $(0, 6, 12, \text{ and } 18 \text{ kg ha}^{-1})$ were applied to the sub-plots. Findings demonstrated that delayed tasselling (56.4 days), increased ears plant⁻¹ (1.70), plant height (211.8 cm), maturity days (108.2), weight of ear (198.5 g), grain rows ear⁻¹ (12.9), grains ear⁻¹ (511.1), thousand grain weight (292.5 g), biological and grain yield (13604.2 kg ha⁻¹ and 4844.2 kg ha⁻¹), grain zinc content (28.3 mg kg⁻¹) was associated with *Trichoderma harzianum* application. Likewise, results revealed that 18 kg ha⁻¹ positively delayed tasselling (57.6 days), increased ears $plant^{-1}$ (1.86), plant height (213.5 cm), days to maturity (110.7), ear weight (204 g), grain rows ear⁻¹ (12.7), grains ear⁻¹ (524.9), thousand grain weight (312.6 g), biological and grain yield (13721 kg ha⁻¹ and 5148 kg ha⁻¹) over control plots. Nutrient content was improved with 18 kg ha⁻¹ zinc that was statistically at par with 16 kg ha⁻¹ zinc. Thus, applying Trichoderma harzianum combined with zinc at 18 kg ha⁻¹ improved the yield and yield characteristics of maize, making it a recommended practice for climatic conditions of Peshawar.

Keywords:

Biofertilizer, chemical analysis, maize, supplementation, yield enhancement

Introduction

Maize (Zea mays L.) is one of the most vital cereal crops both globally and in Pakistan. The leading countries of maize cultivation include China, Brazil, and the United States. It provides an energy value of 365 kcal per 100 grams (Ranum et al., 2014). Often called the "queen of cereals," maize holds significant importance due to its nutritional value, high grain yield, and substantial energy influence (USDA, 2018). In Pakistan, maize cultivation spans 1.016 million hectares, yielding a yearly production of 3.037 million tons. Around two-thirds of the maize-growing regions are irrigated, while the rest depend on rainwater. The production, yield, and cultivated area of maize in Pakistan have shown notable trends in recent years. In 2023, maize production reached approximately 11 million tonnes, reflecting well-above-average output due to favorable growing conditions, expanded cultivation, and improved yields. This output has positioned maize as a critical cereal crop within Pakistan's agricultural economy (Rizwanullah et al., 2023). According to the Pakistan Bureau of Statistics, maize contributes approximately 2.7% to the country's agricultural sector and 0.5% to its GDP, underscoring its significant role in Pakistan's economy. The majority of maize production is concentrated in the provinces of Khyber Pakhtunkhwa (KPK) and Punjab. However, recent years have seen the expansion of maize cultivation into Sindh and, on a smaller scale, Balochistan (Ali et al., 2020). This trend highlights the crop's growing importance across the country.

Trichoderma is a genus of fungi integral to ecosystem of soil, functioning as natural decomposers due to their capacity to absorb nutrients, rapidly colonize, and modify the rhizosphere. These fungi exhibit strong antagonism against plant pathogenic bacteria and are highly resilient in harsh environmental conditions (Harman, 2006). Several *Trichoderma* species are utilized as biofertilizers and growth stimulants because of their proven role in enhancing crop growth, productivity, and nutritional quality, particularly in maize (Colla et al., 2015). *Trichoderma harzianum* is widely distributed fungi, naturally present in most soils and organic matter, making them easily extractable. Their mechanisms include the synthesis of phytohormones and improved availability of essential minerals such as phosphates, which are critical for plant metabolism. These functions contribute to better plant growth, enhanced fruit quality, and increased crop yields (Sharma et al., 2017). Study by Raharjo et al. (2023) showed that *Trichoderma harzianum* enhances maize growth, boosts fruit quality, and increases crop yields by producing phytohormones. According to Sharma and Gothalwal, (2017) this fungus also enhances the accessibility of phosphates and other vital minerals necessary for crop metabolic processes.

Zinc (Zn) is a vital microelement that positively augments yield in crop production systems, particularly in maize (Suganya et al., 2020). Zinc plays an important role in improving plant water regulation and stomatal regulation by maintaining ionic balance within the plant system. It also participates in numerous metabolic processes. The incorporation of zinc fertilizers in maize not only increases zinc accumulation in plant tissues but also enhances overall productivity (Cakmak et al., 2008). Although required in trace amounts, zinc is indispensable for the optimal functioning of several physiological processes, including the maintenance of membrane integrity (Hafeez et al., 2013). Zinc is essential for various plant functions, such as supporting photosynthesis, aiding nitrogen assimilation, mitigating reactive oxygen species (ROS), activating carbonic anhydrase, and improving tolerance to abiotic and biotic stress (Bindraban et al., 2015). Deficiency of zinc adversely impacts these physiological processes, leading to compromised plant health, reduced growth, lower yields, and inferior crop quality, and in severe cases, crop failure (Bhatt et al., 2020). Notably, maize is particularly sensitive to zinc deficiency, underlining the necessity of adequate Zn supplementation in its cultivation. Therefore, the study aims to explore the role of Trichoderma in improving maize productivity while assessing zinc's critical contribution to physiological processes, stress tolerance, and overall plant health. This integrated approach seeks to optimize maize cultivation practices and contribute to sustainable agricultural productivity.

MATERIALS AND METHODS

A trial was conducted at the Agronomy Research Farm of The University of Agriculture, Peshawar, in the kharif season of 2023. The weather data of growing season is presented in Figure 1. The trial was arranged in a randomized complete block design (RCBD) with a split-plot arrangement and replicated three times. Each plot measured 3 m × 5 m. Maize variety 'Azam' was sown at the rate of 30 kg ha⁻¹ with the help of corn planter. Standard cultural practices, including weeding, irrigation, and hoeing, were carried out as required. The main plots were assigned treatments of *Trichoderma harzianum*, while the sub-plots were allocated varying zinc doses. *Trichoderma harzianum* was applied to the seed after sowing at 680 g ha⁻¹ by mixing it with moist soil and broadcasting it uniformly. The two factors: Factor A consisted of *Trichoderma harzianum* application, with T₁ representing plots treated with *Trichoderma harzianum* and T₀ representing untreated plots. Factor B included zinc doses at four levels: $Z_0 = 0$ kg ha⁻¹, $Z_1 = 6$ kg ha⁻¹, $Z_2 = 12$ kg ha⁻¹ and $Z_3 = 18$ kg ha⁻¹.



Figure 1. Weather data of the year 2023 during growing season.

Assessment

Data collection encompassed multiple growth, yield, and yield-related parameters. Days to tasseling were observed from planting to when 80% of the plants displayed prominent tassels, while days to maturity were recorded from planting to when 75% of the plants showed signs of maturity, including drying leaves and a color change from green to brown. Plant height (cm) was assessed from the bottom to the top of five chosen plants, and the average was calculated. Ears per plant were determined by counting ears on five representative plants per subplot. Ear weight (g) was measured by weighing five ears on a balance and averaging the results. The number of grain rows per ear was obtained by counting rows on five randomly selected ears, while grains per ear were calculated by summing the grains from the same ears and averaging. Thousand grain weight (g) was documented by weighing 1,000 grains from each subplot using weight balance.

Biological yield (kg ha⁻¹) was assessed by harvesting and drying the biomass from three central rows in each subplot and using equation (1). Grain yield (kg ha⁻¹) was calculated after threshing, cleaning, and weighing the grains from three central rows and applying equation (2).

Biological yield (kg ha⁻¹) = $\frac{\text{Biological yield in three central rows}}{\text{R-R distance (m) × R length (m) × Row number}} \times 10000 \text{m}^2$ (1)

Grain yield (kg ha⁻¹) = $\frac{\text{Grain yield in three central rows}}{\text{R}-\text{R} \text{ distance (m)} \times \text{R length (m)} \times \text{Row number}} \times 10000 \text{m}^2 (2)$

Zinc concentration (g kg⁻¹) in grains

To determine the zinc concentration in grains, 5 grams of finely grounded sample was placed in a flask, and 10 mL of concentrated sulfuric acid (H₂SO₄) was added in place of nitric acid (HNO₃), allowing it to

pg. 166

react for 24 hours. Subsequently, 3.8 mL of perchloric acid was introduced, and the mixture was heated on a plate until the generation of white fumes was observed. Once the fumes dissipated, the flask was removed from the heat source. To dilute the digest, 100 mL of distilled water was added, and the solution was filtered through Whatman-42 filter paper into a 100 mL volumetric flask. The concentration of micronutrients in the extract was then quantified using an atomic absorption spectrophotometer and the equation (3) was applied to obtained data.

Zinc content (mg kg⁻¹) = $\frac{\text{Obstained values} \times \text{volume made}}{\text{weight of sample} \times \text{volume taken}}$ (3)

Statistical analysis

The data were statistically examined using Statistix 8.1 software. Differences between treatments were evaluated using the least significant difference test at a significance level of 5%.

RESULTS

Agronomic parameters

The findings specified that Trichoderma harzianum and zinc had an optimistic influence on the agronomic parameters of maize (Table 1). However, the interactive effect of zinc and Trichoderma harzianum was not significant for all the agronomic parameters except grain and biological yield. Plots treated with *Trichoderma harzianum* recorded the highest days to tasseling (56.4), while control plots had fewer days (55.1). Among zinc treatments, the maximum days to tasseling (57.6) were observed with zinc at 18 kg ha⁻¹, which at statistical parity with 12 kg ha⁻¹ zinc (56.6). The lowest days to tasseling (53.5) were recorded in plots without zinc application (0 kg ha⁻¹). Plots treated with Trichoderma harzianum showed more ears per plant (1.70) compared to untreated plots (1.53). Similarly, the more ears per plant (1.86) was observed with 18 kg ha⁻¹ zinc, which was comparable to 12 kg ha⁻¹ of zinc application (1.70), while the lowest (1.36) was found in plots without zinc application. Plant height was significantly influenced ($p \le 0.05$) by both *Trichoderma harzianum* and zinc treatments with taller stature plants (211.8 cm) were associated with *Trichoderma harzianum* incorporation; whereas plants were short (205.8 cm) in untreated plots. Among the zinc treatments, the tallest plants (213.4 cm) were associated with zinc commenced at 18 kg ha⁻¹, followed by 12 kg ha⁻¹ zinc (209.8 cm). The shortest plants (203.8 cm) were noted in control. Similarly, maturity duration was positively influenced ($p \le 0.05$) with *Trichoderma harzianum* Treated plots required more days to maturity (108.2), while control plots matured earlier (107.1). Zinc application at 18 kg ha⁻¹ resulted in the longest maturity duration (109.5 days), which was statistically identical to 12 kg ha⁻¹ zinc application. The shortest maturity duration (103.8 days) was recorded in plots without zinc application. The results indicated that ear weight was affected by supplementing Trichoderma harzianum and zinc. The maximum ear weight (204 g) was achieved with zinc applied at 18 kg ha⁻¹, while the minimum (179 g) was recorded in the absence of zinc (0 kg ha⁻¹). Plots treated with *Trichoderma harzianum* showed greater ear weight (183.1 g) compared to those untreated (198.5 g).

Zinc levels	Days to	Ears	Plant	Days to	Ear weight	
(Zn)	Tasseling	(plant ⁻¹)	height (cm)	maturity	(g)	
0 kg ha ⁻¹	53.5 c	1.36 b	204.0 c	103.8 c	179 c	
6 kg ha ⁻¹	55.3 b	1.55 b	208.1 b	106.5 b	190 b	
12 kg ha ⁻¹	56.6 a	1.7 a	209.6 b	109.7 a	190.5 b	
18 kg ha ⁻¹	57.6 a	1.86 a	213.5 a	110.7 a	204 a	
LSD(0.05)	1.0	0.10	5.0	0.9	15.1	

Table 1. Agronomic performance of maize as influenced by various zinc levels and Trichoderma
harzianum.

Trichoderma harzianum (T)					
With (+)	56.4 a	1.70 a	211.9 a	108.2 a	198.5 a
Without (-)	55.1 b	1.53 b	205.8 b	107.1 b	183.1 b
LSD(0.05)	1.2	0.27	2.6	1.1	10.1
Zn×T	NS	NS	NS	NS	NS

Means within the same category that share the same letters do not differ significantly at the 0.05 probability level. Asterisks (*) indicate a significant effect.

The evaluation of grain rows per ear demonstrated a beneficial impact of *Trichoderma harzianum* and zinc application. The maximum grain rows per ear (12.7) were achieved with 18 kg ha⁻¹ zinc, while the minimum (12.2) was observed without zinc application (0 kg ha⁻¹) (Table 2). Similarly, plots treated with *Trichoderma harzianum* produced more grain rows per ear (12.9) that was compared to untreated plots (12.5). In terms of grains per ear, the highest count (524.9) was recorded at 18 kg ha⁻¹ zinc, followed by 12 kg ha⁻¹ zinc (506.4), which was comparable to 6 kg ha⁻¹ zinc (496.1), whereas the lowest grains per ear (481.8) were noted with no zinc application. Treatment with *Trichoderma harzianum* further enhanced grains per ear (511.1) compared to untreated plots (493.5). Thousand grain weight also varied significantly, with the highest weight (312.6 g) observed at 18 kg ha⁻¹ zinc, while the lowest weight (261.3 g) occurred without zinc application. Furthermore, plots receiving *Trichoderma harzianum* application had a greater thousand grain weight (292.5 g) than those without treatment (283.4 g).

The biological and grain yield data indicated that the applying *Trichoderma harzianum* and zinc had a positive effect, with a significant interaction observed (Table 2). The highest biological yield (13,064.2 kg ha⁻¹) was reported with the supplementation of *Trichoderma harzianum*, while the lowest (12,702 kg ha⁻¹) was seen in the control plots. Likewise, the highest biological yield (13,721 kg ha⁻¹) was achieved with 18 kg ha⁻¹ zinc, while the lowest was recorded with no zinc application (0 kg ha⁻¹). Improved grain yield (5,148 kg ha⁻¹) was observed with 18 kg ha⁻¹ zinc, which was statistically comparable to 12 kg ha⁻¹ zinc (5,003 kg ha⁻¹), followed by 6 kg ha⁻¹ zinc (4,729.6 kg ha⁻¹), and the lowest grain yield (4,456.8 kg ha⁻¹) was measured with no zinc application. The interaction effect showed that the highest biological yield was achieved with the combination of *Trichoderma harzianum* and 18 kg ha⁻¹ zinc (Figure 2a). For grain yield, higher values (4,679.2 kg ha⁻¹) in the absence of *Trichoderma harzianum* and 18 kg ha⁻¹ zinc resulted in the highest grain yield (Figure 2b).

narzianum.							
Zinc levels	Grain	Grains	Thousand	Biological	Grain yield		
(Zn)	rows	(ear ⁻¹)	grain weight	yield (kg ha ⁻	(kg ha ⁻¹)		
	(ear ⁻¹)		(g)	1)			
0 kg ha ⁻¹	12.2 b	481.8 c	261.3 d	12042.1 d	4456.8 d		
6 kg ha ⁻¹	12.4 b	496.1 b	280.4 c	12539.3 c	4729.6 c		
12 kg ha ⁻¹	12.4 b	506.4 b	297.4 b	13230 b	5003 b		
18 kg ha ⁻¹	12.7 a	524.9 a	312.6 a	13721 a	5148 a		
LSD(0.05)	0.12	14.0	9.1	357	161		

Fable 2. Agronomic per	rformance of maize	as influenced by	y various zinc	levels and T	richoderma
	1	•			

Trichoderma					
harzianum (1	Γ)				
With (+)	12.9 a	511.1 a	292.5 a	13064.2 a	4844 a
Without (-)	12.5 b	493.5 b	283.4 b	12702 b	4679 b
LSD(0.05)	0.26	11.4	8.2	257	108
Zn×T	NS	NS	NS	*	*

Means within the same category that share the same letters do not differ significantly at the 0.05 probability level. Asterisks (*) indicate a significant effect.



Figure 2. Interactive effect (Zn×T) of various zinc levels and *Trichoderma harzianum* on biological yield (a) and grain yield (b).

Nutrient analysis of maize

Zinc in grains of maize was positively influenced by zinc application rates and the incorporation of *Trichoderma harzianum* (Figure 3). However, the interactive effects of these two factors were non-significant for both grain and plant zinc and nitrogen content. The highest grain zinc content (26.1 mg kg⁻¹) was recorded at 12 kg ha⁻¹ of zinc application, compared to the control (16.2 mg kg⁻¹). Application of *Trichoderma harzianum* also significantly improved grain zinc content, with *Trichoderma harzianum* treatments recording 28.3 mg kg⁻¹ compared to 21.8 mg kg⁻¹ in the absence of the fungus.



Figure 3. Grain zinc content (mg kg⁻¹) of maize as influenced by various zinc levels and *Trichoderma harzianum*.

DISCUSSION

Zinc, a micronutrient and *Trichoderma harzianum*, a biofertilizer, have been extensively studied for their individual and combined effects on crop productivity and economic benefits. This study evaluated their influence on maize agronomic performance and quality. The application of *Trichoderma harzianum* delayed tasseling compared to untreated plots, likely due to its pathogen resistance properties, which may extend the crop's active growth period as the plant allocates resources to defense mechanisms (Harman et al., 2006). Similarly, higher zinc levels prolonged tasseling, potentially because Zn plays a major role in biofortification and enzymatic activity, which require additional time for nutrient synthesis and seed development (Yadavi et al., 2014). For ears per plant, treatments with *Trichoderma harzianum* resulted in more ears compared to untreated plants, reflecting its role in enhancing protein synthesis and stimulating beneficial enzymatic activities, such as β -1,3 glucanase and exochitinase in maize plants (Harman et al., 2006). Zinc incorporation also improved ears per plant, with the highest values observed with 18 kg ha⁻¹ of zinc application. This outcome can be attributed to Zn's involvement in main physiological processes, including protein and carbohydrate synthesis and cell elongation, which promote leaf expansion and early ear formation (Yadavi et al., 2014).

The results demonstrated that plots treated with *Trichoderma harzianum* produced taller plants compared which may be accredited to the ability of *Trichoderma harzianum* to enhance plant growth by stimulating the synthesis of chlorophyll pigments, nucleic acids and phytohormones during maize development (Akladious and Abbas, 2014). The tallest plants were recorded at the highest zinc dose (18 kg ha⁻¹). This can be explained by zinc's role in activating plant hormones and its involvement as a co-factor in various enzymatic classes, which support growth and metabolic activities (Venkatachalam et al., 2003). Moreover, zinc's contribution to weed suppression, by promoting plant height, suggests that increased Zn levels enhance plant height. The analysis also revealed that *Trichoderma harzianum* delayed maize maturity, whereas earlier maturity was observed in untreated plots. Similarly, delayed maturity occurred with 18 kg ha⁻¹ Zn application; while hastened maturity was witnessed at 0 kg ha⁻¹ Zn. Zinc deficiency may disrupt pollination and reproductive processes, potentially due to reduced synthesis of indole acetic acid, which is critical for plant development (Maqsood et al., 2009). Adequate zinc supply appears to support essential biological and chemical processes, thereby prolonging the crop's maturity period and optimizing growth.

Plots treated with *Trichoderma harzianum* exhibited higher ear weight compared to untreated plots, which aligns with findings by Contreras-Cornejo et al. (2009) that stated *Trichoderma harzianum* enhances plant growth by releasing bioactive complexes that stimulate root development and nutrient uptake. Similarly, the highest ear weight was recorded at 18 kg ha⁻¹ zinc application, while the lowest ear weight was observed in unfertilized plots. Zinc, although required in trace amounts, is essential for numerous physiological processes, including membrane integrity and enzyme activation, as noted by Hafeez et al. (2013). The supplementation of *Trichoderma harzianum* increased grain rows per ear, likely due to its role in promoting plant growth through phytohormone stimulation and enhanced nutrient availability, as described by Sharma and Gothalwal (2017). Maximum grain rows per ear were achieved with 18 kg ha⁻¹ zinc, which may be attributed to zinc's involvement in critical processes such as photosynthesis, nitrogen assimilation, chlorophyll synthesis, and stress tolerance, ultimately influencing leaf size and early ear development (Bindraban et al., 2015).

The number of grains per ear was significantly enhanced by the application of *Trichoderma harzianum* and zinc. The highest grain count per ear was seen in plots having *Trichoderma harzianum*, while a less grain count was recorded in untreated plots. This improvement is likely attributed to the stimulation of aerial plant growth, along with increased production of nucleic acids, chlorophyll, proteins, and phytohormones during maize development, as reported by Akladious and Abbas (2014). Similarly, the greatest number of grains per ear was observed with 18 kg ha⁻¹ zinc application, whereas the lowest was

recorded at 0 kg ha⁻¹ zinc. Zinc plays a vital role in enzyme activation, ion transport, and overall plant metabolism, enabling optimal grain production under adequate zinc supply. Treated plots with *Trichoderma harzianum* exhibited a higher thousand-grain weight, possibly due to enhanced root and shoot biomass production, as suggested by Okoth et al. (2011). Zinc application at 18 kg ha⁻¹ caused improvements in thousand-grain weight, likely due to improved pollen viability and grain formation. Biological yield was similarly impacted, with higher yields recorded in plots treated with *Trichoderma harzianum* that enhances rootlet development, organic matter decomposition, and nutrient supply, as noted by Gomes et al. (2016). Zinc supplementation also influenced biological yield, with the highest yield achieved at 18 kg ha⁻¹, reflecting the micronutrient's biofortification and bioremediation properties that enhance crop productivity under sufficient zinc levels.

The application of Trichoderma harzianum and zinc significantly affected grain yield. Plots treated with Trichoderma harzianum yielded higher grain outputs, while untreated plots showed lower yields. This rise can likely be attributed to the capability of Trichoderma species to boost the host plant's resilience against biotic and abiotic challenges by stimulating root growth, improving nutrient absorption, and triggering defense responses to combat oxidative impairment (Ahmad et al., 2015). Similarly, the highest grain yield was recorded with 18 kg ha⁻¹ of zinc, while the lowest yield was seen in plots with no zinc application (0 kg ha⁻¹). Zinc promotes plant growth by stimulating internode elongation, which contributes to improved development and productivity (Amanullah et al., 2011). A lack of adequate zinc availability remains a key issue in plant nutrition, leading to reduced quality and yield. Both Trichoderma harzianum and zinc also positively influenced the harvest index of maize. Comparable harvest indices were observed with and without Trichoderma harzianum, likely due to its role in stimulating plant growth through phytohormone production and enhancing the accessibility of phosphates and other vital elements for plant metabolism (Sharma and Gothalwal, 2017). The highest harvest index was noted with 18 kg ha⁻¹ of zinc, which surpassed the control plots. This improvement is attributed to zinc's role in plant nutrition, particularly in preventing deficiencies that negatively impact yield and nutrient content. Zinc deficiencies are especially detrimental to maize, highlighting the importance of proper zinc supplementation (Mattiello et al., 2015).

Zinc concentrations in grains were positively influenced by the supplementation of *Trichoderma harzianum* and zinc fertilizers. Data revealed that plots treated with *Trichoderma harzianum* exhibited higher Zn concentrations (g kg⁻¹) compared to untreated plots, likely due to *Trichoderma harzianum* enhancing root development through the creation of indole-3-acetic acid, which increases nutrient uptake (Hexon et al., 2009). Similarly, the highest Zn concentration was observed with 18 kg ha⁻¹ zinc supplementation, while the lowest was noted in untreated plots. This aligns with previous studies showing that zinc fertilizers address inherent Zn deficiencies in maize (Cakmak et al., 2008). Adequate zinc supplementation mitigates soil Zn deficiencies, which otherwise reduce the Zn content in cereal grains and degrade their nutritional value (Welch et al., 2004).

Conclusions

The results demonstrated that *Trichoderma harzianum* supplementation boosted maize yield and its associated components. Furthermore, the addition of zinc at 18 kg ha⁻¹ optimized both agronomic and quality traits. Plots treated with *Trichoderma harzianum* surpassed the control plots, showing higher grain yields and improved concentrations of zinc in grains. Based on these observations, it is recommended to apply *Trichoderma harzianum* alongside 18 kg ha⁻¹ of zinc to enhance maize productivity and its characteristics in the tested area.

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pg. 172

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