

INTEGRATED TRICHODERMA, ZINC, AND BORON APPLICATION IMPROVES NUTRIENT USE EFFICIENCY, ANTIOXIDANT METABOLISM, AND YIELD IN WHEAT

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Abstract

In the calcareous soils micronutrients deficiency is a major constraint. It can limit wheat growth and grains nutritional value. Therefore, in this study we investigated the effects of zinc, boron, and *Trichoderma* inoculation in different combinations on wheat growth, yield, soil enzymatic activities, and micronutrient concentration under field conditions. Treatments consisted of full (6 kg Zn ha⁻¹ and 2 kg B ha⁻¹) and half (3 kg Zn ha⁻¹ and 1 kg B ha⁻¹) recommended doses applied with or without *Trichoderma*. Growth attributes, yield components, soil biochemical properties, antioxidant responses, nutrient uptake, and root colonization were evaluated. The integrated application of Zn, B, and *Trichoderma* significantly improved wheat performance compared with the untreated control. Full Zn combined with *Trichoderma* inoculation increased plant height, tillers m⁻², grains spike⁻¹, 1000-grain weight, biological yield, and grain yield by 12.07%, 19.98%, 17.84%, 12.12%, 5.58%, and 9.25%, respectively. Soil biological activity was markedly enhanced, with increases of 17.77%, 17.65%, 13.30%, and 21.94% in urease, phosphatase, FDA hydrolysis, and dehydrogenase activities, respectively. Total soluble protein and free amino acid contents increased by 19.48% and 13.78%, respectively. Integrated micronutrient and microbial application also alleviated oxidative stress, reducing H₂O₂, malondialdehyde, proline, SOD, POD, CAT, and APX by 19.96%, 26.64%, 28.89%, 19.07%, 14.37%, 17.77%, and 26.30%, respectively. Furthermore, Zn concentrations increased by 47.22%, 59.27%, and 55.69% in leaves, grains, and straw, while root colonization increased by 56.05%. In conclusion, *Trichoderma* with Zn and B fertilization is an effective strategy for improving wheat productivity, soil biological health, micronutrient biofortification, and nutrient-use efficiency in micronutrient-deficient soils.

Key words: boron; soil enzymes; oxidative stress; zinc; grain yield; *Trichoderma*

Introduction

Wheat is a staple food crop globally and a key source of calories and protein, forming the backbone of food security in Pakistan. Despite its importance, wheat productivity is often limited by soil-borne pathogens and deficiencies of essential micronutrients, particularly zinc (Zn) and boron (B), which are prevalent in calcareous and low-organic-matter soils (Rehman *et al.*, 2020, Zulfiqar *et al.*, 2020, Ali *et al.*, 2022). The role of Zn is critical role in enzyme activation, protein metabolism, chlorophyll synthesis, and auxin production. These can influence vegetative growth as well as biomass accumulation. Furthermore, B is an important element for cell wall integrity, reproductive development, sugar transport, pollen viability, and formation of grain. Deficiency of such micronutrient, i.e., Zn and B leads to poor spike development, kernel abortion, and grain yield in crops (Wang *et al.*, 2015, Brdar-Jokanović, 2020, Rerkasem *et al.*, 2020, Vera-Maldonado *et al.*, 2024).

Soil-borne infections have a detrimental impact on wheat growth and yield in addition to nutritional constraints. *Trichoderma* is a helpful soil fungus that promotes root growth, increases nutrient availability, and has antagonistic activity against diseases. Soil-borne infections have a detrimental impact on wheat growth and yield in addition to nutritional constraints. *Trichoderma* is a helpful soil fungus that promotes root growth, increases nutrient availability, and has antagonistic activity against diseases (Puyam, 2016, Dutta *et al.*, 2023, Asghar *et al.*, 2024). Soil-borne infections have a detrimental impact on wheat growth and yield in addition to nutritional constraints. *Trichoderma* is a helpful soil fungus that promotes root growth, increases nutrient availability, and has antagonistic activity against diseases (Zulfiqar *et al.*, 2020, Ali *et al.*, 2022, 2023).

Due to widespread micronutrient deficiencies, low soil fertility, soil born diseases and a lack of uptake of biological inputs, wheat yields in the area are still below potential (Ali *et al.*, 2022, Nadia *et al.*, 2023, Farouk *et al.*, 2024). The impact of such combined treatments on Zn and

B uptake and wheat productivity has not been well investigated, and farmers lack precise, site-specific instructions for the integrated use of micronutrients and beneficial microorganisms. Therefore, in order to create sustainable and efficient nutrient management techniques, it is imperative to assess how *Trichoderma*, zinc, and boron interact with wheat growth, yield, and micronutrient accumulation. (Ali *et al.*, 2022, 2023, Yadav *et al.*, 2023).

In comparison to individual treatments or an untreated control, it is predicted that the combination application of *Trichoderma*, zinc, and boron will greatly enhance wheat growth, yield, and the uptake of Zn and B. In particular, zinc is expected to support vegetative growth and grain formation, boron is expected to improve reproductive development and spike fertility, and *Trichoderma* is expected to improve root development and nutrient absorption. When applied together, these elements are expected to have a synergistic effect that maximizes wheat yield and micronutrient content.

Material and Methods

Experimental Site Description: A field experiment was carried out at Jamra Farm Takht Bhai, District Mardan, Khyber Pakhtunkhwa province of Pakistan, during winter 2022-2023.

Soil sampling and physicochemical characterization: Before initiating the experiment, soil sample was taken at several locations between 0 and 20 cm deep, a composite was created for a variety of fundamental soil parameters. After that, the composite sample was taken to the lab to be further processed and evaluated according to the below-mentioned standard procedures for soil analysis. The soil texture was determined following particle size analysis hydro meter method (Bouyoucos, 1962). The assessment of fully soluble salts in the soil involved measuring soil electrical conductivity (EC) in a 1:5 (soil: water) mixture after 30 minutes of thorough mixing (Rhoades, 2018). Soil pH was assessed by preparing a 1:5 suspension of soil in water. After thorough mixing for 30 minutes, the pH was measured using a pH meter equipped with German Type B-124 glass and calomel electrodes (Page *et al.*, 1982). For analysis of soil organic matter, one gram of the soil sample was treated with 1N $K_2Cr_2O_7$ and then mixed with 20 ml concentrated H_2SO_4 . To achieve acid dilution, 200 ml of distilled water was added, along with 2-3 drops of orthophenothroline indicator. The resulting solution was titrated against 0.25N $FeSO_4$ until a light maroon color appeared at the endpoint (Nelson & Sommers, 1996). AB-DTPA extraction method was followed for analysis of soil zinc concentration (Hanlon, 1998). However, colorimetric methods with azomethine-H was used for soil boron analysis (Gaines & Mitchell, 1979). The soil characteristics were pHs = 8.54, EC_e = 4.26 dS/m, Organic matter = 0.41%, Zn = 0.18 $\mu\text{g/g}$ and B = 0.15 $\mu\text{g/g}$.

Experimental design and treatments: The experiment was conducted in a randomized complete block design (RCBD) with a split-plot arrangement and three replications. Each block was divided into two main plots, to which *Trichoderma* treatments (with and without

Trichoderma inoculation) were randomly assigned. Each main plot was further divided into five subplots receiving the micronutrient treatments: control, Zn at 3 kg ha^{-1} , Zn at 6 kg ha^{-1} , B at 0.5 kg ha^{-1} , and B at 1.0 kg ha^{-1} . Thus, each block contained 10 subplots, resulting in a total of 30 experimental units. Each subplot measured 3 $\times$ 3 m (9 m^2). Micronutrient treatments were randomly assigned to the subplots within each main plot.

***Trichoderma* inoculation:** One g of the formulation was suspended in 9 mL of sterile distilled water and serially diluted under aseptic conditions. Appropriate dilutions were spread on potato dextrose agar (PDA) plates and incubated at $28 \pm 2^\circ\text{C}$ for 3–5 days. The viable population was quantified as colony-forming units per gram (CFU g^{-1}) after colonies exhibiting typical *Trichoderma* growth were enumerated. The inoculum used for field application contained approximately 1×10^8 CFU g^{-1} and was applied at a rate of 1.70 kg ha^{-1} to the designated treatment plots. Before wheat was sown, the inoculum was applied consistently across the soil's surface and then combined with the top 5–10 cm of soil to guaranty even dispersion.

Zinc and boron application: Zinc and boron treatments were applied on an elemental nutrient basis. Zinc was supplied as zinc sulfate, whereas boron was supplied as boric acid. The Zn treatments provided 3 and 6 kg Zn ha^{-1} , while the B treatments supplied 0.5 and 1.0 kg B ha^{-1} . The required quantity of each fertilizer was calculated according to its elemental Zn or B content. The micronutrient treatments were applied to their respective subplots before sowing by broadcasting and were distributed uniformly within each subplot. The control plots received no additional Zn or B.

Wheat variety and seed sowing: The wheat variety Abasin-2021 was used as the test crop owing to its adaptability to the local agro-climatic conditions and high yield potential. The experiment was conducted in plots measuring 3 m $\times$ 3 m (9 m^2) and arranged according to a randomized complete block design with a split-plot arrangement. Wheat was sown manually in rows on 26 November 2022 at a seed rate of 125 kg ha^{-1} . Rows were maintained at a spacing of 25 cm, while plants within rows were spaced approximately 8–10 cm apart after establishment.

Fertilizer application: Nitrogen (N), phosphorus (P), and potassium (K) fertilizers were applied at the recommended rates of 120, 90, and 60 kg ha^{-1} , respectively. Phosphorus was supplied through diammonium phosphate (DAP; 18% N and 46% P_2O_5), potassium through sulfate of potash (SOP; 50% K_2O), and the remaining nitrogen through urea (46% N). To supply 90 $\text{kg P}_2\text{O}_5 \text{ ha}^{-1}$, 195.65 kg DAP ha^{-1} was applied, which also contributed 35.22 kg N ha^{-1} . This N contribution was deducted from the total N requirement, leaving 84.78 kg N ha^{-1} to be supplied through 184.31 kg urea ha^{-1} . Potassium was supplied through 120 kg SOP ha^{-1} to provide 60 $\text{kg K}_2\text{O ha}^{-1}$. Nitrogen was supplied in three equal portions of 40 kg N ha^{-1} . At sowing, the N supplied by DAP was supplemented with 10.40 kg urea ha^{-1} to complete the first 40 kg N ha^{-1} dose. The remaining two N

doses were supplied through 86.96 kg urea ha⁻¹ at the tillering stage and 86.96 kg urea ha⁻¹ at the booting stage. The full quantities of DAP and SOP were applied at sowing. For each 9 m² subplot, 176.09 g DAP, 108.00 g SOP, and 9.36 g urea were applied at sowing, followed by 78.26 g urea at tillering and 78.26 g urea at booting.

Irrigation: After crop establishment, five irrigations were applied at the crown root initiation, tillering, booting, flowering, and grain-filling stages. Each post-sowing irrigation supplied approximately 3 acre-inches of water, resulting in a total post-sowing seasonal irrigation depth of approximately 15 acre-inches (about 381 mm). The pre-sowing irrigation was not included in this seasonal irrigation-depth calculation. Irrigation water was applied uniformly to all experimental plots.

$$\text{Biological yield (kg ha}^{-1}\text{)} = \frac{\text{Biological yield of randomly selected rows}}{\text{Row} - \text{Row distance} \times \text{Row length} \times \text{No. of rows}} \times 10000$$

$$\text{Grain yield (kg ha}^{-1}\text{)} = \frac{\text{Grain yield of randomly selected rows}}{\text{Row} - \text{Row distance} \times \text{Row length} \times \text{No. of rows}} \times 10000$$

Soil enzymatic activities: Rhizosphere soil samples were collected from each experimental subplot at heading from the 0–20 cm soil depth. Fresh soil samples were passed through a 2-mm sieve and stored at 4°C until enzyme analysis.

Urease activity was determined following the hydrolysis of urea and expressed as NH₄⁺-N released per unit soil. 5 g of fresh soil was placed in an incubation flask and treated with 9 mL of tris (hydroxymethyl) aminomethane (THAM) buffer (pH 9.0) and 1 mL of 0.2 M urea solution. The mixture was incubated at 37°C for 2 h. After incubation, the released NH₄⁺-N was extracted and quantified, and urease activity (Tabatabai & Bremner, 1972). Alkaline phosphatase activity was measured based on the release of p-nitrophenol from p-nitrophenyl phosphate substrate (Eivazi & Tabatabai, 1977). One gram of fresh soil was mixed with 4 mL of modified universal buffer (pH 11.0) and 1 mL of 0.05 M p-nitrophenyl phosphate solution. The mixture was incubated at 37°C for 1 h. After incubation, 1 mL of 0.5 M CaCl₂ and 4 mL of 0.5 M NaOH were added to terminate the reaction and develop the color. The suspension was filtered, and the absorbance of the filtrate was measured at 400 nm using a UV–Visible spectrophotometer. Fluorescein diacetate (FDA) hydrolysis activity was quantified as an indicator of total microbial activity through the measurement of fluorescein released from FDA (Frlolov *et al.*, 2022). Fresh soil (2 g) was mixed with phosphate buffer (pH 7.6), followed by the addition of FDA substrate solution. The samples were incubated at 30°C for 20 min with periodic mixing. The enzymatic reaction was terminated by adding acetone, and the suspension was centrifuged or filtered to obtain a clear extract. Absorbance was measured at 490 nm. Dehydrogenase activity, reflecting overall microbial oxidative activity, was determined using triphenyl tetrazolium chloride (TTC) reduction and expressed as triphenyl formazan (TPF) produced per unit soil (Casida *et*

Assessment of growth and yield attributes: Growth and yield attributes were recorded at physiological maturity. Plant height was measured from the soil surface to the tip of the spike (excluding awns) using five randomly selected plants from each plot. Productive tillers (spikes m⁻²) were counted from three randomly selected one-meter row sections and converted to a square meter basis. Grains per spike were determined by counting grains from five randomly selected spikes per plot. Thousand-grain weight was recorded by weighing 1,000 randomly selected grains using an electronic balance. Biological yield was determined from the total aboveground biomass harvested from the net plot area and expressed as kg ha⁻¹ after conversion. Grain yield was measured after threshing the harvested crop from each plot and subsequently converted to kg ha⁻¹ using the respective conversion formula:

al., 1964). Five grams of fresh soil was mixed with 5 mL of 1% TTC solution and incubated at 37°C for 24 h. The triphenyl formazan (TPF) formed during incubation was extracted with methanol, and the extract was filtered until clear. Absorbance was measured at approximately 485 nm using a UV–Visible spectrophotometer.

Determination of biochemical attributes: Superoxide dismutase (SOD) activity was determined based on its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) and was measured spectrophotometrically at 560 nm (Durak *et al.*, 1993). Peroxidase (POD) activity was assayed using guaiacol (C₇H₈O₂) as a substrate, and the increase in absorbance due to tetraguaiacol formation was recorded at 470 nm (Elavarthi & Martin, 2010). Catalase (CAT) activity was determined by monitoring the decomposition of hydrogen peroxide (H₂O₂) at 240 nm (Aebi, 1984). Ascorbate peroxidase (APX) activity was measured by following the oxidation of ascorbic acid (C₆H₈O₆) in the presence of H₂O₂ at 290 nm (Nakano & Asada, 1981). Hydrogen peroxide content was quantified using potassium iodide (KI) reagent and measured at 390 nm, whereas malondialdehyde (MDA) content was determined using the thiobarbituric acid (TBA) reaction at 532 nm, with correction at 600 nm (Dhindsa *et al.*, 1981, Cakmak *et al.*, 1993). Proline content was estimated following extraction with sulfosalicylic acid and reaction with acid ninhydrin, with absorbance recorded at 520 nm (Bates *et al.*, 1973). Free amino acids were quantified using the ninhydrin reagent method, and absorbance was measured at 570 nm (Moore & Stein, 1954). Fresh leaf tissue (0.5 g) was homogenized in 5 mL of ice-cold 50 mM potassium phosphate buffer (pH 7.0). The homogenate was centrifuged at 12,000 × g for 15 min at 4°C, and the resulting supernatant was used for protein determination. An aliquot (0.1 mL) of the supernatant was mixed with 5 mL of Bradford reagent, and the mixture was incubated at room temperature for 5 min. Absorbance was

measured at 595 nm using a UV–Visible spectrophotometer for total soluble protein (Bradford, 1976).

Determination of zinc and boron: At harvest, representative samples of leaves, grains, and straw were collected, oven-dried at 65°C until constant weight, ground, and digested using a di-acid mixture of nitric acid (HNO₃) and perchloric acid (HClO₄) (Miller, 1997). Zinc concentration in plant tissues was determined using an atomic absorption spectrophotometer (AAS) (Hanlon, 1998). Boron concentration in leaves, grains, and straw was determined colorimetrically using the azomethine-H reagent and measured at 420 nm with a UV–Visible spectrophotometer (Roig-Navarro, 1996).

$$\text{Root colonization (\%)} = \frac{\text{Number of root segments showing fungal structure}}{\text{Total number of root segments examined}} \times 100$$

Statistical Analysis

Prior to statistical analysis, the data were examined for normality and homogeneity of variance using the Shapiro–Wilk and Levene’s tests, respectively. Data that satisfied the assumptions of analysis of variance (ANOVA) were subjected to ANOVA. The effects of *Trichoderma* inoculation and micronutrient treatments were evaluated at a significance level of $p \leq 0.05$. Means were separated using Fisher’s least significant difference (LSD) test at the 5% probability level using OriginPro software (OriginLab Corporation, 2021).

Results

Effect of treatments was significant on plant height, tillers m⁻², grains spike⁻¹, 1000-grain weight, biological yield, grain yield, zinc concentration in soil, and total soluble protein content of wheat. Results showed that Zinc Full and Boron Full with *Trichoderma* resulted in significant increases in plant height (12.07% and 9.24%, respectively) compared to the control. However, 1/2 Zinc Half (3.45%) and 1/2 Boron Half (1.60%) with *Trichoderma* resulted in non-significant enhancement in plant height compared to control. Without *Trichoderma* Zinc Full, Boron Full, 1/2 Zinc Half and 1/2 Boron Half showed significant enhancement in plant height, i.e., 12.19, 11.07, 9.53 and 7.71% than the control (Table 1).

Under *Trichoderma* inoculation, full zinc significantly increased tillers m⁻² by 19.98%, followed by full boron and 1/2 zinc, which also showed significant increases of 12.97% and 9.52%, respectively, over the control. However, 1/2 boron showed a non-significant increase of 3.03% compared with the control. Under no-*Trichoderma* conditions, full zinc, full boron, 1/2 zinc, and 1/2 boron all significantly increased tillers m⁻² by 35.66%, 27.66%, 20.96%, and 8.15%, respectively, compared with the control.

Full zinc application significantly increased grains spike⁻¹ by 17.84% in the presence of *Trichoderma*. Full boron and 1/2 zinc treatments also significantly increased grains spike⁻¹ by 9.30% and 6.90%, respectively, compared with the control. However, 1/2 boron showed a non-significant increase of 4.05%. Without *Trichoderma*, all nutrient treatments significantly increased grains spike⁻¹ compared with the control. The increases were 12.74%,

Fungal root colonization: Root colonization was assessed in plants grown with and without *Trichoderma* inoculation. Fresh fine roots were collected from each experimental plot, thoroughly washed with distilled water to remove adhering soil, and cut into approximately 1-cm segments. Root segments were cleared in 10% (w/v) KOH at 90°C for 30 min, washed thoroughly with distilled water, and stained with 0.05% trypan blue. The stained root segments were examined under a light microscope at 100× magnification for the presence of fungal hyphae and other fungal structures (Romano *et al.*, 2020). For each replicate, 50 root segments were examined, and fungal root colonization was calculated as:

10.09%, 7.79%, and 5.49% for full zinc, full boron, 1/2 zinc, and 1/2 boron, respectively.

In the presence of *Trichoderma*, full zinc application significantly increased 1000-grain weight by 12.12% compared with the control. Likewise, full boron and 1/2 zinc treatments significantly increased 1000-grain weight by 6.82% and 3.41%, respectively, over the control treatment. However, 1/2 boron showed a non-significant increase of 1.21% compared with the control.

With *Trichoderma* inoculation, full zinc application significantly increased biological yield by 5.58% compared with the control. Similarly, full boron and 1/2 zinc treatments significantly increased biological yield by 3.72% and 3.15%, respectively, over the control treatment. However, 1/2 boron showed a non-significant increase of 1.03% compared with the control. Without *Trichoderma*, full zinc and full boron treatments significantly increased biological yield by 6.95% and 5.84%, respectively, compared with the control. In contrast, 1/2 zinc and 1/2 boron treatments showed non-significant increases of 2.10% and 0.49%, respectively, over the untreated control.

In the presence of *Trichoderma*, full zinc application significantly increased grain yield by 9.25% compared with the control. Likewise, full boron and 1/2 zinc treatments significantly increased grain yield by 6.46% and 4.30%, respectively, over the control treatment. However, 1/2 boron showed a non-significant increase of 1.77% compared with the control. Without *Trichoderma*, all nutrient treatments significantly increased grain yield relative to untreated control. The increases observed were 18.28%, 16.21%, 14.13%, and 10.73% for full zinc, full boron, 1/2 zinc, and 1/2 boron treatments, respectively.

Application of full zinc significantly increased zinc concentration in soil by 42.91% compared with the control in the presence of *Trichoderma*. Similarly, full boron and 1/2 zinc treatments significantly increased soil zinc concentration by 29.92% and 12.20%, respectively, over the control treatment. However, 1/2 boron showed a non-significant increase of 6.30% compared with the control. Without *Trichoderma*, all nutrient treatments significantly increased zinc concentration in soil relative to the untreated control. The increases recorded were 32.40%, 25.14%, 18.44%, and 8.94% for full zinc, full boron, 1/2 zinc, and 1/2 boron treatments, respectively.

In the presence of *Trichoderma*, full zinc application significantly increased total soluble protein by 19.48% compared with the control. Similarly, full boron and 1/2 zinc treatments significantly increased total soluble protein by 17.34% and 10.93%, respectively, over the control treatment. However, 1/2 boron showed a non-significant increase of 7.23% compared with the control. Without *Trichoderma*, full zinc and full boron treatments significantly increased total soluble protein by 13.68% and 10.59%, respectively, compared with the untreated control. In contrast, 1/2 zinc and 1/2 boron treatments showed non-significant increases of 5.63% and 2.41%, respectively, over the control.

In the presence of *Trichoderma*, full zinc application significantly increased urease activity by 17.77% compared with the control. Likewise, full boron and 1/2 zinc treatments significantly increased urease activity by 10.76% and 7.37%, respectively, over the control treatment. However, 1/2 boron showed a non-significant increase of 3.80% compared with the control. Without *Trichoderma*, all nutrient treatments significantly increased urease activity relative to the untreated control. The increases observed were 26.34%, 19.41%, 15.15%, and 8.46% for full zinc, full boron, 1/2 zinc, and 1/2 boron treatments, respectively (Fig. 1A).

The presence of *Trichoderma* significantly influenced phosphatase activity in response to zinc and boron applications. Full zinc treatment significantly increased phosphatase activity by 17.65%, while full boron and 1/2 zinc treatments also resulted in significant increases of 12.45% and 9.57%, respectively, compared with the control. Conversely, the increase observed with 1/2 boron treatment (3.89%) was non-significant relative to the control. Compared with the untreated control, full zinc, full boron, 1/2 zinc, and 1/2 boron treatments significantly increased phosphatase activity by 23.76%, 13.99%, 11.63%, and 9.03%, respectively (Fig. 1B).

Application of zinc and boron noticeably affected FDA activity in both *Trichoderma*-inoculated and uninoculated

soils. In the presence of *Trichoderma*, full zinc treatment significantly increased FDA activity by 13.30% compared with the control. Likewise, full boron and 1/2 zinc treatments significantly increased FDA activity by 11.74% and 9.12%, respectively, over the control treatment. However, the increase recorded with 1/2 boron treatment (5.39%) was non-significant compared with the control. In soils without *Trichoderma*, all nutrient treatments significantly improved FDA activity relative to the untreated control. The maximum increase was observed with full zinc application (42.99%), followed by full boron (30.19%), 1/2 zinc (25.42%), and 1/2 boron (16.56%) (Fig. 1C).

Compared with the control treatment, full zinc application significantly increased dehydrogenase activity by 21.94%, followed by full boron and 1/2 zinc treatments, which significantly increased activity by 15.68% and 12.07%, respectively. In contrast, 1/2 boron treatment showed a non-significant increase of 5.20% relative to the control. A similar trend was observed in treatments without *Trichoderma* inoculation. Full zinc application caused the highest significant increase in dehydrogenase activity (38.27%), whereas full boron, 1/2 zinc, and 1/2 boron treatments significantly increased activity by 30.98%, 23.58%, and 14.51%, respectively, compared with the untreated control (Fig. 1D).

Superoxide dismutase (SOD) activity was significantly influenced by zinc and boron application in both *Trichoderma*-inoculated and uninoculated treatments. In the presence of *Trichoderma*, full zinc, full boron, 1/2 zinc, and 1/2 boron treatments significantly reduced SOD activity by 19.07%, 15.91%, 11.27%, and 7.59%, respectively, compared with the control. Similarly, without *Trichoderma*, SOD activity was significantly reduced by 12.49%, 9.87%, and 6.87% following full zinc, full boron, and 1/2 zinc applications, respectively, whereas the reduction caused by 1/2 boron (3.40%) was non-significant relative to the untreated control.

Table 1. Effect of zinc, boron, and *Trichoderma* application on plant height, tillers m⁻², grains spike⁻¹, 1000-grain weight, biological yield, grain yield, zinc concentration in soil, and total soluble protein content of wheat.

Zinc	Boron	Plant height (cm)	Tillers m ⁻²	Grains spike ⁻¹	1000-Grains weight (g)
Control	<i>Trichoderma</i>	90.22 ± 0.19 bcd	318.67 ± 4.16 cd	44.47 ± 0.81 d	44.00 ± 0.26 cd
Zinc full	<i>Trichoderma</i>	101.11 ± 0.84 a	382.33 ± 13.80 a	52.40 ± 0.87 a	49.33 ± 1.62 a
Boron full	<i>Trichoderma</i>	98.55 ± 1.07 a	360.00 ± 7.00 b	48.60 ± 1.04 b	47.00 ± 0.36 b
1/2 Zinc half	<i>Trichoderma</i>	93.33 ± 0.88 b	349.00 ± 5.20 b	47.53 ± 0.50 b	45.50 ± 0.70 bc
1/2 Boron half	<i>Trichoderma</i>	91.67 ± 0.58 bc	328.33 ± 5.51 c	46.27 ± 0.31 c	44.53 ± 0.25 cd
Control	No <i>Trichoderma</i>	79.33 ± 5.57 g	229.00 ± 4.58 h	37.67 ± 0.95 h	37.07 ± 2.68 g
Zinc full	No <i>Trichoderma</i>	89.00 ± 0.67 cde	310.67 ± 1.15 d	42.47 ± 0.50 e	42.97 ± 0.46 de
Boron full	No <i>Trichoderma</i>	88.11 ± 0.19 def	292.33 ± 10.50 e	41.47 ± 0.50 ef	41.73 ± 0.42 ef
1/2 Zinc half	No <i>Trichoderma</i>	86.89 ± 0.70 ef	277.00 ± 3.00 f	40.60 ± 0.53 fg	41.27 ± 0.12 ef
1/2 Boron half	No <i>Trichoderma</i>	85.44 ± 0.77 f	247.67 ± 13.61 g	39.73 ± 0.23 g	40.83 ± 0.31 f
Zinc	Boron	Biological yield (kg ha ⁻¹)	Grain yield (kg ha ⁻¹)	Zinc soil (mg kg ⁻¹)	Total soluble protein (mg g ⁻¹ FW)
Control	<i>Trichoderma</i>	8796 ± 1.30 d	3270 ± 22.53 d	8.50 ± 0.40 d	18.55 ± 0.23 d
Zinc full	<i>Trichoderma</i>	9287 ± 49.95 a	3573 ± 82.84 a	12.1 ± 0.56 a	22.16 ± 0.24 a
Boron full	<i>Trichoderma</i>	9123 ± 38.36 b	3481 ± 0.00 b	11.0 ± 0.52 b	21.77 ± 0.03 a
1/2 Zinc half	<i>Trichoderma</i>	9074 ± 12.82 b	3411 ± 36.48 c	9.50 ± 0.20 c	20.58 ± 0.22 b
1/2 Boron half	<i>Trichoderma</i>	8887 ± 73.40 c	3328 ± 20.40 d	9.00 ± 0.20 c	19.89 ± 0.68 c
Control	No <i>Trichoderma</i>	8170 ± 20.65 g	2681 ± 32.29 h	6.00 ± 0.21 h	15.96 ± 0.37 g
Zinc full	No <i>Trichoderma</i>	8738 ± 38.98 d	3172 ± 31.50 e	7.90 ± 0.10 e	18.15 ± 0.11 de
Boron full	No <i>Trichoderma</i>	8647 ± 47.51 e	3116 ± 14.97 ef	0.75 ± 0.15 ef	17.66 ± 0.25 e
1/2 Zinc half	No <i>Trichoderma</i>	8341 ± 109.88 f	3060 ± 13.00 f	7.10 ± 0.25 f	16.86 ± 0.39 f
1/2 Boron half	No <i>Trichoderma</i>	8210 ± 7.12 g	2969 ± 59.53 g	6.50 ± 0.10 g	16.35 ± 0.10 fg

Different lowercase letters within the same column indicate statistically significant differences among treatments [Fisher LSD test at p≤0.05]

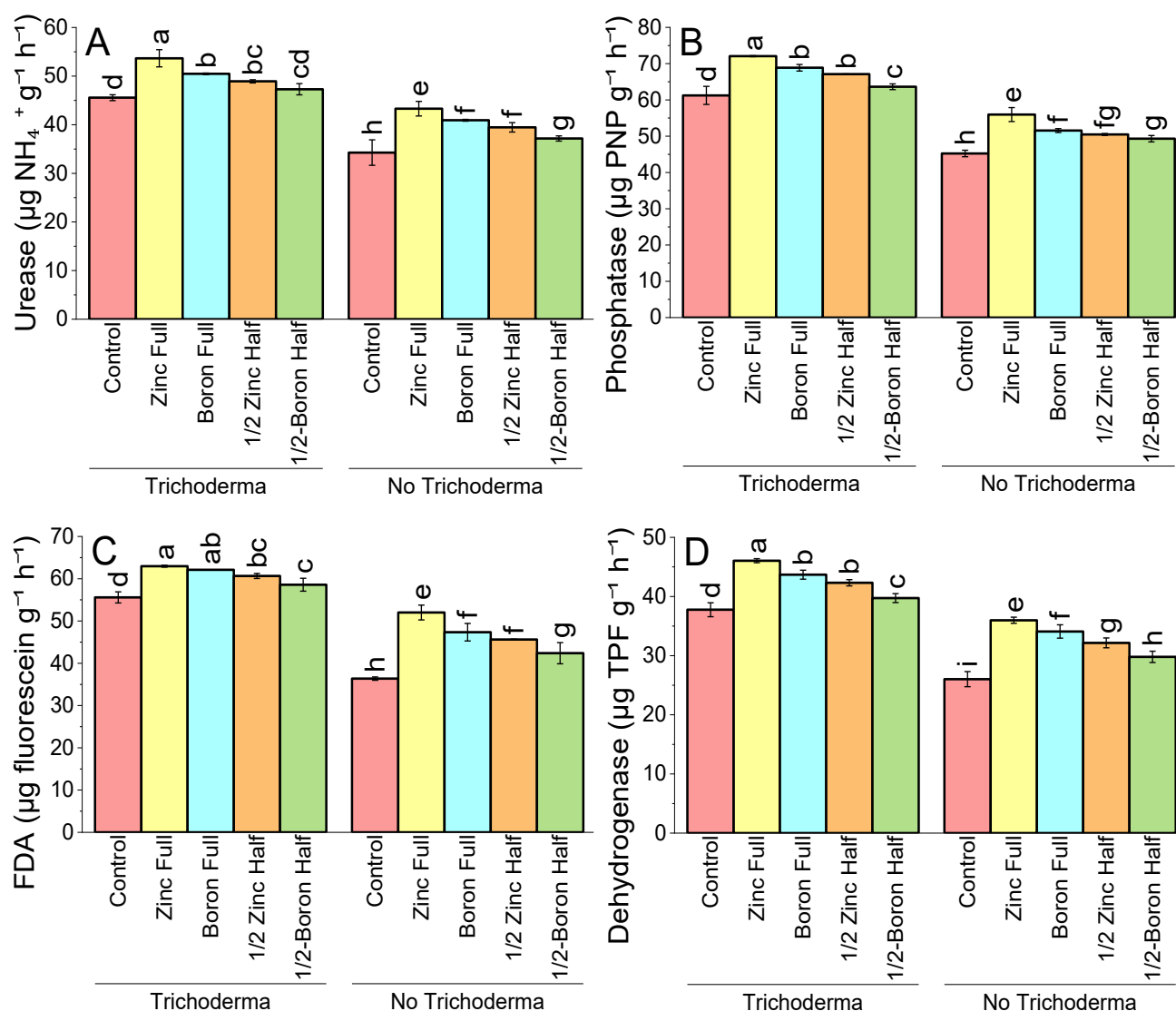


Fig. 1. Effect of zinc, boron, and Trichoderma application on soil enzymatic activities, including urease (A), phosphatase (B), fluorescein diacetate (FDA) hydrolysis (C), and dehydrogenase activity (D) under experimental conditions. Bars represent mean $\pm$ standard deviation (SD). Different lowercase letters indicate significant differences among treatments according to Fisher's LSD test at $p \leq 0.05$.

Peroxidase (POD) activity also declined following nutrient application. Under Trichoderma inoculation, full zinc, full boron, and 1/2 zinc treatments significantly reduced POD activity by 14.37%, 9.18%, and 5.70%, respectively, compared with the control treatment. However, the reduction observed with 1/2 boron (1.88%) was non-significant. In uninoculated treatments, POD activity was significantly reduced by 13.57%, 8.66%, and 5.89% with full zinc, full boron, and 1/2 zinc applications, respectively, while 1/2 boron showed a non-significant reduction of 4.61% over the control.

Catalase (CAT) activity responded significantly to nutrient supplementation in the presence of Trichoderma. Full zinc, full boron, 1/2 zinc, and 1/2 boron treatments significantly reduced CAT activity by 17.77%, 14.79%, 12.44%, and 2.54%, respectively, compared with the control. Likewise, in treatments without Trichoderma, CAT activity significantly decreased by 13.78%, 10.10%, and 7.57% with full zinc, full boron, and 1/2 zinc applications, respectively, whereas the reduction recorded with 1/2 boron (4.43%) remained non-significant.

Ascorbate peroxidase (APX) activity showed a similar trend. In Trichoderma-inoculated treatments, full zinc, full boron, 1/2 zinc, and 1/2 boron significantly reduced APX activity by 26.30%, 19.84%, 11.83%, and 6.12%, respectively, relative to the control. In the absence of Trichoderma, full zinc, full boron, and 1/2 zinc treatments significantly reduced APX activity by 8.09%, 6.72%, and 5.42%, respectively, while the reduction caused by 1/2 boron (3.33%) was non-significant compared with the untreated control.

Hydrogen peroxide (H_2O_2) content decreased markedly following nutrient application. In the presence of Trichoderma, full zinc, full boron, and 1/2 zinc treatments significantly reduced H_2O_2 content by 19.96%, 11.19%, and 7.39%, respectively, compared with the control treatment, whereas the reduction observed with 1/2 boron (3.18%) was non-significant. Under uninoculated conditions, all nutrient treatments significantly reduced H_2O_2 content by 27.42%, 21.88%, 16.87%, and 4.59%, respectively, over the control.

Malondialdehyde (MDA) content was significantly reduced by nutrient supplementation. In Trichoderma-treated plants, full zinc, full boron, and 1/2 zinc applications significantly reduced MDA content by 26.64%, 17.27%, and 6.98%, respectively, while the reduction caused by 1/2 boron (3.30%) was non-significant compared with the control. Similarly, full zinc, full boron, and 1/2 zinc treatments significantly reduced MDA concentration in the absence of Trichoderma by 22.98%, 19.00%, and 11.79%, respectively, whereas the decrease with 1/2 boron (4.29%) remained non-significant.

Proline accumulation was also significantly affected by zinc and boron application. In Trichoderma-inoculated treatments, full zinc, full boron, and 1/2 zinc significantly reduced proline content by 28.89%, 18.24%, and 8.72%, respectively, compared with the control treatment, while the reduction with 1/2 boron (2.90%) was non-significant. Similarly, without Trichoderma, full zinc, full boron, and 1/2 zinc significantly reduced proline content by 22.32%, 18.05%, and 11.20%, respectively, whereas the reduction observed with 1/2 boron (4.12%) was non-significant relative to the control.

Free amino acid contents increased significantly following nutrient application. In the presence of Trichoderma, full zinc, full boron, and 1/2 zinc treatments significantly increased free amino acid contents by 13.78%, 11.74%, and 7.31%, respectively, over the control treatment, whereas the increase recorded with 1/2 boron (3.52%) was non-significant. Similarly, under uninoculated conditions, free amino acid contents significantly increased by 14.82%, 10.27%, and 7.97% following full zinc, full boron, and 1/2 zinc applications, respectively, while the increase caused by 1/2 boron (3.94%) remained non-significant compared with the untreated control (Table 2).

In the presence of Trichoderma, full zinc application significantly increased zinc concentration in leaves, grains, and straw by 47.22%, 59.27%, and 55.69%, respectively, compared with the control treatment. Likewise, full boron and 1/2 zinc treatments significantly increased leaf zinc concentration by 32.42% and 19.23%, grain zinc concentration by 42.29% and 25.56%, and straw zinc concentration by 39.50% and 25.54%, respectively, over the control. Similarly, 1/2 boron treatment also significantly increased zinc concentration in leaves, grains, and straw by 7.31%, 12.12%, and 10.26%, respectively, compared with the control.

A comparable trend was observed for boron concentration. In Trichoderma-inoculated treatments, full zinc significantly increased boron concentration in leaves, grains, and straw by 38.34%, 55.40%, and 51.23%, respectively, compared with the control. Similarly, full boron and 1/2 zinc treatments significantly enhanced boron concentration in leaves by 31.26% and 17.35%, grains by 44.50% and 26.10%, and straw by 40.91% and 25.06%, respectively. Furthermore, 1/2 boron treatment significantly increased boron concentration in leaves, grains, and straw by 7.45%, 10.81%, and 10.69%, respectively, over the control treatment.

Soil boron concentration and root colonization percentage also responded positively to nutrient application in the presence of Trichoderma. Compared with the control, full zinc, full boron, 1/2 zinc, and 1/2 boron treatments significantly increased soil boron concentration by 66.20%, 50.70%, 30.99%, and 15.49%, respectively. Likewise, root colonization increased significantly by 56.05%, 43.14%, 31.35%, and 15.19% following full zinc, full boron, 1/2 zinc, and 1/2 boron applications, respectively, compared with the control treatment.

Table 2. Effect of zinc, boron, and Trichoderma application on SOD, POD, CAT, APX, H₂O₂, MDA, proline, and free amino acids in wheat leaves.

Zinc	Boron	SOD (U mg ⁻¹ protein)	POD (U mg ⁻¹ protein)	CAT (U mg ⁻¹ protein)	APX (U mg ⁻¹ protein)
Control	Trichoderma	80.44 ± 2.59 e	91.86 ± 0.61 e	64.75 ± 0.58 f	54.38 ± 1.01 e
Zinc full	Trichoderma	67.55 ± 0.24 h	80.32 ± 2.33 h	54.98 ± 0.51 i	43.06 ± 0.72 i
Boron full	Trichoderma	69.40 ± 0.54 h	84.14 ± 0.72 g	56.41 ± 0.64 h	45.38 ± 1.17 h
1/2 Zinc half	Trichoderma	72.29 ± 1.46 g	86.91 ± 0.62 f	57.58 ± 0.55 h	48.63 ± 0.88 g
1/2 Boron half	Trichoderma	74.77 ± 1.52 f	90.17 ± 0.26 e	63.14 ± 0.92 g	51.25 ± 1.19 f
Control	No Trichoderma	94.47 ± 0.57 a	108.36 ± 2.80 a	76.38 ± 0.92 a	61.34 ± 0.29 a
Zinc full	No Trichoderma	83.97 ± 0.31 c	95.41 ± 1.76 d	67.14 ± 0.56 e	56.75 ± 0.27 d
Boron full	No Trichoderma	85.98 ± 0.96 d	99.72 ± 1.18 c	69.38 ± 0.83 d	57.48 ± 0.38 cd
1/2 Zinc half	No Trichoderma	88.39 ± 1.45 c	102.33 ± 0.39 b	71.01 ± 0.70 c	58.19 ± 0.24 bc
1/2 Boron half	No Trichoderma	91.36 ± 1.14 b	103.58 ± 0.40 b	73.15 ± 0.93 b	59.37 ± 0.99 b
Zinc	Boron	H ₂ O ₂ (μmol g ⁻¹ FW)	MDA (nmol g ⁻¹ FW)	Proline (μmol g ⁻¹ FW)	Free amino Acids (mg g ⁻¹ FW)
Control	Trichoderma	13.07 ± 0.33 f	9.71 ± 0.04 ef	6.95 ± 0.04 ef	13.27 ± 0.20 d
Zinc full	Trichoderma	10.90 ± 0.16 h	7.66 ± 0.12 i	5.39 ± 0.32 i	15.39 ± 0.13 a
Boron full	Trichoderma	11.75 ± 0.22 g	8.28 ± 0.16 h	5.87 ± 0.13 h	15.03 ± 0.20 a
1/2 Zinc half	Trichoderma	12.17 ± 0.15 g	9.07 ± 0.36 g	6.39 ± 0.26 g	14.31 ± 0.31 b
1/2 Boron half	Trichoderma	12.67 ± 0.12 f	9.39 ± 0.08 fg	6.75 ± 0.09 f	13.75 ± 0.25 c
Control	No Trichoderma	17.89 ± 0.05 a	12.16 ± 0.33 a	8.80 ± 0.11 a	10.32 ± 0.02 h
Zinc full	No Trichoderma	14.04 ± 0.12 e	9.88 ± 0.11 de	7.19 ± 0.17 de	12.11 ± 0.45 e
Boron full	No Trichoderma	14.68 ± 0.27 d	10.21 ± 0.16 d	7.45 ± 0.10 d	11.50 ± 0.14 f
1/2 Zinc half	No Trichoderma	15.30 ± 0.27 c	10.87 ± 0.28 c	7.91 ± 0.18 c	11.21 ± 0.12 f
1/2 Boron half	No Trichoderma	17.10 ± 0.48 b	11.66 ± 0.13 b	8.45 ± 0.27 b	10.74 ± 0.30 g

Different lowercase letters within the same column indicate statistically significant differences among treatments [Fisher LSD test at $p \leq 0.05$].

Table 3. Effect of zinc, boron, and Trichoderma application on zinc and boron concentrations in wheat leaves, grains, and straw, soil boron concentration, and Trichoderma root colonization.

Zinc	Boron	Zinc in leaves (mg kg ⁻¹)	Boron in leaves (mg kg ⁻¹)	Zinc in grains (mg kg ⁻¹)	Boron in grains (mg kg ⁻¹)
Control	Trichoderma	28.44 ± 0.82 e	16.25 ± 0.41 e	24.33 ± 0.61 e	10.27 ± 0.24 e
Zinc full	Trichoderma	41.87 ± 1.14 a	22.48 ± 0.56 a	38.75 ± 1.02 a	15.96 ± 0.35 a
Boron full	Trichoderma	37.66 ± 0.95 b	21.33 ± 0.47 b	34.62 ± 0.88 b	14.84 ± 0.31 b
1/2 Zinc half	Trichoderma	33.91 ± 0.73 c	19.07 ± 0.38 c	30.55 ± 0.73 c	12.95 ± 0.26 c
1/2 Boron half	Trichoderma	30.52 ± 0.66 d	17.46 ± 0.35 d	27.28 ± 0.65 d	11.38 ± 0.22 d
Control	No Trichoderma	22.18 ± 0.57 i	12.83 ± 0.29 i	18.11 ± 0.43 i	7.44 ± 0.16 i
Zinc full	No Trichoderma	34.75 ± 0.88 c	18.91 ± 0.42 c	31.94 ± 0.79 c	13.41 ± 0.29 c
Boron full	No Trichoderma	31.62 ± 0.71 d	17.54 ± 0.36 d	28.77 ± 0.67 d	12.28 ± 0.25 d
1/2 Zinc half	No Trichoderma	28.40 ± 0.63 e	15.98 ± 0.31 e	25.39 ± 0.58 e	10.95 ± 0.21 e
1/2 Boron half	No Trichoderma	25.67 ± 0.52 f	14.44 ± 0.28 f	22.64 ± 0.51 f	9.41 ± 0.18 f

Zinc	Boron	Zinc in straw (mg kg ⁻¹)	Boron in straw (mg kg ⁻¹)	Boron in soil (mg kg ⁻¹)	Root colonization (%)
Control	Trichoderma	18.91 ± 0.48 e	8.14 ± 0.19 e	0.71 ± 0.02 e	52.33 ± 1.53 d
Zinc full	Trichoderma	29.44 ± 0.76 a	12.31 ± 0.28 a	1.18 ± 0.04 a	81.67 ± 2.08 a
Boron full	Trichoderma	26.38 ± 0.69 b	11.47 ± 0.25 b	1.07 ± 0.03 b	74.91 ± 1.82 b
1/2 Zinc half	Trichoderma	23.74 ± 0.54 c	10.18 ± 0.21 c	0.93 ± 0.02 c	68.74 ± 1.69 c
1/2 Boron half	Trichoderma	20.85 ± 0.49 d	9.01 ± 0.18 d	0.82 ± 0.01 d	60.28 ± 1.44 d
Control	No Trichoderma	14.05 ± 0.31 i	5.72 ± 0.11 i	0.49 ± 0.01 i	0.00 ± 0.00 f
Zinc full	No Trichoderma	24.16 ± 0.61 c	10.26 ± 0.23 c	0.86 ± 0.02 d	0.00 ± 0.00 f
Boron full	No Trichoderma	21.73 ± 0.50 d	9.44 ± 0.20 d	0.79 ± 0.02 e	0.00 ± 0.00 f
1/2 Zinc half	No Trichoderma	18.86 ± 0.42 e	8.55 ± 0.17 e	0.68 ± 0.01 f	0.00 ± 0.00 f
1/2 Boron half	No Trichoderma	16.47 ± 0.37 f	7.36 ± 0.15 f	0.57 ± 0.01 g	0.00 ± 0.00 f

Different lowercase letters within the same column indicate statistically significant differences among treatments [Fisher LSD test at $p \leq 0.05$]

In the absence of Trichoderma, full zinc application significantly increased zinc concentration in leaves, grains, and straw by 56.67%, 76.37%, and 71.96%, respectively, over the untreated control. Similarly, full boron and 1/2 zinc treatments significantly increased zinc concentration in leaves by 42.56% and 28.04%, grains by 58.86% and 40.20%, and straw by 54.66% and 34.24%, respectively, compared with the control. Likewise, 1/2 boron treatment significantly increased zinc concentration in leaves, grains, and straw by 15.73%, 25.01%, and 17.22%, respectively.

Boron concentration in leaves, grains, and straw was also significantly enhanced in uninoculated treatments. Full zinc application significantly increased boron concentration in leaves, grains, and straw by 47.39%, 80.24%, and 79.37%, respectively, relative to the untreated control. Likewise, full boron and 1/2 zinc treatments significantly increased boron concentration in leaves by 36.71% and 24.55%, grains by 65.05% and 47.18%, and straw by 65.03% and 49.48%, respectively. Similarly, 1/2 boron significantly increased boron concentration in leaves, grains, and straw by 12.55%, 26.48%, and 28.67%, respectively, compared with the untreated control. Moreover, soil boron concentration significantly increased by 75.51%, 61.22%, 38.78%, and 16.33% following full zinc, full boron, 1/2 zinc, and 1/2 boron applications, respectively, relative to the untreated control (Table 3).

Discussion

Growth and yield enhancement through micronutrient-microbe interactions: The similar physiological and biochemical roles of zinc, boron, and Trichoderma within the plant-soil system are responsible for the improvement in wheat growth and production following their application. Many enzymes involved in photosynthesis, protein

synthesis, carbohydrate metabolism, and auxin biosynthesis use zinc as a structural and catalytic component. (Ray *et al.*, 2024, Mosa *et al.*, 2025). Increased cell division, cell elongation, and root proliferation were probably caused by increased zinc availability stimulating the formation of indole-3-acetic acid (IAA). (Tymoszuk *et al.*, 2024). Increased biomass production and reproductive development were supported by improved root architecture, which also improved the efficiency of water and nutrient intake. Because of its critical function in cell wall stabilization, membrane integrity, and glucose transport, boron helped to promote growth (Martello *et al.*, 2025, Wang *et al.*, 2025). During vegetative growth, boron-mediated cross-linking of pectic polysaccharides strengthens cell walls and promotes tissue expansion. Additionally, boron plays a direct role in the processes of fertilization, pollen germination, and pollen tube elongation. Therefore, increased grain formation and filling were probably caused by better reproductive success and assimilating transport toward growing grains. A synergistic relationship between microbial activity and nutrient availability is suggested by the improved micronutrient performance in the presence of Trichoderma. (Sobrinho *et al.*, 2025). Organic acids, siderophores, phytohormones, and nutrient-solubilizing metabolites are produced by Trichoderma species that are known to invade the rhizosphere and root cortex. These substances both promote root growth and enhance nutrient mobility and availability in the rhizosphere (Sinha *et al.*, 2024). Increased root surface area and root hair density enhance the efficiency of nutrient usage and boost crop output by improving nutrient interception and absorption.

Soil biological processes and nutrient cycling: The stimulation of rhizosphere biological processes and nutrient turnover is reflected in the improvement of soil

enzymatic activity after the integrated application of zinc, boron, and *Trichoderma*. Microorganisms and plant roots are the main producers of soil enzymes, which are markers of the degree of nutrient cycling (Jabborova *et al.*, 2026).

Accelerated nitrogen transformation and ammonification processes in the soil are indicated by elevated urease activity. By more effectively hydrolyzing urea-derived nitrogen, increased urease activity probably increased nitrogen availability and supported the synthesis of proteins and amino acids in plant tissues (Marzouk *et al.*, 2024). Similarly, increased phosphatase activity indicates more organically bound phosphorus mineralization, which increases the availability of orthophosphate ions for plant uptake. Because it represents total microbial respiratory activity and metabolic intensity, elevated dehydrogenase activity is especially significant (Pathania *et al.*, 2024). A physiologically active rhizosphere that can maintain nutrient transformations necessary for plant growth is indicated by higher dehydrogenase activity. In a similar vein, elevated FDA hydrolysis activity indicates activation of various bacteria populations and hydrolytic enzymes involved in the breakdown of organic materials (Fernández-Gatell *et al.*, 2021). By secreting extracellular enzymes and altering root exudation patterns, *Trichoderma* probably directly and indirectly influenced these reactions, fostering the growth of advantageous microbial communities.

Regulation of oxidative metabolism and cellular homeostasis: Instead of impairing antioxidant defense, the decrease in antioxidant enzyme activity after zinc and boron supplementation should be seen as proof of a lower oxidative burden. Antioxidant enzymes such superoxide dismutase, peroxidase, catalase, and ascorbate peroxidase are activated when plants with nutritional imbalances generate excessive amounts of reactive oxygen species (ROS). (Dumanović *et al.*, 2021, Kabir *et al.*, 2021). Sufficient zinc intake minimizes ROS production by enhancing membrane integrity and lowering electron leakage from mitochondria and chloroplasts. Additionally, zinc preserves the structural integrity of bio membranes and shields proteins that contain sulfhydryl. Boron further reduces oxidative damage by aiding in cellular structure and membrane stabilization (Behtash *et al.*, 2024, Sidhu *et al.*, 2024). The observed decrease in antioxidant enzyme activity can be explained by decreased ROS generation, which lessens the need for enzymatic detoxification systems. Reductions in the amounts of malondialdehyde and hydrogen peroxide provide additional evidence for this process. Reduced malondialdehyde content indicates less lipid peroxidation and membrane damage, whereas lower hydrogen peroxide levels show less ROS generation (Fernando *et al.*, 2024, Su *et al.*, 2024). These findings collectively imply that combined micronutrient and microbial therapies enhanced cellular homeostasis and shielded physiological functions from oxidative disruptions.

Nitrogen metabolism and protein biosynthesis: Improved nutrition absorption and metabolic efficiency probably led to increased soluble protein and free amino acid buildup. Many enzymes involved in nitrogen intake, nitrate reduction, amino acid synthesis, and protein assembly require zinc as a cofactor (Saqib *et al.*, 2026). Therefore, more effective conversion of inorganic nitrogen into physiologically useful molecules is made possible by improved zinc nutrition.

Trichoderma may also promote nitrogen metabolism by promoting root development and improving the uptake of mineral nutrients (Ayyandurai *et al.*, 2024, Sobrinho *et al.*, 2025). While increased glucose production provides the energy and carbon skeletons needed for protein creation, improved food availability promotes amino acid biosynthesis (Amoah *et al.*, 2025). Boron-mediated improvements in phloem transport may additionally facilitate movement of assimilates and nitrogenous compounds throughout the plant, contributing to greater protein accumulation and improved physiological performance (Liu *et al.*, 2025, Wang *et al.*, 2026).

Conclusion

The combined application of *Trichoderma* (1.70 kg ha⁻¹) and zinc at 6 kg ha⁻¹ was the most effective treatment for improving wheat growth, soil enzyme activities, yield, and soil biological activity. The enhanced performance was attributed to improved rhizosphere colonization, and root development, mediated by *Trichoderma*. The integrated treatments also stimulated soil enzymatic activities and reduced oxidative stress by lowering H₂O₂, MDA, and proline accumulation, thereby maintaining cellular homeostasis. Although Zn and B were applied separately, changes in rhizosphere conditions, root activity, and nutrient acquisition may have influenced the availability and uptake of the non-applied micronutrient. This response was more pronounced in *Trichoderma*-inoculated treatments. However, the mechanisms responsible for the observed Zn–B interaction were not directly investigated in the present study; therefore, these cross-nutrient responses should be interpreted cautiously and require further investigation in future.

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