IMPROVING PHYSIOLOGY, GROWTH, PRODUCTIVITY, AND GRAIN BIO-FORTIFICATION OF WHEAT WITH SOIL AND FOLIAR APPLIED MICRONUTRIENTS

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Abstract. Micro-nutrient, especially iron (Fe) and zinc (Zn), deficiency is prevalent in a large segment of the world's population. Therefore, it is essential to provide these nutrients to humans for better health. Cereals are rich in nutrients; therefore, it is essential to increase the grain nutrient concentration to fulfil human needs. The present study was conducted to find the optimum combination of different micro-nutrients to improve the physiology, yield, and grain nutrient concentration of wheat. The study was comprised of diverse treatments: T_1: control, T_2: foliar water spray, T_3: Zn soil application, T_4: Zn + B soil application, T_5: Zn + Mn soil application, T_6: Zn + B + Mn soil application, T_7: B + Mn soil application, T_8: Zn foliar spry, T_9: Zn + B foliar spray, T_{10}: Zn + Mn foliar spray, T_{11}: Zn + B + Mn foliar spray, T_{12}: Zn soil application + foliar spray, T_{13}: Zn + B soil application + foliar spray, T_{14}: Zn + Mn soil application + foliar spray, T_{15}: Zn + B + Mn soil application + foliar spray, T_{16}: B + Mn soil application + foliar spray. The results indicate that different combinations of soil and foliar applied micro-nutrients significantly improved plant physiological functioning, yield, and grain bio-fortification of wheat. The maximum improvement in relative water contents (RWC), chlorophyll content, antioxidant activities, grain yield, and biomass were observed with T_{15} (Zn + B + Mn soil application + foliar spray). The application of different nutrient combinations also enhanced the grain nutrients concentration. However, co-applying soil and foliar spray of B, Mn, and Zn effectively improved their grain concentrations than their individual applications. Therefore, a combination of soil and foliar applied Zn, B, and Mn could be an important practice to improve wheat growth, productivity, and bio-fortification.
Keywords: *antioxidant activities, bio-fortification, chlorophyll, foliar spray, micro-nutrients, yield*

Introduction

Malnutrition is considered as a major reason for the decline in human health particularly in poor and developed countries (Jankowska et al., 2012). It has been reported that around 75% of the world's population are facing a deficiency of micro-nutrients particularly, zinc (Zn), iron (Fe), and copper (Cu) (Hotz and Brown, 2004; Stein, 2010). The deficiency of these micro-nutrients is more prevalent in children and pregnant women which is causing serious health implications (Garcia-Oliveira et al.,

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2018). Globally more than 2 billion people are facing Zn and Fe deficiency which is causing many problems including anemia, stunted growth, infectious diseases, and disrupted brain development (Cakmak, 2010; WHO, 2020). Thus, it is essential to provide these nutrients to humans for their health (Cakmak, 2010). Fortification is considered as effective and quick way to provide these nutrients to humans. However, it needs proper care and is not acceptable to large segments of the population, alternate to this, bio-fortification is an effective way to provide nutrients to humans (Yaseen et al., 2018). This involves increasing the grain nutrient concentration by breeding and agronomic ways and it is economical and accepted all around the world (Sperotto et al., 2012).

Wheat is a staple food for many nations around the globe, and it is among the three main cereal crops bridging the food gap (Billen et al., 2014). However, its average yield is low in many countries owing to poor nutrient management. Thus, a balanced nutrition approach is essential to improve the yield and quality of wheat crops. This approach involves integrated nutrient management along with a foliar spray of nutrients to ensure better productivity and provide nutrients to people suffering from nutrient deficiency (Chattha et al., 2017; Hassan et al., 2021). It has been reported that differences in wheat around the globe are due to growing conditions, management practices, rainfall, varieties, and soil fertility (Iwańska et al., 2020). Soil fertility plays an important role in crop production and deficiency of nutrients causes a substantial reduction in wheat yield and quality (Schjoerring et al., 2019). The modern varieties of wheat achieved a higher yield potential in recent times, nonetheless, their sensitivity has been substantially increased against environmental conditions (Schauberger et al., 2018).

The deficiency of micro-nutrients in plants is also caused by soil conditions. For instance, lower availability of micro-nutrients, soil pH, and high concentrations of bi-carbonate ions and phosphorus contribute to a reduction in the availability of micronutrients to plants (Mathpal et al., 2015; Ram et al., 2024). The micro-nutrients play a crucial role in plant physiological, and biochemical functions, yield formation, and responses to stress conditions (Putra et al., 2012; Ram et al., 2024). The enrichment of wheat-based food products is essential to remediate malnutrition problems in humans (Zhao et al., 2011; Yaseen et al., 2018). The application of fertilizers has gained momentum as a short-term approach to increase grain quality (Yaseen et al., 2018). Nonetheless, microbes applied to soil are mostly fixed in the soil and they have limited availability to plants (Watersa and Renuka, 2011). Therefore, in such conditions, the foliar spray of micro-nutrients is an effective way to apply nutrients to plants to increase the grain nutrient concentration (Pandey and Gupta, 2013). The application of foliar spray of micro-nutrients has shown promising results in increasing grain nutrient concentration (Zn, B, Cu, and Fe) to provide a balanced diet to people (Zhao and McGrath, 2009).

Different studies have documented that foliar spray of micro-nutrients significantly improved the growth, productivity, and grain bio-fortification of wheat and rice crops (Mathpal et al., 2015; Chattha et al., 2017, 2023; Hassan et al., 2019). Likewise, Pataco et al. (2017) found that exogenous application of Fe and Zn significantly increased their concentration in grain. Zinc has higher mobility in the phloem; therefore, the foliar spray is considered as an effective way to increase the grain Zn concentration. The studies reported that foliar spray of Zn can increase the grain Zn concentration by 3-4 folds depending on soil and climatic conditions (Cakmak, 2008; Hassan et al., 2019, 2021). Manganese (Mn) and boron (B) are also important nutrients that play a critical

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role in wheat growth (Broadley et al., 2012). Manganese can inhibit the activity of pathogen (*Gaumannomyces graminis*) and both B and Mn also play an important role in tillers production and final yield (Barłóg and Grzebisz, 2008). Though, many studies determined the impacts of soil and foliar applied micro-nutrients on growth and productivity of wheat. However, there is no comprehensive research about the effect of different combinations of Zn, B, and Mn) on the physiology, productivity, and grain bio-fortification of wheat. Therefore, this study was performed to find the optimum combination of foliar spray of micro-nutrients to improve the physiology, productivity, and grain bio-fortification of wheat.

Materials and methods

Experiment site

This study was conducted during 2023-2024 to determine the impacts of different combinations of micro-nutrient applications on the physiology, productivity, and grain quality of wheat. The study was conducted at the Agronomic area of the University of Agriculture Faisalabad and the study site has a semi-arid climate with hot summer and dry winter. Moreover, further, climatic conditions during the growing season are presented in *Table 1*.

Table 1. *Weather conditions during growth period*

Months	Mean maximum temperature (°C)	Mean minimum temperature (°C)	Total rainfall (mm)	Relative humidity (%)
November	27.2	13.8	3.8	68.6
December	23.0	7.2	0	70.1
January	13.3	5.3	0	82.3
February	24.6	7.5	7.5	58.1
March	28.1	11.4	5.8	55.5
April	35.8	15.8	21.2	49.5

The soil samples were collected from diverse locations and composite samples were made. Thereafter, different soil properties were determined with the standard methods of Homer and Pratt (1961). The experiment soil had loamy soil with alkaline pH (7.65), organic matter (0.88%), total nitrogen (N: 0.041%), and available phosphorus (P) and potassium (K) 12.76 and 165 mg kg^{-1}.

Treatment details

The study was performed in a randomized complete block design (RCBD) having three replications with net plot size of 6 m × 1.8 m. The study comprised 16 different combinations of micro-nutrients and details are given in *Table 2*.

Crop management

The wheat crop was sown by zero tillage method, for this seed was directly sown in the field with a seed drill to ensure the optimum seed and soil contact and uniform depth. The wheat seeds were cleaned and then sown by maintaining and row spacing of

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23 cm. The fertilizers urea (46%), di-ammonium phosphate (46% P and 18%), and sulfate of potash (50%K) were applied at the rate of 140:80:65 kg ha^{-1} to fulfill crop needs. The complete dosage of P, K, and 50% N was applied as basal dosage while the remaining N was applied in two splits (tillering and milking stage). The micro-nutrients (Zn, B, and Mn) were applied at tillering stage at the 5, 2.5, and 2 kg ha^{-1} respectively while a foliar spray of micro-nutrients (0.5%: Zn, B, and Mn) was done at flag leaf stage. The irrigations were applied according to the crop's needs and prevailing weather conditions. Furthermore, all aspects of crop management, including weed and insect pest control, were managed following the direction of the agriculture department to ensure optimal wheat growth.

Table 2. *Different combinations of micro-nutrients used in the study*

T_1	Control
T_2	Foliar water spray
T_3	Zn soil application
T_4	Zn + B soil application
T_5	Zn + Mn soil application
T_6	Zn + B + Mn soil application
T_7	B + Mn soil application
T8	Zn foliar spray
T_9	Zn + B foliar spray
T_{10}	Zn + Mn foliar spray
T_{11}	Zn + B + Mn foliar spray
T_{12}	Zn soil application + foliar spray
T_{13}	Zn + B soil application + foliar spray
T_{14}	Zn + Mn soil application + foliar spray
T_{15}	Zn + B + Mn soil application + foliar spray
T_{16}	B + Mn soil application + foliar spray

In soil application, Zn, B, and Mn were applied at rates of 5, 2.5, and 2 kg ha^{-1} respectively, whereas foliar spray was applied at the rate of 0.5%

Observations and measurements

Physiological traits

The fresh leaf samples (5 g) were taken and weighed for determination of fresh weight (FW) and then the same samples were soaked in water and weighed (TW). Thereafter, samples were oven-dried and weighed again (DW), and finally, relative water contents (RWC) were assessed with the following equation: RWC = (FW-DW)/(TW-DW) × 100. To determine chlorophyll contents; fresh leaf samples (0.5 g) were collected and homogenized with acetone and concentrations of chlorophyll-a and chlorophyll-b were determined by measuring absorbance at 663 and 645 nm. Finally, total chlorophyll concentration was determined by adding chlorophyll-a and chlorophyll-b (Lichtenthaler, 1987). For determination of catalase (CAT) fresh samples (0.5 g) were taken and homogenized by using potassium phosphate buffer (PPB). Then 100 µL plant extract was taken and mixed with 100 µL of H_2O_2, and 1000 buffer along

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and absorbance was taken at 240 nm. For determination of peroxidase activity; fresh leaf samples (0.5 g) were ground with PPB. Thereafter, 100 µL of each extract enzyme, guaiacol, and H_2O_2 were mixed and centrifuged at 15,000 rpm and absorbance was recorded at 470 nm (Zhang, 1992). In the case of ascorbate peroxidase (APX); fresh samples were ground with PPB. Then 100 µL of each enzyme extract, ascorbate, and H_2O_2 were mixed and absorbance was taken at 290 nm. For determination of superoxide dismutase (SOD), fresh samples were collected and extracted with 5 ml of PPB and centrifuged at 10,000 rpm for 10 min. Then, 100 µl of each enzyme extract and Triton, 50 µl NBT and riboflavin, and 400 µl H_2O_2 were mixed and absorbance was noted at 560 nm.

Growth and yield traits

An area of one square meter was marked in each plot and spike-bearing tillers were counted. Further, ten plants from each plot were taken and spikelets, grains per spike, spike length, and grains/spike were counted and the average was taken. The complete plots were harvested to determine grain and biological yield and a sub-sample of 1000 grains was taken to determine thousand grain weight. Moreover, the harvest index (HI) was worked out as a ratio of grain and biomass yield.

Quality traits

The wheat grains were collected dried and digested with a mixture of di-acids ($HClO_4$: HNO_3) and concentrations of Zn and Mn were determined with atomic absorption spectrophotometry (Prasad, 2006). On the other hand, the concentration of B in wheat grains was assessed by the methods of John et al. (1975), with a spectrophotometer at 420 nm.

Statistical analysis

The data collected on different traits were analyzed by analysis of variance (ANOVA) with the computer-based software Statistix 8.1. The means were separated by using least significant difference (LSD) test at $p \leq 0.05$ (Steel et al., 1997). The figures were prepared with Sigma-plot 10 and PCA analysis was done with R studio (RStudio Team, 2020).

Results

Relative water and chlorophyll contents

The results regarding the impact of different combinations of micro-nutrients on RWC and chlorophyll contents are presented in *Figure 1*. Different combinations of micro-nutrients showed a noteworthy impact on both RWC and chlorophyll contents. The maximum RWC (90.67%) were recorded in T_{15} (Zn + B + Mn Soil application + foliar spray) that remained the same with T_{14} (Zn + Mn soil application + foliar spray) and lowest RWC (62.33%) were observed in T_1 (control) followed by a foliar spray of water (*Fig. 1*). Moreover, maximum chlorophyll contents (1.73 mg g^{-1} FW) were observed in T_{15} (Zn + B + Mn Soil application + foliar spray) followed by T_{14} and T_{16} and lowest chlorophyll contents (1.02 mg g^{-1} FW) were observed in T_1 followed by T_2 and T_3 (*Fig. 1*).

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Antioxidant activities

The results about antioxidant activities as affected by diverse combinations of micro-nutrients are given in *Table 3*. The results indicate that application of different combinations of micro-nutrients application significantly increased antioxidant activities (*Table 3*). The maximum activity of APX (6.84 U/mg protein) and CAT (5.86 U/mg protein) was observed in T_{15} (Zn + B + Mn soil application + foliar spray) followed by T_{14} (Zn + Mn soil application + foliar spray) and T_{16} (B + Mn soil application + foliar spray) (*Table 1*). The lowest activity of both APX and CAT was observed in the control (T_1) that remained same with T_2 (*Table 3*). The application of different combinations of micro-nutrients also significantly increased the activity of both POD and SOD. Their maximum activity was observed in T_{15} (Zn + B + Mn soil application + foliar spray) that remained the same with T_{14} (Zn + Mn soil application + foliar spray) and the lowest activity of both POD and SOD was observed in control (T_1) that was at par with T_2 (*Table 3*). The results indicated that no spray of micro-nutrients reduced the activities of all the antioxidants while water spray caused a slight increase in the activity of all the antioxidants (*Table 3*).

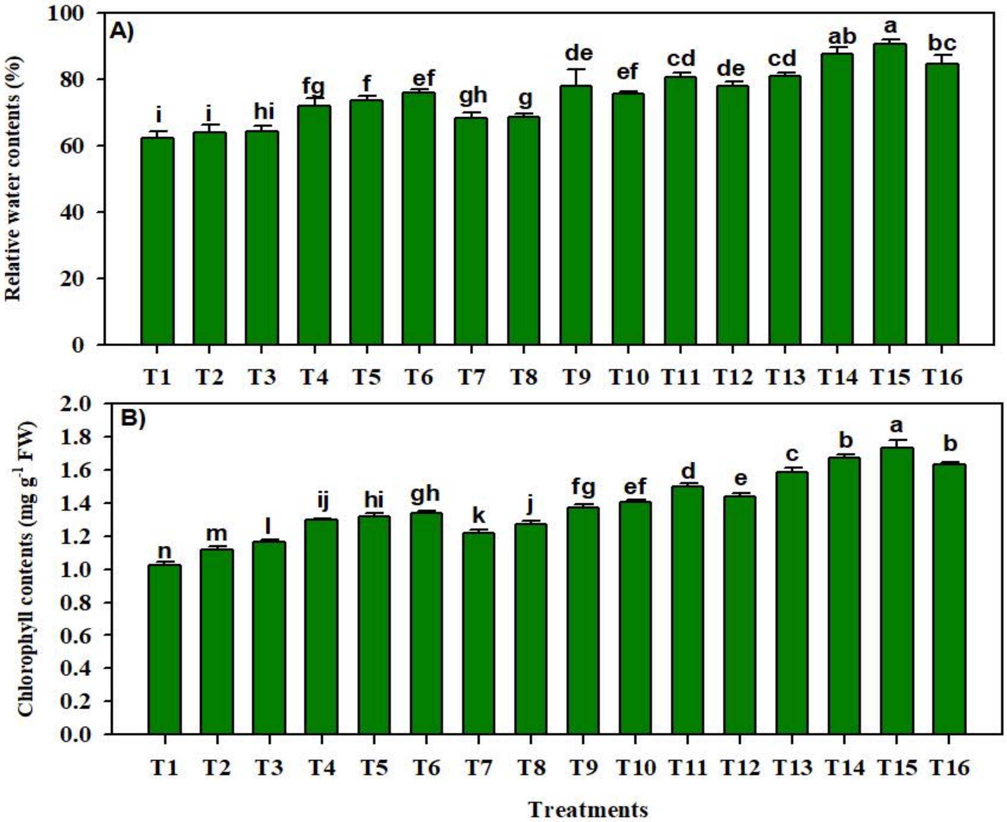

Figure 1. *Effect of different combination of micro-nutrients on the relative water and chlorophyll contents of wheat. T_1: control, T_2: foliar water spray, T_3: Zn soil application, T_4:Zn + B soil application, T_5: Zn + Mn soil application, T_6: Zn + B + Mn soil application, T_7: B + Mn soil application, T_8: Zn Foliar spry, T_9: Zn + B foliar spry, T_{10}: Zn + Mn foliar spray, T_{11}: Zn + B + Mn foliar spray, T_{12}: Zn soil application + foliar spray, T_{13}: Zn + B soil application + foliar spray, T_{14}: Zn + Mn soil application + foliar spray, T_{15}: Zn + B + Mn soil application + f oliar spray, T_{16}: B + Mn soil application + foliar spray. PH: plant height, PT: productive tillers, SL: spike length, SLPS: spikelets per spike, GPS: grains per spike. The data is mean (n = 3) and same letters on bars do not indicate significant difference among means*

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***Table 3.** Effect of different combination of micro-nutrients on antioxidant activities of wheat crop*

Treatments	APX	CAT	POD	SOD
T_1	4.77k	3.51k	3.97m	3.28m
T_2	4.84jk	3.74j	4.07m	3.35lm
T_3	4.91j	3.84ij	4.22l	3.42kl
T_4	5.53g	4.24g	4.77i	3.68i
T_5	5.63g	4.49g	4.99h	3.93h
T_6	5.77f	4.62eg	5.18g	4.04g
T_7	5.09i	3.99hi	4.49k	3.48jk
T_8	5.24h	4.05h	4.66j	3.53j
T_9	5.87f	4.74e	5.32f	4.18f
T_{10}	6.05e	4.91f	5.39f	4.28e
T_{11}	6.33d	5.20b	5.75d	4.48cd
T_{12}	6.12e	5.00f	5.52e	4.40d
T_{13}	6.47c	5.32b	5.93c	4.54c
T_{14}	6.60b	5.74ab	6.17a	4.75ab
T_{15}	6.84a	5.86a	6.24a	4.86a
T_{16}	6.54bc	5.63b	6.05b	4.69b
LSD ≤ 0.05P	**0.121**	**0.167**	**0.104**	**0.098**

T_1: control, T_2: foliar water spray, T_3: Zn soil application, T_4: Zn + B soil application, T_5: Zn + Mn soil application, T_6: Zn + B + Mn soil application, T_7: B + Mn soil application, T_8: Zn Foliar spry, T_9: Zn + B foliar spry, T_{10}: Zn + Mn foliar spray, T_{11}: Zn + B + Mn foliar spray, T_{12}: Zn soil application + foliar spray, T_{13}: Zn + B soil application + foliar spray, T_{14}: Zn + Mn soil application + foliar spray, T_{15}: Zn + B + Mn soil application + foliar spray, T_{16}: B + Mn soil application + foliar spray. The data is mean (n = 3) and same letters do not indicate significant difference among means

Yield and yield components

Different combinations of foliar and soil-applied micro-nutrients significantly affected the yield and yield components of wheat crops (*Tables 2* and *3*). The results indicate that taller plants (121 cm) with more tillers (420 m^2) and longer spikes (12.04 cm) were observed in T_{15} (Zn + B + Mn soil application + foliar spray) followed by T_{14} (Zn + Mn soil application + foliar spray) and T16 (B + Mn soil application + foliar spray).

Moreover, shorter plants (82 cm) with minimum tillers (82 m^2) and shorter spikes (8.04 cm) were observed in control (*Table 4*). The results also indicate a substantiated impact of soil and foliar applied micro-nutrient combination on TGW, grain, and biomass yield (*Table 5*). The results indicate that maximum TGW (46.73 g), grain yield (6.13 t ha^{-1}), biological yield (14.98 t ha^{-1}) and harvest index (40.96%) was recorded in T_{15} (Zn + B + Mn soil application + foliar spray) and lowest TGW (27.97 g), grain yield (3.48 t ha^{-1}), biological yield (11.39 t ha^{-1}) and harvest index (30.55%) was observed in control (*Table 5*).

Grain quality traits

The results indicated that foliar and soil-applied micro-nutrients remarkably increased the grain B, Mn, and Zn concentrations (*Fig. 2*). The maximum concentration

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of grain B was observed in T_{13} (Zn + B soil application + foliar spray) and remained at par with T_{14} (Zn + Mn soil application + foliar spray) and the lowest grain B concentration was observed in control (*Fig. 2*).

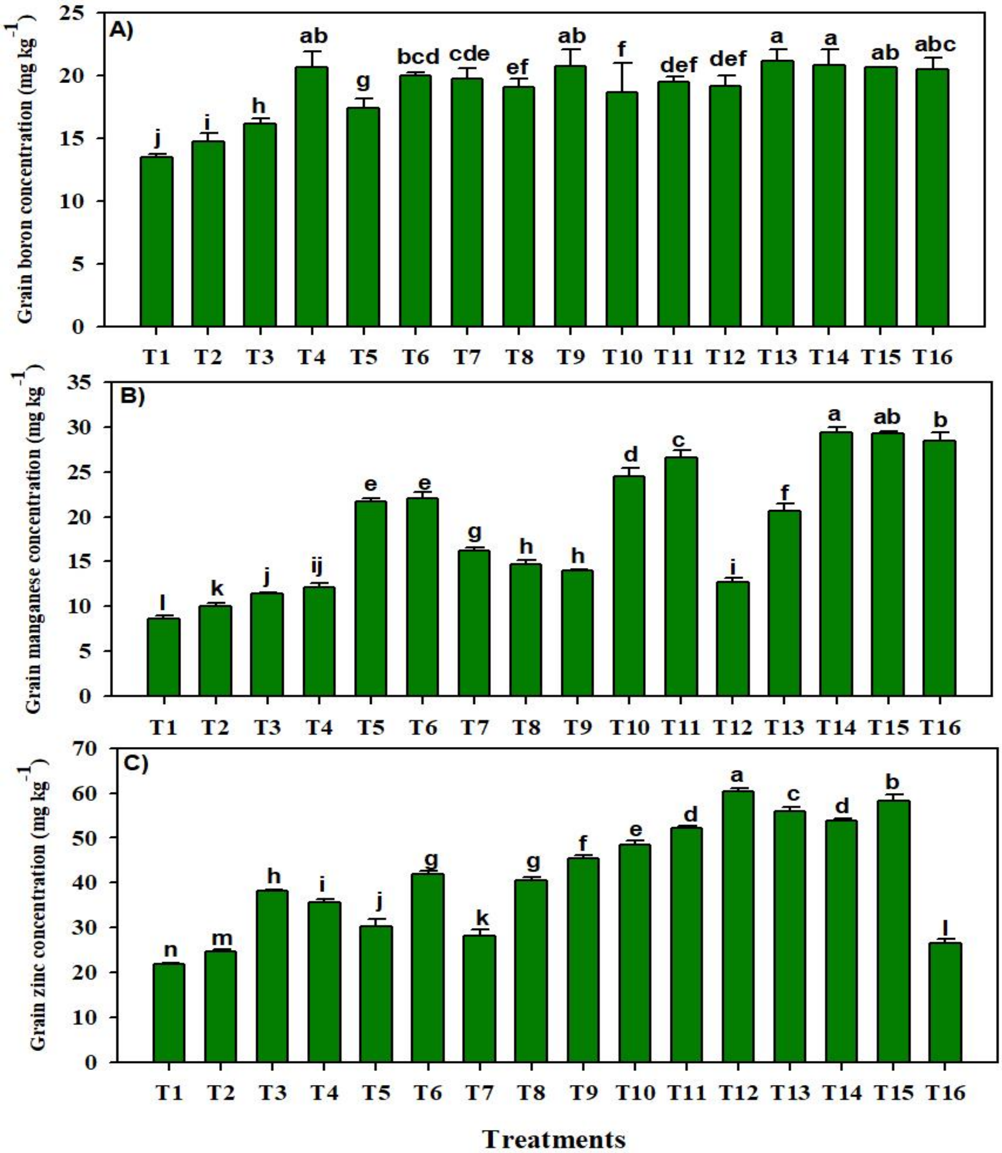

Figure 2. *Effect of different combination of micro-nutrients on grain boron, manganese and zinc concentration of wheat.* T_1*: control,* T_2*: foliar water spray,* T_3*: Zn soil application,* T_4*: Zn + B soil application,* T_5*: Zn + Mn soil application,* T_6*: Zn + B + Mn soil application,* T_7*: B + Mn soil application,* T_8*: Zn Foliar spry,* T_9*: Zn + B foliar spry,* T_{10}*: Zn + Mn foliar spray,* T_{11}*: Zn + B + Mn foliar spray,* T_{12}*: Zn soil application + foliar spray,* T_{13}*: Zn + B soil application + foliar spray,* T_{14}*: Zn + Mn soil application + foliar spray,* T_{15}*: Zn + B + Mn soil application + foliar spray,* T_{16}*: B + Mn soil application + foliar spray. PH: plant height, PT: productive tillers, SL: spike length, SLPS: spikelets per spike, GPS: grains per spike. The data is mean (n = 3) and same letters on bars do not indicate significant difference among means*

The maximum grain Mn concentration noted in T_{14} (Zn + Mn soil application + foliar spray) remained the same with T_{15} (Zn + B + Mn soil application + foliar spray)

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and T_{16} (B + Mn soil application + foliar spray) and the lowest grain Mn concentration was noted in control. In the case of Zn, maximum grain Zn concentration was noted in T_{12} (Zn soil application + foliar spray) followed by T_{15} (Zn + B + Mn soil application + foliar spray) and T_{16} (B + Mn soil application + foliar spray) and lowest grain Zn concentration was noted in control (*Fig. 2*).

Table 4. *Effect of different combination of micro-nutrients on the growth and yield traits of wheat*

Treatments	PH (cm)	PT	SL (cm)	SLPS	GPS
T_1	84i	313.67k	8.04j	12.65i	26.67j
T_2	83i	322.67jk	8.68i	13.32hi	28.00ij
T_3	82i	332.33j	9.03h	14.00ghi	29.00ij
T_4	88h	355.67gh	9.74g	15.00fgh	32.33gh
T_5	90gh	361.00g	10.02f	15.65efg	34.00fg
T_6	94g	363.33fg	10.16f	16.66def	36.33ef
T_7	93g	343.00i	9.26h	15.31efg	28.67ij
T_8	91gh	347.33hi	9.56g	14.00ghi	30.00hi
T_9	98f	372.67ef	10.22f	17.00de	37.00ef
T_{10}	100f	376.00e	10.61e	17.67cd	38.67de
T_{11}	109d	397.67c	11.07cd	18.32bcd	41.67bcd
T_{12}	105e	386.33d	10.93d	18.33bcd	40.33cd
T_{13}	111cd	407.00bc	11.22c	19.00abc	42.33bc
T_{14}	117ab	416.67ab	11.87ab	19.67ab	46.33a
T_{15}	121a	420.33a	12.04a	20.32a	47.67a
T_{16}	115bc	409.67b	11.63b	19.30abc	44.67ab
LSD ≤ 0.05P	**3.92**	**10**	**0.272**	**1.88**	**3.14**

T_1: control, T_2: foliar water spray, T_3: Zn soil application, T_4:Zn + B soil application, T_5: Zn + Mn soil application, T_6: Zn + B + Mn soil application, T_7: B + Mn soil application, T_8: Zn Foliar spry, T_9: Zn + B foliar spry, T_{10}: Zn + Mn foliar spray, T_{11}: Zn + B + Mn foliar spray, T_{12}: Zn soil application + foliar spray, T_{13}: Zn + B soil application + foliar spray, T_{14}: Zn + Mn soil application + foliar spray, T_{15}: Zn + B + Mn soil application + foliar spray, T_{16}: B + Mn soil application + foliar spray. PH: plant height, PT: productive tillers, SL: spike length, SLPS: spikelets per spike, GPS: grains per spike. The data is mean (n = 3) and same letters do not indicate significant difference among means

Principal component analysis

The results of PCA showed that two components collectively showed a total variance of 92.70% in which Dim-1 and Dim-2 have a shared of 89.2% and 3.40%, respectively (*Fig. 3*). The results showed that all the treatments effectively improved the plant growth, yield and quality traits. According to results, all the studied traits showed a positive relationship with each other (*Fig. 3*).

Discussion

Micro-nutrients play an important role in plants' growth and development. The increase in the nutritional value of crops is essential because many people are facing serious malnutrition around the globe. Agronomic bio-fortification has emerged as an

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excellent strategy to reduce the malnutrition problem in humans (Ram et al., 2024). Thus, this study was conducted to determine the effect of different combinations of micro-nutrients (B, Mn, and Zn) on the physiology, yield, and grain bio-fortification of wheat. The application of different nutrient combinations significantly improved RWC and chlorophyll contents (*Fig. 1*). The improvement in RWC with the application of micro-nutrients due to micro-nutrients application could be due to increased root tips which ensures better water uptake, thereby resulting in better RWC (Farooq et al., 2011; Atique-ur-Rehman et al., 2011).

Table 5. *Effect of different combination of micro-nutrients on the growth and yield traits of wheat*

Treatments	TGW (g)	GY (t ha^{-1})	BY (t ha^{-1})	HI (%)
T_1	27.97j	3.48k	11.39l	30.55g
T_2	29.41ij	3.53k	11.65kl	30.29g
T_3	30.60hij	3.77j	12.04jk	31.28g
T_4	35.17fg	4.32h	12.84hi	33.62ef
T_5	35.50f	4.37h	13.06gh	33.47f
T_6	36.33f	4.63g	13.18fgh	35.16de
T_7	32.22ghi	4.07i	12.27j	33.14f
T_8	33.87fgh	4.17i	12.45ij	33.52f
T_9	39.80e	4.87f	13.46efg	36.21cd
T_{10}	40.63de	4.98f	13.56ef	36.75bcd
T_{11}	42.84bcde	5.33d	14.10cd	37.84b
T_{12}	41.91cde	5.11e	13.89de	36.78bc
T_{13}	43.36bcd	5.67c	14.25cd	39.87a
T_{14}	45.23ab	5.95b	14.85ab	40.08a
T_{15}	46.73a	6.13a	14.98a	40.96a
T_{16}	44.76abc	5.77c	14.44bc	39.94a
LSD ≤ 0.05P	**3.27**	**0.11**	**0.488**	**1.60**

T_1: control, T_2: foliar water spray, T_3: Zn soil application, T_4:Zn + B soil application, T_5: Zn + Mn soil application, T_6: Zn + B + Mn soil application, T_7: B + Mn soil application, T_8: Zn Foliar spry, T_9: Zn + B foliar spry, T_{10}: Zn + Mn foliar spray, T_{11}: Zn + B + Mn foliar spray, T_{12}: Zn soil application + foliar spray, T_{13}: Zn + B soil application + foliar spray, T_{14}: Zn + Mn soil application + foliar spray, T_{15}: Zn + B + Mn soil application + foliar spray, T_{16}: B + Mn soil application + foliar spray. TGW: thousand grain weight, GY: grain yield, BY: biological yield, HI: harvest index. The data is mean (n = 3) and same letters do not indicate significant difference among means

The application of different nutrient combinations also significantly improved chlorophyll synthesis. This aligns with earlier findings of Varghese and Duraisami (2005) and Yonghou (2006), who also found that micro-nutrient application effectively increased photosynthetic pigments. The addition of micro-nutrients also improves the plant metabolism which ensures better synthesis of photosynthetic pigments (Maghsoudi et al., 2015; Brdar-Jokanović, 2020).

Micro-nutrients play a crucial role in plant physiology and in the present study application of different micro-nutrients significantly increased the activities of all the antioxidants (*Table 3*). The application of micro-nutrients favors the plants by increasing antioxidant activities which protect them from different stress conditions

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(Silva et al., 2020). Boron is an important micro-nutrient and its application substantially increased the antioxidant activities (APX, CAT, and SOD: Ardıc et al., 2009; Zhu et al., 2019). Manganese works as an important modulator antioxidant defense system and photosynthesis (Santos et al., 2017). In the current study Mn significantly enhanced antioxidant activities which aligns with Tavanti et al. (2020) and Silber et al. (2009) they found a significant increase in antioxidant activities of pepper following MN application. Zinc is an essential nutrient required for enzyme activity, RNA and DNA polymerases (Lee et al., 2020), and detoxification of ROS (Taran et al., 2016). The current study reported a marked increase in antioxidant activities (*Table 3*) which aligns with earlier studies of Sattar et al. (2022) who also found a substantial increase in antioxidant activities with Zn application.

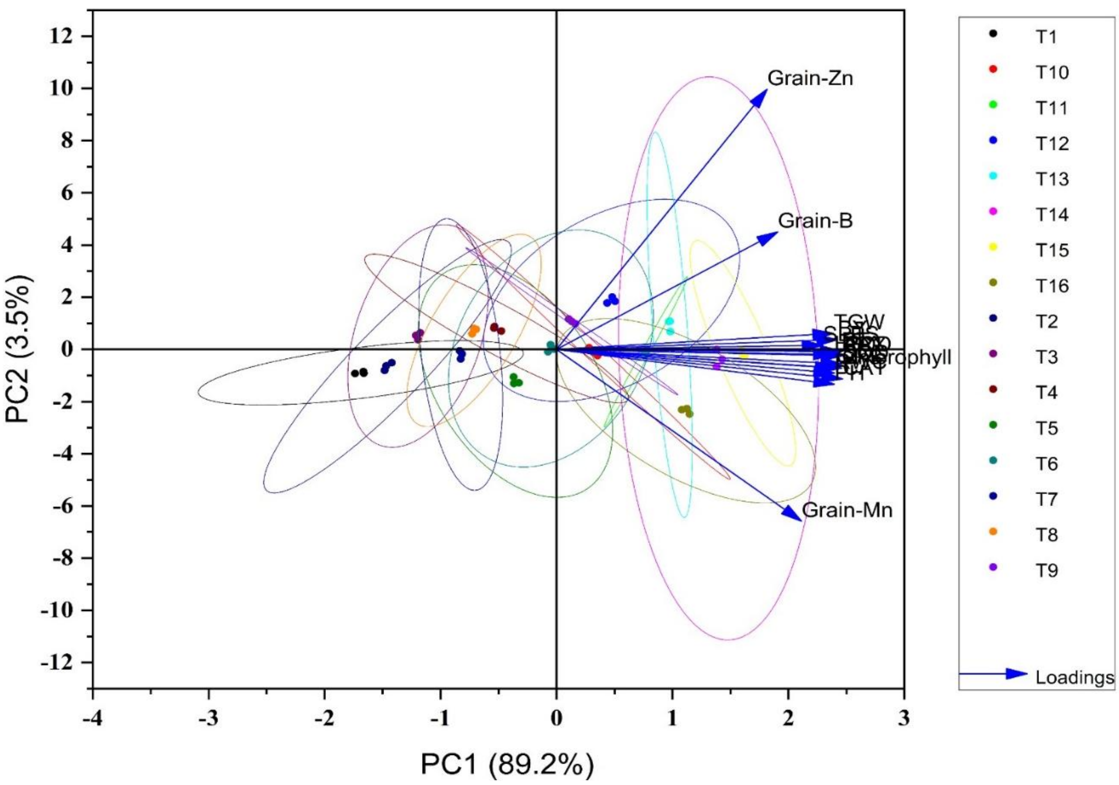

Figure 3. *The principal component analysis indicating the impact of different treatments on growth, physiological, yield and quality traits of wheat. APX: ascorbate peroxidase, CAT: catalase, POD: peroxidase, SOD: superoxide dismutase, RWC: relative water contents, PH: plant height, PT: productive tillers, SL: spike length, SLPS: spikelets per spike, GPS: grains per spike, TGW: thousand grain weight, GY: grain yield, BY: biological yield, HI: harvest index, Grain-Mn: grain manganese, grain-B: grain boron, grain-Zn: grain zinc*

The results indicate growth and yield of wheat showed a positive response to foliar and soil-applied micro-nutrients. The application of different nutrient combinations substantially improved all the studied yield traits and yield of wheat crops (*Tables 2* and *3*). The present results are in agreement with earlier investigations where authors found that B and Zn application appreciably improved the plant yield (Zhang et al., 2009; Sardar et al., 2021). Zinc is involved in chlorophyll formation and synthesis of carbohydrates, and tryptophan is an important source of IAA (Waraich et al., 2011).

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Boron also assists carbohydrate translocation within plants and it also stimulates different hormones for growth and yield (Moniruzzaman et al., 2008; Ain et al., 2021). Thus, increase in wheat yield application of Mn, B, and Zn which was linked with improved plant functioning (Kumar et al., 2018, 2019). The application of micro-nutrients also improves assimilate production which could be another important reason for improved plant growth and yield (Deswal and Vijai, 2018; Almosawy et al., 2019); Alimuddin et al., 2020). The result indicates that micro-nutrient application improved the kernel weight which could be due to improved source-sink relation which ensures better translocation of assimilates to grain. Additionally, better kernel size was also linked with improved chlorophyll contents which ensured better availability of assimilates to plants for a long time. The foliar-applied micro-nutrients is quickly absorbed by plant leaves and then they are translocation of seeds by phloem which results in the production of bold grains with better yield (Shukla et al., 2009; Hassan et al., 2019). Earlier different authors also documented that foliar application improved plant growth and yield by improving photosynthetic efficiency, antioxidant activities, and plant physiological activities (Kadam et al., 2018; Patel et al., 2022; Tuiwong et al., 2022).

The results indicate that different combinations of micro-nutrients significantly increased the grain nutrient concentration. However, the foliar application showed more promising results than the soil application. The foliar spray of B increased the grain B concentration because it regulates the translocation of assimilates to grains. It also maintains membrane integrity supports enzyme activity and encourages the translocation of photosynthates to seed therefore increasing grain B contents (Arif et al., 2006). The application of Zn particularly foliar spray significantly enhanced grain Zn concentrations. This is consistent with earlier findings of different researchers as they reported that foliar spray of Zn effectively increased grain Zn concentration than soil application (Saha et al., 2017; Sher et al., 2022). In foliar spray, Zn directly enters the leaf apoplast through the pores of stomata which enhances the concentration of Zn in developing grains (Gupta et al., 2016). The foliar spray at earlier growth stages also increases the re-translocation of Zn from plant vegetative tissues to grains increasing grain Zn concentration (Wang et al., 2018). Moreover, the foliar application of nutrients also maintains a higher pool of nutrient availability during the later stages of the plant which also increases the grain nutrient concentration (Chattha et al., 2017). The current findings also align with earlier studies where authors reported that a mixture of micro-nutrients improved the grain nutrient concentrations (El-Mogy et al., 2019; Ain et al., 2021). Contrarily, soil-applied nutrients are quickly fixed in soil which reduces the nutrient uptake by roots thereby reducing the grain nutrient concentration (Holloway et al., 2010).

Conclusion

The present study was conducted to determine the impact of different combinations of soil and foliar micro-nutrients on the physiology, yield, and grain bio-fortification of wheat crop. The results indicate a significant impact of different combinations of micro-nutrients on the physiology, yield, and grain bio-fortification of the wheat crop. The maximum improvement in plant growth, yield, and physiological functions was observed with combined soil and foliar application of boron, manganese, and zinc. However, maximum grain boron concentration was noted in T_{13} (Zn + B soil application

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+ foliar spray) while maximum grain manganese and zinc concentration were observed in T_{14} (Zn + Mn soil application + foliar spray) and T_{12} (Zn soil application + foliar spray) respectively. Nevertheless, more studies are needed to investigate the effect of these micro-nutrient combinations under different soil and climatic conditions before making it recommendation. In present study only one cultivar of wheat was used, therefore, more studies are needed to determine the genotype-specification reaction. Additionally, the experiment was conducted for one growing season, however, long term studies are direly needed for practical relevance of these findings.

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