Impact of soil applied humic acid, zinc and boron supplementation on the growth, yield and zinc translocation in winter wheat

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Abstract

The experiment was conducted to optimize the level of humic acid, zinc and boron for better growth, enhanced yield and grain quality of wheat crop cultivated under field condition. Growth characteristics, yield and its components, and zinc concentrations in straw and grain were analyzed under two application rate of humic acid (0 and 10 kg ha^{-1}), three level of boron (B_0 = 0, B_1 = 2 and B_2 = 4 kg ha^{-1}) and zinc (Zn_0 = 0, Zn_1 = 5 and Zn_2 =10 kg ha^{-1}). Results revealed that application of Zn at 10 kg ha^{-1} and B at 4 kg ha^{-1} combine with humic acid (10 kg ha^{-1}) significantly increased yield and its components (except flag leaf area under humic acid application), and Zn contents in straw. The maximum plant height, spike length and grain filling rate were recorded at Zn_2 and B_2 with humic acid application. Maximum grain yield was produced for Zn_2 and B_2 under humic acid application, which was associated with higher 1000-grain weight and biological yield. Maximum biological and straw yields were produced by application of Zn_2 and B_2 under humic acid fertilization. Maximum Zn contents in straw have been recorded for Zn_2 and B_2 under humic acid application, while maximum values of crude protein and Zn contents in Zn_2 and B_2 were statically same with Zn_1 and B_1 under humic acid application. Furthermore, the highest boron contents in grain were recorded for Zn_2 and $B_{2,}$ and these values were statistically same with Zn_2 and B_1 under humic acid application. It is concluded that application of Zn_2 and B_2 in combination with humic acid could be a good agronomic practice to enhance the zinc content in straw and grain yield of wheat crop.

Keywords: Micronutrients; productivity; crude protein; grain quality, wheat straw.

Introduction:

The demand of agricultural products is increasing day by day because of uncontrolled increasing trends of world population. To feed the increasing population, the production of edible crops should be increased. Wheat (*Triticum aestivum* L.), a major staple grain, provides a basic source of protein and calories for humans on the global scale. Although, agronomists have achieved the highest yield from last few years, however, average yield of wheat is too low, because of inadequate nutrition for crop (Kosina *et al.*, 2007). Micronutrients deficiencies have gained more concern in recent years, and various researchers reports as a major problem in south Asian countries, where rice-wheat cropping system (RWCS) exists (Johnson *et al.*, 2005). Chemical composition of grains, particularly micronutrients are significantly affected under various growing environments (Akcura *et al.*, 2019).

Zinc (Zn) deficiency in soil is responsible for the reduction in yield as well as nutritional value of cereal crops especially wheat. It is estimated that 50% of cereals around the globe are produced in Zn-deficit soils, and this topic has been achieved great attention in last two decades because of widespread inadequacy of Zn in plants and humans as well. The quantity of Zn up to 40 mg kg^{-1} in cereals' grain is recommended as nutritional requirement for a human body (Cakmak *et al.*, 2010). Low concentration of Zn in cereals' grain causes health hazards for human beings in developing countries, where cereal crops are playing vital role to compensate their daily needs. The microelements play major roles in physiological as well as in biochemical process, and regulate the growth and development, protein synthesis, photosynthetic processes and antioxidant defense system in plants (Hafeez and Saleem, 2013). Ramzan *et al.* (2020) also reported that application of micronutrients, specifically Fe and Zn, is very promising to improve the productivity and grain quality of wheat crop.

Plant requires optimal quantities of boron for better growth and development. However, according to an estimation, 49% of arable land in Pakistan and 31% on the global scale are deficient in boron (Rashid *et al.*, 2004). Boron (B), an essential micronutrient, affects the several biological process in plants including synthesis of cell wall, carbohydrate and nucleic acid metabolism, elongation of cell wall and translocation of assimilates (Herrera-Rodriguez *et al.*, 2010). B supply has no direct-effect on photosynthetic activities, however, it increases the net photosynthetic rates by harvesting different plant pigments such as carotenes and chlorophyll in plants leaves (Ganie *et al.*, 2013). Application of B is responsible to improve the growth of field crops (Khan *et al.*, 2016). Moreover, disrupting cell wall structure, diminished the progress of proton pump ATPase, decreased photosynthetic productivity, and electron transport chain is associated with deficiency of B (Marschener, 1995; Rehman *et al.*, 2018).

Humic substances are produced as a result of chemical and biological degradation of remaining of plants and animals, and provide a major source of organic carbon in the soil. Most prominent biostimulant is humic acid (HA), which has direct and indirect effects on morpho-physiological growth of plants (Peña-Méndez *et al.*, 2005). HA is mainly derived from humic substances and finally decomposed material contains 60% of organic matter in soil (Muscolo *et al.*, 2013). On other hands, HA application to the soil not only improves the various soil properties (such as structure stability, biological activity, effecting soil PH), but also results in better roots development, improve water holding capacity, carbon sequestration, cation exchange capacity, and upgrade the availability of nutrients from rhizosphere (Gümüş and Şeker, 2015). The application of HA in combination of fulvic acid can improve the seed stand establishment, and increase the availability of some macro and micronutrients (such as Zn, Fe, Cu and Mn) from rhizosphere to plants (Sharif *et al.*, 2002).

Considerable number of studies have been conducted to explore the impact of B and Zn supplementation on the growth and productivity of wheat. However, not a single study has been found to evaluate the combine effects of B and Zn with HA application on performance of wheat, when applied to the soil. Therefore, in this study we focused on soil applied B and Zn with humic acid application, and studied their effects on the grain quality, productivity, and concentrations of B and Zn in wheat-grain under field condition. We also evaluated the best levels of B and Zn under humic acid treatments, by studying the growth, yield and quality attributes of wheat crop.

Materials and Methods

Site description

One-year field study was conducted at the Agronomic Research Area, University of Agriculture Faisalabad-Pakistan during 2016-17 cropping year. The seeds of wheat (*Triticum aestivum* L. cv. Galaxy-13) were purchased from Wheat Research Institute (WRI), AARI, Faisalabad, Pakistan. The meteorological data were recorded at latitude 31^{o}N, longitude 73^{o}E and altitude of 184.4m at meteorological observatory cell established at the university. The average relative humidity 54.15%, temperature 19.36^{o}C and average rainfall 10.01 mm were recorded during the growth period (November 2016 to April 2017). The physico-chemical properties of experimental soil (George *et al.*, 2013) were as fellow: organic matter 0.81%. electrical conductivity 1.38 dS m^{-1}, pH 8.2, total nitrogen (N) 0.09%, phosphorus contents (P) 8.1 ppm, potassium contents (K) 200 ppm, hot water-soluble boron (B) 0.35 mg kg^{-1}, DTPA- Extractable zinc (Zn) 0.45 mg kg^{-1}.

Experimental details:

The study involved a randomized complete block design with factorial arrangement, and having three replications for each treatment. The area of plot was 10.8 m^{-2} (1.8m × 6m). The experiment was consisted on two HA levels; H_0 = (Control; 0 kg ha^{-1}) and H_1= (10 kg ha^{-1}), three level of boron ($B_{0\,=}$ 0, $B_{1\,=}$ 2 and $B_{2\,=}$ 4 kg ha^{-1}) and zinc (Zn_0 = 0, Zn_1 = 5 and Zn_2 = 10 kg ha^{-1}) under field condition. The Zn, B and HA were applied into the soil by using $ZnSO_4.7H_2O$, H_3BO_3 and potassium-humate respectively. All the quantity of these nutrients were applied as side dressing at time of sowing.

Crop husbandry:

Field plowing was done twice and followed by planking. The seeds were sown on November 20, 2016 with hand drill with a distance of 22.5 cm among the rows. The seed rate was kept at 120 kg ha^{-1}. At the time of sowing, one-third of N (120 kg ha^{-1} urea), all dose of P (diammonium phosphate, 80 kg ha^{-1}) and total K (murate of potash 60 kg ha^{-1}) were applied to each experimental unit. Remaining doses of nitrogen were applied in two equal splits; first at 25 days after sowing (DAS) and last with second irrigation (80 DAS). During the growth period, five numbers of irrigations were applied.

Observations and measurements:

For the estimation of grain filling rate (g day^{-1}), ten spikes were harvested randomly from each plot before and after anthesis, and placed them into oven to get constant weight. The interval for collecting spikes was 15 days. Then grain filling rate was determined with following formula;

$$\text{Grain filling rate} = \frac{W2 - W1}{T2 - T1} \times 100$$

Here, W_1 and W_2 denote first and second interval dry weight respectively, while T_1 and T_2 are time durations for first and second interval respectively.

At the milking stage, 10 plants from each experimental unit were taken to measure the flag leaf length. At maturity, 10 plants from each plot were selected randomly for measurement of plant height and spike length as well. Plant heights (cm) and spike lengths (cm) were measured by using a meter rod. After harvesting, wheat plants were tied into bundles and sun-dried for one week. The biological yield was recorded by using a spring balance while grain yield was noted by using an electronic balance. From each plot, 1000 grains were manually counted, and their weight was determined with an electronic balance. The wet-digestion protocol was followed for the estimation of Zn contents in straw and grains (Rashid, 1986). Boron contents in the grains were estimated by dry ash (Chapman and Pratt, 1961), and subsequent estimation was done by colorimetry using Azomethine-H (Bingham, 1982). The crude protein contents (%) were determined by Gunning and Hibbard method using H_2SO_4 for digestion followed by distillation of NH_3 in boric acid with the help of Kjeldhal apparatus (Jackson, 1960).

Statistical analysis

Collected data were analyzed statistically according to the ANOVA (analysis of variance) technique proposed by Fisher. Tuckey's HSD test was followed to observe the statistical differences among the variance and means of treatments (Steel *et al.*, 1997).

Results:

Table 1: Analysis of variance for growth, yield contributed attributes and grains quality parameters

Traits	Plant height (cm)	Flag leaf area (cm^2 plant^{-1})	Spike length (cm)	Grain filling rate (g day^{-1})	Biological yield (t ha^{-1})	Straw yield (t ha^{-1})
Zn	75.84***	6.97**	6.27**	5.78**	104.86***	66.22***
B	66.63***	7.21**	5.57**	4.28*	116.19***	66.65***
HA	22.67***	1.92ns	6.15*	4.65*	102.66***	39.18***
Zn × B	2.84*	0.58ns	0.23ns	0.29^{ns}	6.43***	5.47**

Zn × HA	4.35*	0.57ns	1.65ns	1.48ns	28.51***	8.60***
B × HA	3.51*	0.26ns	0.96ns	0.37ns	5.19***	7.33**
Zn × B × HA	1.11ns	0.14ns	0.09ns	0.29ns	1.80ns	3.97**
	1000-grain weight (g)	Grain yield (t ha^{-1})	Straw Zn content (μg mg^{-1})	Grain Zn content (μg mg^{-1})	Grain crude protein (%)	Grain B content (μg mg^{-1})
Zn	45.87***	63.96***	255.12***	58.94***	28.82***	108.78***
B	28.74***	49.98***	310.18***	60.24***	22.44***	119.71***
HA	99.43***	32.76***	120.80***	24.83***	23.51***	18.50***
Zn × B	8.20***	1.32ns	17.11***	6.97***	1.07ns	15.27***
Zn × HA	4.64*	6.20*	28.45***	7.70**	2.57ns	7.74**
B × HA	18.59***	7.61*	19.10***	1.67ns	3.56*	1.85ns
Zn × B × HA	8.89***	2.49ns	3.27*	1.04ns	2.08ns	0.86ns

The values are F-value. Zn, zinc; B, boron; HA, humic acid; ns denotes non-significance at P = 0.05; *, **, *** significance at P < 0.05, P < 0.01, P < 0.001, respectively

The plant height was significantly different under HA application and Zn and B treatments (Figure 1). The interaction between Zn, B and HA rates was also found significant. The maximum values of plant height (115.7 cm) were recorded under HA with Zn and B (10 and 4 kg ha^{-1}) application. The minimum plant height was recorded when Zn and B was applied at 5 and 0 kg ha^{-1}, respectively. A similar trend was also observed for Zn_0, B_1, B_0 and Zn_1 under with and without HA application. Likewise, the highest value of spike length (17.5 cm) was observed for Zn_2 and B_2 under with and without HA application, the value was statistically at par with B_1 and Zn_1. The minimum value was recorded in B_0 and Zn_1 under with and without HA application. The application of Zn and B had a significant effect on flag leaf area (FLA), however, HA application and their interaction showed non-significant effect on FLA (Figure 1). Maximum grain filling rate (0.05 g day^{-1}) was recorded under micronutrient treatment with Zn_2 and B_2 under HA application, which was statistically same with Zn_1, B_0 and B_1.

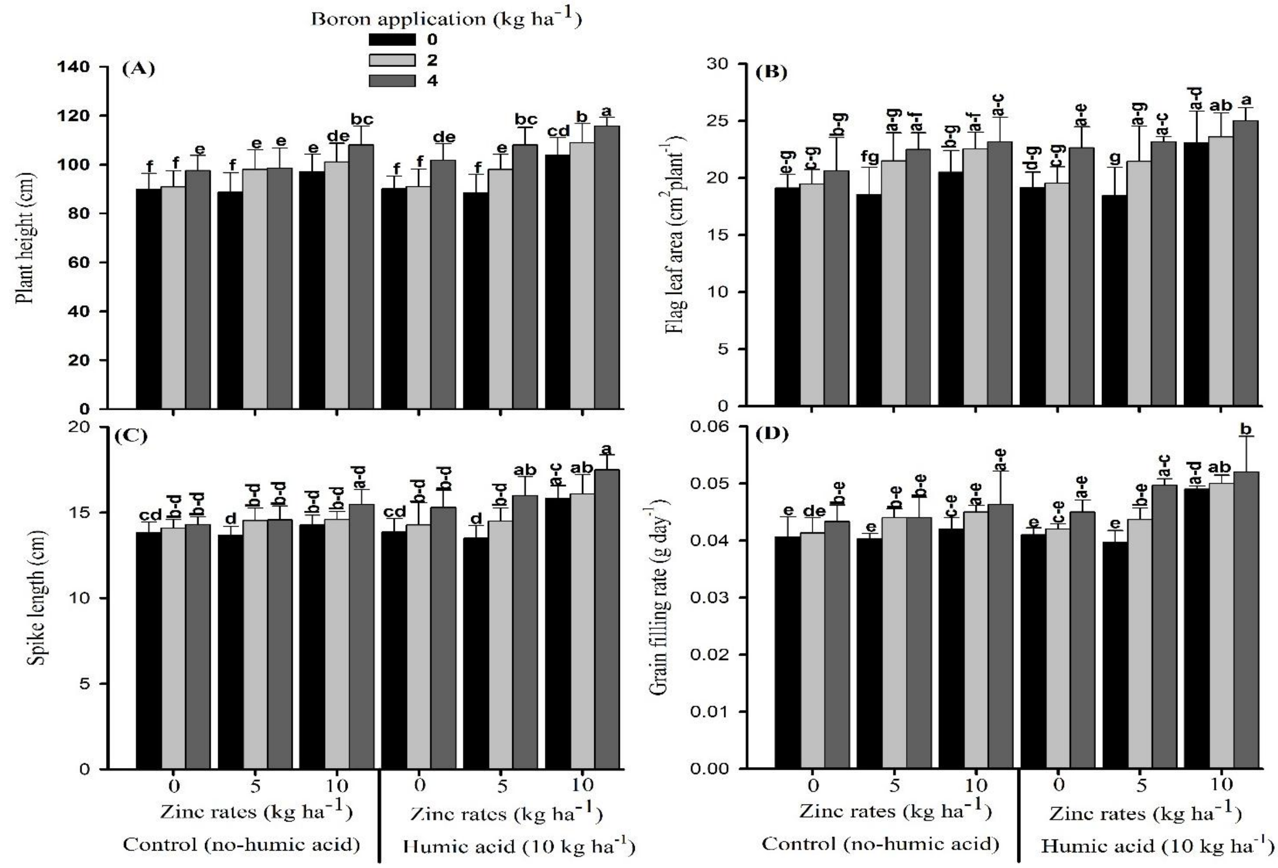

Figure 1: Plant height (A), flag leaf area (B), spike length (C), and grain filling rate (D) under two application rate of humic acid (0 kg ha^{-1} (control) and 10 kg ha^{-1})), three level of boron (B_0 = 0, B_1 = 2 and B_2 = 4 kg ha^{-1}) and zinc (Zn_0 = 0, Zn_1 = 5 and Zn_2 =10 kg ha^{-1}). Above bars, same alphabet are non-significant at 5% level of significance.

There were significant effects of Zn and B and HA application, and their interaction on biological yield, straw yield, 1000-grain weight and grain yield in winter wheat (Figure 2). Among all the treatments, Zn_2 and B_2 produced the highest biological yield of 15ton ha^{-1} under HA application (Figure 2). The minimum biological yield was recorded in B_0 and Zn_0 that was followed by B_0 under Zn_1. The maximum straw yield was recorded under Zn_2 and B_2, under HA fertilization, and it was statistically same with Zn_2 under B_1, and Zn_1 under B_2 (Figure 2). Minimum yield of straw (4.96 kg ha^{-1}) was recorded in Zn_1 and B_0. The highest 1000-grain weight (39.3 g) was noted under Zn_2 and B_2 with HA application (Figure 2). The lowest 1000-grain weight (33.06 g) was noted in Zn_1 and B_0 followed by Zn_0 with B_1, and Zn_0 with B_0. The application of HA with maximum dose of Zn and B were resulted in highest grain yield (5.2tons ha^{-1}) (Figure 2). The treatment of Zn and B (Zn_1 and B_0) showed the lowest grain yield (3.5tons ha^{-1}), that was not differ significantly with control, Zn_0 under B_1, and Zn_2 with B_0.

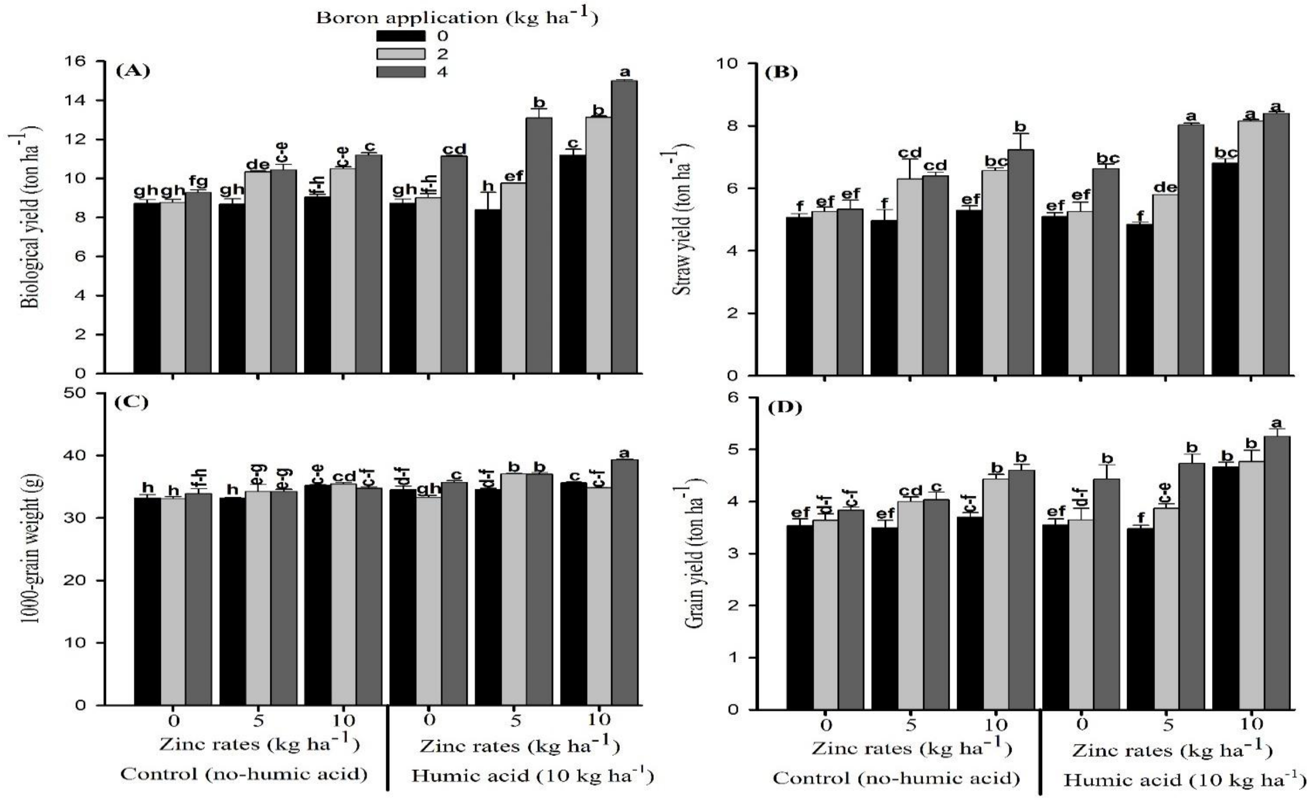

Figure 2: Biological yield (A), straw yield (B), 1000-grain weight (C), and grain yield (D) of wheat under two application rate of humic acid (0 kg ha^{-1} (control) and 10 kg ha^{-1})), three level of boron (B_0 = 0, B_1 = 2 and B_2 = 4 kg ha^{-1}) and zinc (Zn_0 = 0, Zn_1 = 5 and Zn_2 =10 kg ha^{-1}). Above bars, same alphabet is non-significant at 5% level of significance.

The effects of Zn and B and HA application and their interaction were recorded to be significant for quality parameters at 5% probability level (Table 1). The Zn contents in straw (40.5 µg g^{-1}) were observed higher with Zn_2 and B_2 under HA application, while, the minimum were recorded for Zn_1 and B_0 under with and without application of HA, followed by Zn_0 and B_1. The highest Zn contents in grain (46.3 µg g^{-1}) were found for Zn_2 and B_2 under the application of HA followed by Zn_2 under B_1, and Zn_1 under B_2 application (Figure 3). The minimum grain Zn contents (19.3 µg g^{-1}) were found in Zn_1 and B_0 which was statistically at par with Zn_0 under B_0 and B_1 with and without HA application. The highest grain crude protein (12.1%) was recorded under Zn_2 and B_2 with HA application followed by Zn_1 under B_2, and Zn_2 under B_1 and B_0 (Figure 3). The application of HA with Zn_2 and B_2 produced the maximum B content in grain (3.16 µg g^{-1}) which was statistically same with Zn_2 under B_1.

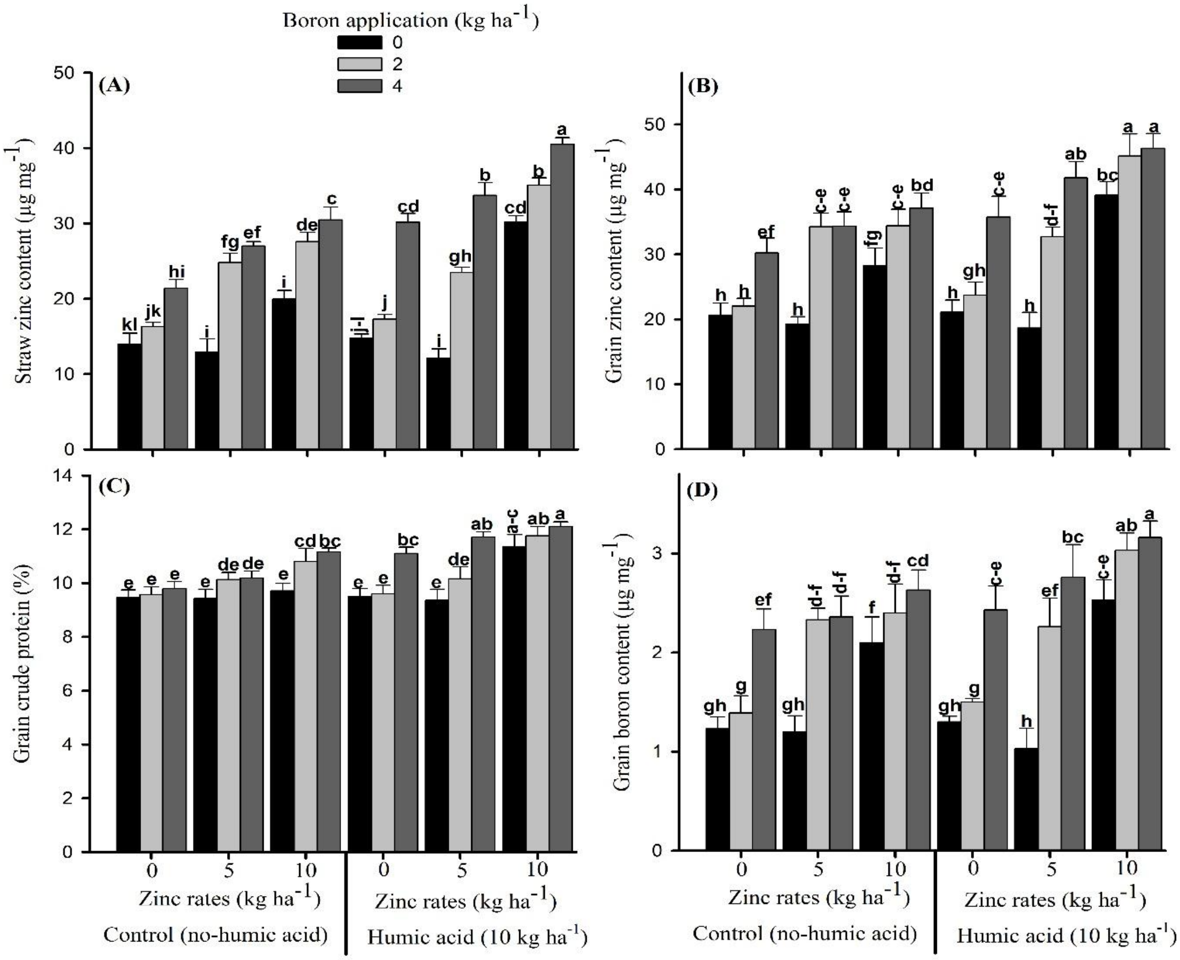

Figure 3: Straw Zn content (A), grain zinc content (B), grain crude protein (C) and grain boron content (D) under two application rate of humic acid (0 kg ha^{-1} (control) and 10 kg ha^{-1})), three level of boron (B_0 = 0, B_1 = 2 and B_2 = 4 kg ha^{-1}) and zinc (Zn_0 = 0, Zn_1 = 5 and Zn_2 =10 kg ha^{-1}). Above bars, same alphabet are non-significant at 5% level of significance.

Discussion

The results supported the hypothesis that soil applied Zn and B and HA enhanced the crop growth, grain mineral contents and productivity of wheat crop. The increased in plant height, spike length and grain filling rate were significantly influenced by sole and combine application of Zn and B and humic acid as well (Table 1). Zinc availability plays a key role during developmental and physiological processes, and the activation of different enzymes, and its deficiency causes severe disorder during physiological processes in plants. Zinc application increases the translocation of photo-assimilate, and improve the auxin (IAA) metabolism as well (Zeidan *et al*., 2010). Zinc availability is also crucial for cell division, cell expansion, improve chlorophyll pigments in leaf, increased internode distance and ultimately resulted in increased the plant height. Significant increase in FLA under Zn, B and HA fertilization in our experiment is due to role of Zn in metabolism of plant hormones like (IAA) and tryptophan (Khan *et al.*, 2006). Zinc is also important for tryptophan, which is prerequisite for IAA production, both the hormones are essential for better leaf growth (Marschener, 1995). The increment in crop growth is due to application of Zn which activates carbonic anhydrase enzyme. The carbonic anhydrase enzyme consists of Zn atom, and thus upgrades the absorption and dehydration of CO_2 and increases the photosynthesis process and biomass production (Hassan *et al.*, 2019). HA application improves the physical properties (such as microbial activity, water holding capacity) and chemical properties (such cation exchange capacity and nitrogenase activity) of soil (Khattak *et al.*, 2013).

The application of HA can increase the plant height, shoot fresh weight and shoot dry weight up to 10%, 25% and 18%, respectively (Tahir *et al.*, 2011). With increasing the rates of micronutrients and HA application, the increased yield and its parameters were reported in this experiment. The increment in biological yield, 1000-grain weight, straw yield and grain yield were associated with maximum grain per spike, higher 1000-grain weight and maximum spike length under supply of Zn, B and HA. The increased number of grains per spike, more spike length, spikelet per spike and 1000-grain weight under Zn application were also due to the better cell elongation, cell division and cell enlargement under Zn treatments (Soleimani, 2006). Zinc is essential for pollen tube formation, fertilization and pollination, and more grain yield under Zn fertilization was reported elsewhere (Pandey *et al.*, 2006). Boron as essential micronutrient, plays a vital role during reproductive phase of plants, and its accessibility for plant ensures the transfer of assimilate for developing grain, and in this way, grain yield is significantly enhanced (Iqbal *et al.*, 2017; Rehman *et al.*, 2018). The improvement in biological and straw yield under application Zn and B was associated with enhancement in morpho-phonological traits of wheat under these treatments. The fertilization of HA with combination of micronutrients enhances the grain and biological yield, spike length, 1000- grain weight of wheat, and these finding are in line with previous study (Radwan *et al.,* 2015). The increment in yield related attributes were due to high photosynthetic activity and stimulation of N metabolism under HA fertilization (Haghighi *et al*., 2012).

The results indicated that quantities of Zn and B in grains and straw, and crude protein contents in wheat grain were improved by fertilization of micronutrients (Zn and B) under application of HA. The application of B significantly enhances the N accumulation in grains, since the role of B in protein synthesis and nucleic acid metabolism is well understood (Ferdoush and Rahman, 2013). There was a positive correlation between B availability and protein content, so fertilization with B can enhance the protein contents (68-74%), increase amino acids, and essential minerals in plant (Iqtidar and Rehman, 1984). Identifying the increased Zn and B contents in straw and grain is might be due to appropriate supply of microelements to developing grains.

Conclusion

In conclusion, higher rates of Zn and B are beneficial for growth, yield and its attributes, Zn and B contents in straw and grains as well. Moreover, the application of HA in the soil can also contribute for the higher yields. Application of B and Zn at 4 and 10 kg ha^{-1} respectively improved the wheat growth, yield and grain quality in combination with HA application at 10 kg ha^{-1}.

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Conflict of Interest

The authors do not declare any conflict of interest regarding current manuscript.

Authors’ contribution

Maqsood ul Hussan, Muhammad Farrukh Saleem and Muhammad Bilal Hafeez designed and conducted the experiment. Maqsood ul Hussan, Muhammad Bilal Hafeez and Sadam Hussain collected the data. Maqsood ul Hussan, Muhammad Farrukh Saleem, Muhammad Bilal Hafeez and Shahbaz Khan analyzed the collected data and prepared first draft. Naeem Ahmad, Yasir

Ramzan and Majid Nadeem helped during the final preparation of draft. Shahbaz Khan submitting the final draft as a corresponding author.

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