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Foliar application of micronutrients improves the growth, yield, mineral contents, and nutritional quality of broccoli (*Brassica oleracea* L.)

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Abstract: Broccoli is rich in vitamins, antioxidants, minerals, free from calories and fat necessary for a healthy life. The production of broccoli is poor as compared to the developed world because of different biotic and abiotic stress. The nutritional stress is increasing with the passage of time because of low soil fertility and numerous stresses occur in the root zone of plants which reduce the uptake of minerals. Therefore, the current study was conducted on the foliar application of Zn and B to enhance broccoli production with excellent nutritional properties important for human health. Foliar application of Zn + B increased the plant height, stem length, and diameter, number of leaves, leaf length and width, root fresh and dry weight, number of florets, head diameter, and weight, chlorophyll a and b, total chlorophyll, carotenoids, nitrogen and potassium concentration in leaves and head. Stem length and diameter, number of leaves, leaf length and width, root length, number of florets, head diameter, chlorophyll a, phosphorus concentration in leaves and head, and potassium n head. So, the current study improved the growth, yield, and quality of broccoli under foliar application of Zn and B alone and their mixture. It has been concluded that the production and quality of broccoli are enhanced under foliar application of different micronutrients.

Key words: Boron, broccoli head, mineral content, zinc, vitamin C

1. Introduction

Broccoli (*Brassica oleracea* L. var. Italica) belongs to the Brassicaceae family and is famous as a cole crop. It is a highly nutritious vegetable crop among other cole crops and is widely grown in the Mediterranean region (Ain et al., 2016). Broccoli is a rich source of many vitamins, antioxidants, calories free, fat-free, and minerals important to reduce the chances of cancer problems (Thamburaj and Singh, 2003; Goncalves et al., 2011). Moreover, it is also a rich source of bioactive compounds important for a healthy life (Jeffery and Araya, 2009). Broccoli production is still limited because of poor management of nutriment applications. Different physiological disorders i.e. sword leaves, browning of heads, and hollow stems occur due to boron deficient conditions (Ali et al., 2019). Therefore, sufficient application of mineral nutrition especially Zn and B are imperative to increase crop productivity (Rivera-Martin et al., 2020; Sardar et al., 2021a). The application of different micronutrients may be involved in the numerous developmental mechanisms of plants (Ali et al., 2008).

Zn is an imperative micronutrient involved in plant growth and development by improving chlorophyll synthesis, simulation of different enzymes, and production of growth hormones (Broadley et al., 2007). Moreover, Zn is involved in the regulation of plant hormones especially indole acetic acid which improves plant growth. Calcareous, sandy, and alkaline soils had low organic matter content and, in such soils, Zn deficiency may occur (Anjum et al., 2019; Sardar et al., 2021a). Zn is necessary for improved photosynthetic pigments, carbohydrate synthesis, and protein metabolism. It acts as a cofactor of myrosinase and favors sulforaphane synthesis in broccoli (Pérez et al., 2014). Therefore, managed application of Zn is effective for the increase of crop production. Environmental stresses alter plant nutrition resulting in poor production of plants in different parts of the world (Han et al., 2020). The rapid uptake of Zn in plants had the potential to alleviate different environmental stresses. The production of reactive oxygen species (ROS) is controlled by applying Zn. This micronutrient could protect the membrane from oxidative damage under abiotic stresses (Fatemi et al., 2020).

Boron is an important micronutrient involved in plant growth and development, sugar translocation, and

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plant metabolism (Vasconcelos et al., 2011). The boron requirement is going to increase because it is effective for the increase of plant growth (Tran et al., 2020). Boron plays a vital role in different physiological aspects i.e. metabolism of nucleic acid, protein synthesis, hormone production, regulation of photosynthesis, carbohydrate synthesis, and membrane functioning (Day and Aasim, 2020). Numerous plant researchers proved that boron application is suitable for quality production (Alloway 2004; Vasconcelos et al., 2011). Boron spray plays a beneficial contribution to attaining higher crop production with excellent quality and also acts as a catalyst for the activation of numerous enzymes and other physiological processes (Alloway, 2004). Moreover, the improved vitamin C level of broccoli was recorded under boron application (Tudu et al., 2020).

For growing broccoli crops, chemical fertilizers play a major role in broccoli production with a larger head. For quality production, farmers did not have any recommendations for chemical fertilizers of micronutrients, especially zinc and boron. Environmental and soil pollution is increasing because of bombardment and improper application of chemical fertilizers. Broccoli is very responsive to different fertilizers. However, foliar application is the rapid and cheapest way of nutrient application on plant leaves (Shahzad et al., 2021; Sardar et al., 2021b). Leaves have the potential to absorb different nutrients and improve food synthesis. The proper management and application of mineral nutrition are vital for vegetable crops by improving tolerance against adverse environmental conditions for increased quality production. Therefore, the application of boron and zinc is effective to increase the quality of products with higher nutritional compounds of broccoli by improving physiological traits. The higher nutritional properties of broccoli are very important for human health.

2. Materials and methods

2.1. Experimental setup of broccoli cultivation

The current study was conducted in the vegetable and floriculture research area of the Department of Horticulture, Bahauddin Zakariya University Multan, Pakistan. The experiment was conducted in a wirehouse (a type of greenhouse with semicontrolled conditions i.e. natural light conditions (14h daylight/10h dark period). The wirehouse was covered with a polythene sheet on rainy days to cover the site. The seeds of broccoli plants (F1 hybrid) were obtained from the More Green nursery (Lahore, Punjab, Pakistan) and sown in the seedling trays containing peat moss medium. The experiment was carried out in pot culture. Each pot (30 cm in height and 25 in cm diameter) was filled with 7.5 kg of sandy loam soil from the vegetable and flower research area. The broccoli seedlings after attaining 10 cm height with 4-5 true leaves were transplanted in the already filled plastic pots in November. Weeds were removed manually after emergence. The experiment was laid out in a completely randomized design (CRD) with six replications and each treatment contained fifteen plants. The physicochemical properties of soil samples were determined before planting (Table 1). The pH, EC, available phosphorous, available potassium, organic matter, B, total nitrogen, and Zn of soil was tested according to Method 21a; U.S. Salinity Lab. Staff (1954), Method 4b; U.S. Salinity Lab. Staff (1954), Watanabe and Olsen (1965), Method 18a; U.S. Salinity Lab. Staff (1954), Walkley and Black (1934), Ponnamperuma et al. (1981), Jackson (1962), and Baker and Amacher (1982) respectively. Metrological data were collected during the study experiment (Table 2).

Three different treatments were included in the experiment i.e. T_0 (control) (pots that receive only distilled water spray), T_1 (Zn 0.5%), T_2 (B 0.5%), and T_3 (Zn 0.5 % + B 0.5%) were applied on broccoli plants at different growth stages till harvesting. All the plants including control plants received an equal amount of half-strength Hoagland solution. Tween 20 (0.01% as nonionic surfactant) was mixed in solutions before spray in all the treatments excluding control (T_0). 1st foliar application of treatments was performed after 30 days of transplanting, 2nd and spray of micronutrients were made with 15 days of interval. During the 2nd year, the same interval was used to apply foliar application on broccoli plants. The leaves and heads of broccoli were analyzed morphologically and biochemically after harvesting. At maturity, the plants were harvested from each pot for plant height, stem length and diameter, leaf length and width, root length, and head diameter were measured through a digital vernier caliper. The number of leaves, number of florets, root fresh and

Table 1. Soil properties before planting the seedlings of broccoli.

Soil analysis	Year I	Year II
Saturation (%)	24	27
pH	8.20	8.21
EC (ds/m)	5.54	5.57
Sand (%)	62.13	62.14
Silt (%)	18.23	18.25
Clay (%)	19.64	19.71
Organic matter (%)	1.0	1.3
N (%)	0.63	0.67
P (mg/kg)	11.6	11.8
K (mg/kg)	221.21	221.29
Zn (mg/kg)	0.86	0.91

Table 2. Metrological data were collected during the experiment.

Months	Year I					Year II				
	October	November	December	January	February	October	November	December	January	February
Min temp. (°C)	19	13	4	3	6	21	12	6	4	8
Max temp. (°C)	35	31	25	20	23	36	29	21	15	20
Average temp. (°C)	27	22	14.5	11.5	14.5	28.5	20.5	13.5	9.5	14
Rainfall (mm)	1.7	2.3	6.9	6.1	9	1.8	2.6	6.1	6	7
Humidity (%)	61	68	78	79	68	65	66	60	71	62
Wind speed km/h	4.7	5.5	2.4	6	8.85	4.82	5.7	2.1	4	6.4

dry weight and head weight were recorded using a digital weighing balance. After recording the growth-related data, the leaves and head samples were saved for biochemical and mineral analysis such as photosynthetic pigments, carotenoids, TSS, acidity, Vitamin C, and minerals.

The contents of chlorophyll a and b and carotenoids in 'Broccoli' head and leaves were determined according to the method outlined by Nagata and Yamashita (1992). One g of fresh leaf and the head sample was placed in a test tube with 5 mL of 80% acetone for 24 h at 0–4 °C. The next day, the extracted material was centrifuged at 8000 × g for 10 min. The supernatant was inserted into a spectrophotometer set at a wavelength of 663 and 645 nm, respectively. Absorbance for total chlorophyll, chlorophyll a and b, and carotenoids were noted at the spectrophotometer (Shimadzu, Kyoto, Japan) and concentration was calculated by the below-listed formula.

Total chlorophyll (mg/g FW) = $20.2\ A^{645} + 8.02\ A^{663}$

Chl 'a' (mg/g FW) = $100 \times [(A^{663} \times 0.0127 - A^{645} \times 0.00269)] / 0.5$

Chl 'b' (mg/g FW) = $100 \times [(0.0229 \times A^{645} - 0.00468 \times A^{663})] / 0.5$

Carotenoids (mg/g FW) = $4.7\ A^{440} - (1.38 A^{663} + 5.48\ A^{645})$

The N (nitrogen) content in the leaves and broccoli head was estimated by following the method as described by Jackson (1962). The samples taken for total nitrogen determination (0.5 g dry head and leaves) were first digested in 68% H_2SO_4 containing a mixture of K2SO4: CuSO4: FeSO4 (10: 05: 01) and distilled in micro Kjeldhal's apparatus (Haoon 1100F) using 40% NaOH. The distillate was then titrated against N/10 H2SO4 until light pink color. The nitrogen was calculated in percentage. For potassium (K^+) content determination the samples (0.5 g) from each treatment were first digested according to Chapman and Parker (1961) in a mixture of H_2O_2 (1 mL) and H_2SO_4 (5mL) in a digestion block. The samples were heated at 350 °C till the digested material become colorless. The extract was filtered and readings were noted by using a flame photometer (Sherwood 410 Scientific Ltd., Cambridge, UK). The P (phosphorous) content was measured by the molybdenum-reduced molybdophosphoric blue color method as described by Chapman and Parker (1961). The prepared sample was inserted in a spectrophotometer (Model U2020, IRMECO Uv-Vis, Germany) set at a wavelength of 420nm. The values were noted by using a standard curve. For Zn and B determination the air-dried samples of broccoli head and leaves were first wet digested in a mixture of HNO_3 and $HClO_4$ (2:1) till the digested material become colorless. The digested samples were filtered and used for the determination of Zn and B by using an Atomic Absorption Spectrophotometer (2-8200 Series Polarized Zeeman, Hitachi, Ibaraki, Japan) and expressed as mg/g of dry mass. All analyses were performed in triplicate.

The vitamin C content of broccoli head at harvest was determined by using the 2, 6- dichlorophenol-indophenol method. For vitamin C determination, 10 mL of broccoli head (freshly harvested) extract after filtration was poured into a flask, and the volume was made up to 100 mL by adding 0.4% oxalic acid. Thereafter, the samples were titrated against freshly prepared 2, 6-dichlorophenol indophenol dye, until the light pink endpoint appeared. Finally, its concentration was expressed on a fresh weight basis as mg 100 g^{-1} FW as described by Sardar et al. (2021a).

For total soluble solids (TSS) the broccoli fresh head (10g) was crushed in 10 mL distilled water and juice was filtered through Whatman filter paper. The extracted juice was homogenized and the concentration of total soluble solids was determined by using a digital refractometer (Atago, Japan) expressed as °Brix (Ali et al., 2021). For titratable acidity (TA) determination the extracted juice (10 mL) was diluted with deionized water and titrated against 0.1 N NaOH solution using 2–3 drops of phenolphthalein indicator until the pink colour endpoint was achieved. The titratable acidity was expressed as a citric acid percentage (Ali et al., 2021).

2.2. Data analysis

The data were processed with a statistical computer-based software "Statistix 8.1® (Tallahassee Florida, USA) by using an analysis of variance under CRD. The graphs were prepared with the Microsoft excel program. Treatment means were separated by using the LSD test at 5% probability.

3. Results

3.1. Growth and yield traits

Plant growth and development were significantly affected by micronutrient (Zn and B) foliar spray. The foliar application of T_3 (Zn 0.5 % + B 0.5%) resulted in taller plant height, while shorter plant height was recorded in T_0 (control) plants of broccoli (Figure 1). Stem length and diameter were recorded maximum with the application of T_1 (Zn 0.5%) and in T_3 (Zn 0.5 % + B 0.5%), while minimum stem length and diameter were recorded from T_0 (control) broccoli plants as shown in Figure 1. The number of leaves was found to be highest in the plants where T_1 (Zn 0.5%) and T_3 (Zn 0.5 % + B 0.5%) were applied, while a lower number of leaves were observed from control (T_0) broccoli plants. The maximum leaf length and width were recorded from the application of T_1 (Zn 0.5%) and T_3 (Zn 0.5 % + B 0.5%), while the minimum leaf length and width were observed in T_0 (control) plants than in other nutrients application (Figure 1). The root length of broccoli plants was longer under the foliar spray of Zn (T_1) than other treatments (T_0, T_2, and T_3). The treatment T_3 (Zn 0.5 % + B 0.5%) showed higher root fresh and dry weights, while control plants had lower root fresh and dry weights as compared to other treatments (T_1 and T_2). The number of florets and head diameter was greater under the foliar spray of T_1 (Zn 0.5 %) and T_3 (Zn 0.5 % + B 0.5%), while a lower number of florets and head diameter were observed in control plants (water spray only). The greater head weight of broccoli was recorded from T_3 (Zn 0.5 % + B 0.5%) spray than control (T_0) and all other studied nutrient applications (Figure 1).

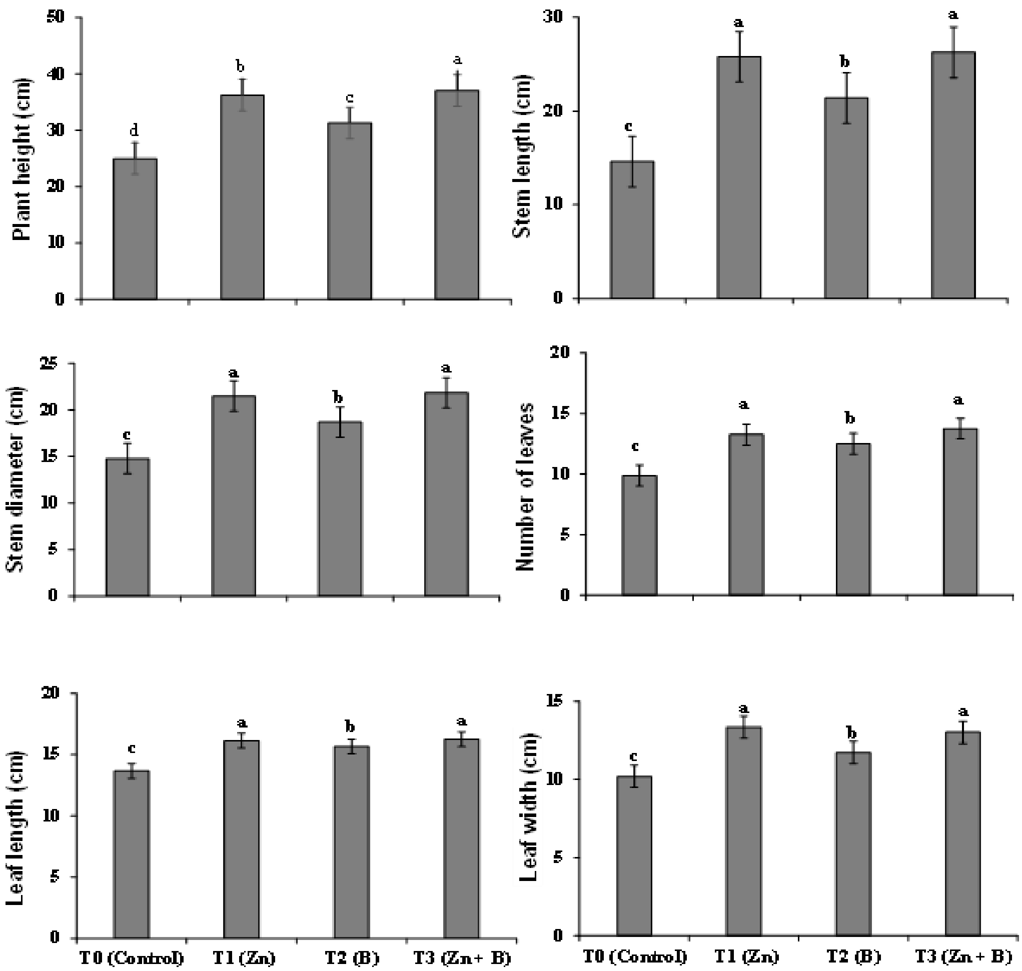

Figure 1. Effect of foliar application of boron and zinc on plant height, stem diameter, leaf length, stem length, number of leaves, and leaf width of broccoli. Vertical bar represents ± SE of means. n = 6. T_0 (control) water spray only T1 = (Zn 0.5%), T2 = (B 0.5%), T3 = (Zn 0.5% + B 0.5%).

3.2. Photosynthetic pigments level in broccoli leaves and head

The photosynthetic pigments were significantly ($p \leq 0.05$) increased after foliar application of micronutrients. Chlorophyll a was higher in the plant leaves with the application of T_1 (Zn 0.5 %) and T_3 (Zn 0.5 % + B 0.5%), while lower chlorophyll a was recorded in leaves of control (water spray only) plants. Chlorophyll b, total chlorophyll, and carotenoids were found higher in plant leaves with the foliar application of T_2 (Zn 0.5%), while the lowest amount of chlorophyll b, total chlorophyll, and carotenoids were recorded in the leaves of control (water spray only) plants of broccoli crop (Figure 2).

Chlorophyll a and b, total chlorophyll, and carotenoids of harvested broccoli head were significantly higher in micronutrient sprayed plants as compared to control (untreated). The highest amount of chlorophyll a and b, total chlorophyll, and carotenoids were recorded in the head of plants with the application of T_3 (Zn 0.5 % + B 0.5%), while lower the amount of chlorophyll a and b, total chlorophyll and carotenoids were observed from the head of control plants (Figure 3).

3.3. Minerals contents in broccoli leaves and head

Leaf micronutrient concentrations (N, P, K, Zn, and B) of broccoli plants were significantly increased after foliar application of micronutrients (Figure 4). The highest nitrogen (N) and potassium (K) concentrations were found in plants sprayed with T_3 (Zn 0.5 % + B 0.5%), while the minimum N and K were measured from control (T_0) plants. Moreover, Zn (T_1) spray also exhibited a greater level of phosphorous (P) in broccoli leaves, while lower P was estimated in control plants (Figure 4).

Foliar spray of micronutrients (Zn and B) increased the nutritional quality of broccoli florets (Figure 5). The results

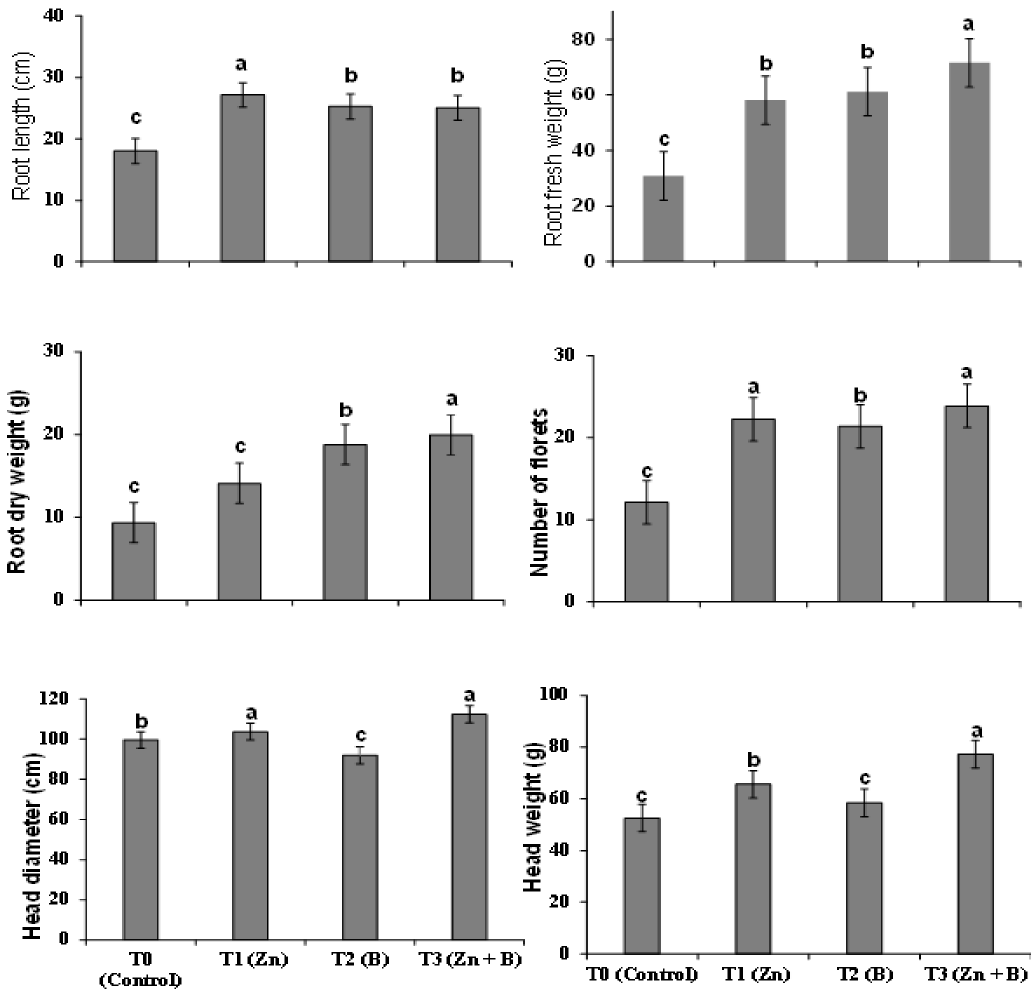

Figure 2. Effect of foliar application of boron and zinc on root dry weight, number of florets, head diameter and weight of broccoli. Vertical bar represents ± SE of means. n = 6. T_0 (control) water spray only T1 = (Zn 0.5%), T2 = (B 0.5%), T3 = (Zn 0.5% + B 0.5%).

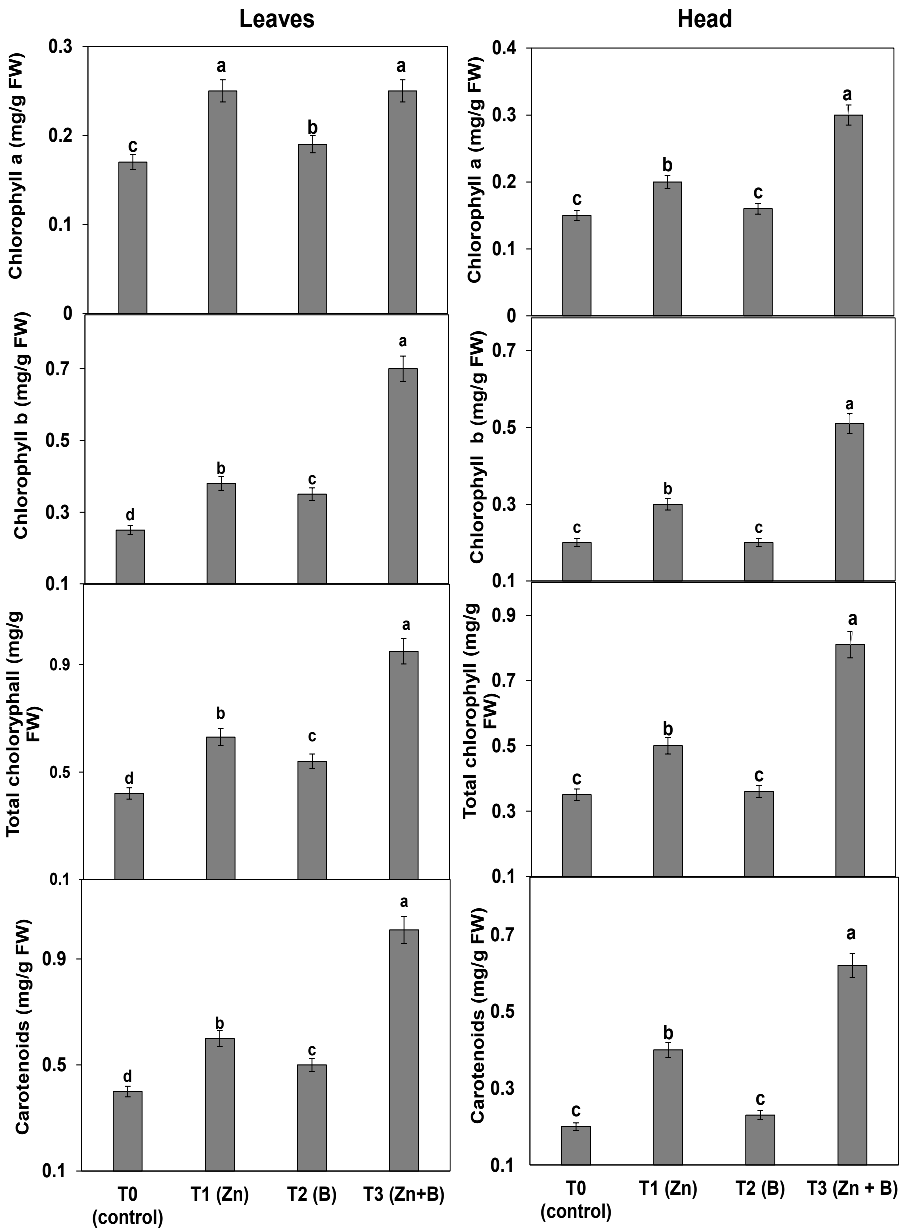

Figure 3. Effect of foliar application of boron and zinc on photosynthetic pigments of broccoli leaves and head. Vertical bar represents ± SE of means. n = 6. T_0 (control) water spray only T1 = (Zn 0.5%), T2 = (B 0.5%), T3 = (Zn 0.5% + B 0.5%).

indicated that broccoli florets sprayed with T_1 (Zn 0.5%) had the highest N contents as compared to T_0, T_1, and T_3. Furthermore, the plants sprayed with T_3 (Zn 0.5% + B 0.5%) exhibited a higher amount of P and K in the broccoli heads while lower P and K were observed in control (water spray only) (Figure 5). Higher Zn and B were accumulated in broccoli head with the foliar spray of T_3 (Zn 0.5% + B 0.5%) (Figure 6).

The TSS, TSS: titratable acidity, and vitamin C content in broccoli florets were increased significantly after foliar application of micronutrients (Figure 6). Maximum ascorbic acid (vitamin C) was determined in Zn 0.5% + B 0.5% (T_3) treated plants than T_0 (control), T_1 and T_2. Maximum TSS and TSS: TA ratio were found in broccoli florets treated with T_3 and T_1 respectively while the minimum was found in T_1 (control) plants (Figure 6). However, titratable acidity (TA) was recorded higher in T_1 treated plants as compared to other treatments (T_0, T_2, and T_3) (Figure 6).

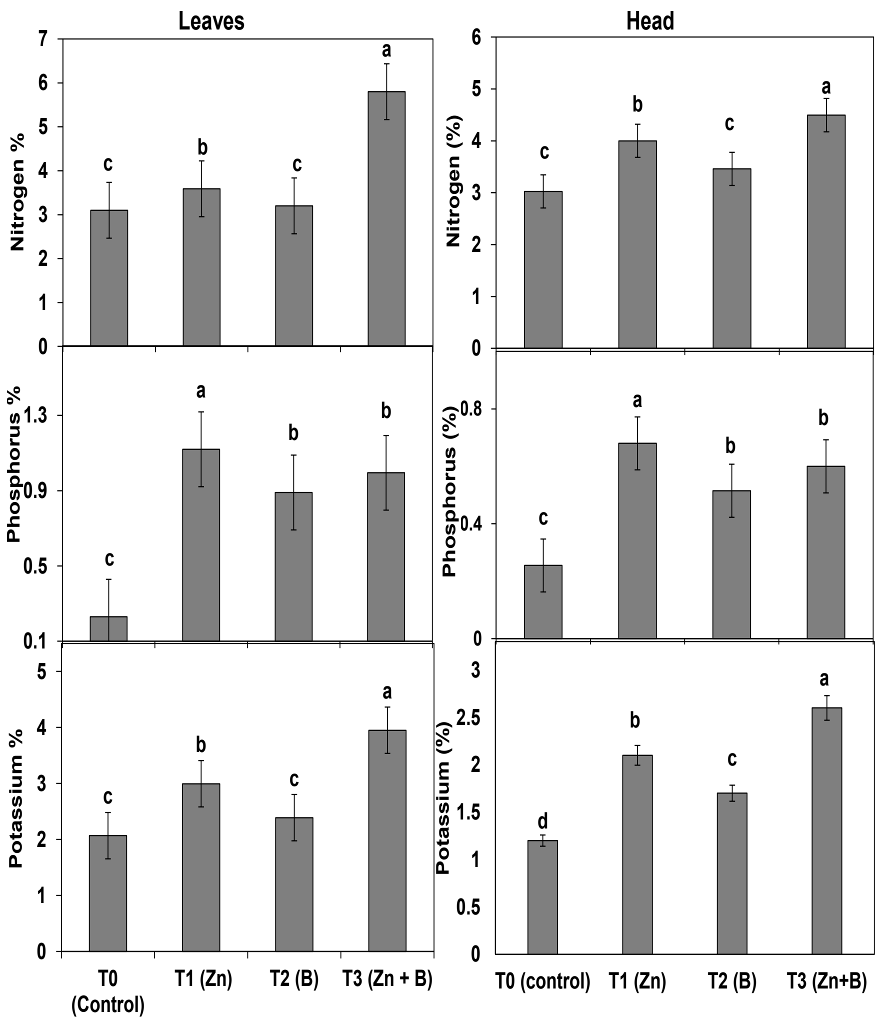

Figure 4. Effect of foliar application of boron and zinc on minerals contents in broccoli leaves and head. Vertical bar represents ± SE of means. n = 6. T_0 (control) water spray only T1 = (Zn 0.5%), T2 = (B 0.5%), T3 = (Zn 0.5% + B 0.5%).

4. Discussion

Growth and yield traits of broccoli showed a positive response to the foliar spray of Zn and B. These nutrient sprays improved the plant height, stem length and diameter, number of leaves, leaves length and width, root length, fresh and dry weight of roots, number of florets, head diameter and head weight in the present study. The current study is in agreement with earlier work because Zn and B sprays are effective for the increase of plant yield (Zhang et al., 2009; Abd EL-ALL, 2014; Sardar et al., 2021a). Zn is mainly involved in the synthesis of carbohydrates and the formation of chlorophyll content. Moreover, it is also effective for the synthesis of tryptophan which is considered an important source of IAA (Indole acetic acid) production (Waraich et al., 2011). Boron assists in the translocation of carbohydrates within plants (Moniruzzaman et al., 2008). Moreover, it also stimulates the activation of numerous hormones necessary for better growth and yield of broccoli (Ain et al., 2016). Therefore, increased growth and yield were recorded from the mixture of Zn and Boron spray in the present study. In previous work, different growth and yield stages were also responded to better by foliar spray of B and Zn (Narayanamma et al., 2007; Kumar et al., 2018; Gad and Abd El-Moez, 2011).

The mixture of Zn and B spray enhances the photosynthetic pigments i.e. chlorophyll a and b, total chlorophyll, carotenoids and leaf greenness. The current study is in agreement with the earlier work of Varghese and Duraisami (2005) and Yonghou (2006). They studied that mineral nutrients spray is effective for the increase of photosynthetic pigments which results in improved plant yield. Moreover, different plant researchers also observed the increase of photosynthetic pigments in the plant by

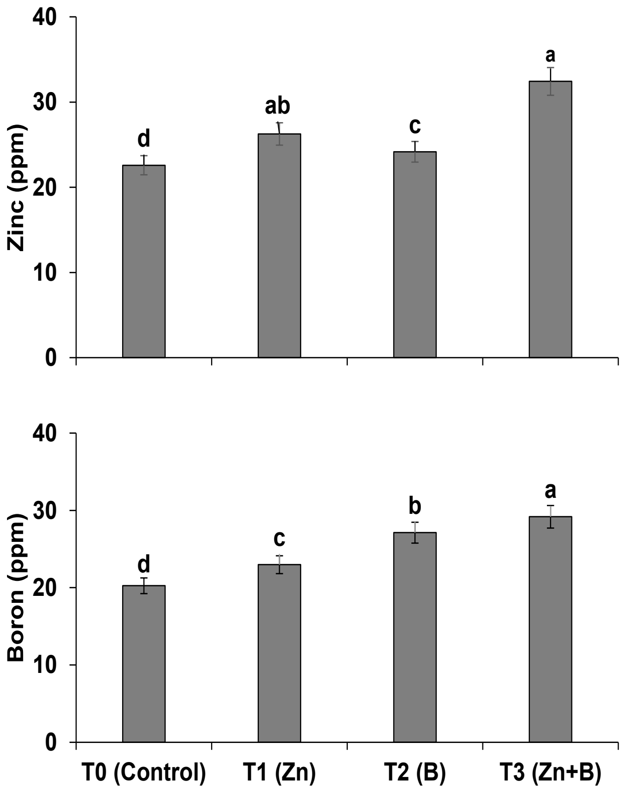

Figure 5. Effect of foliar application of boron and zinc on concentration of zinc and boron content absorbed in broccoli head. Vertical bar represents ± SE of means. n = 6. T_0 (control) water spray only T1 = (Zn 0.5%), T2 = (B 0.5%), T3 = (Zn 0.5% + B 0.5%).

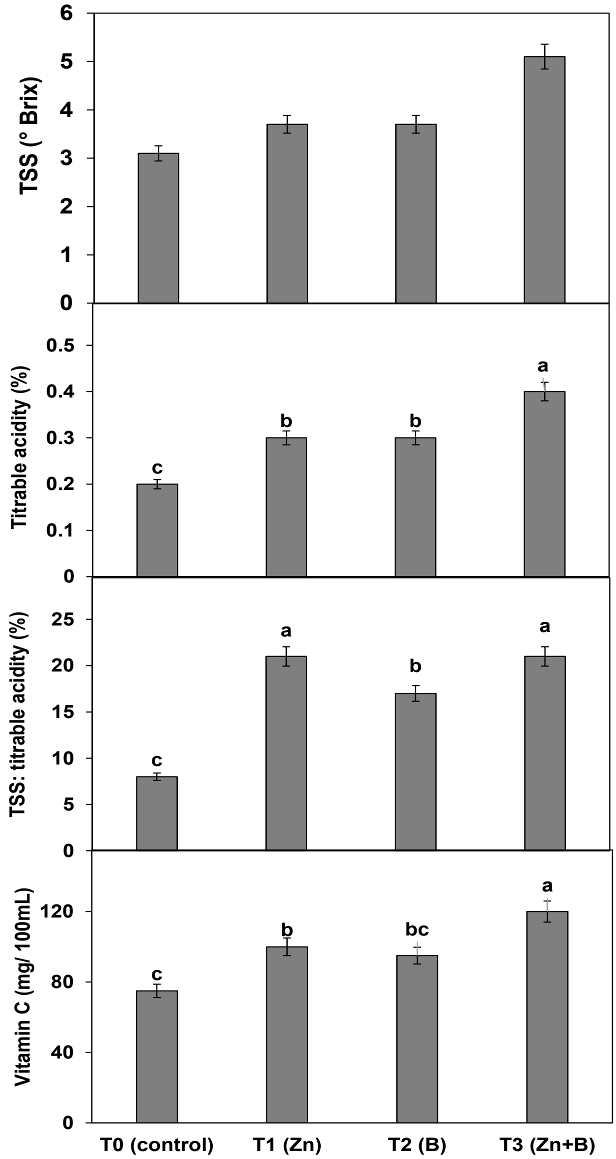

Figure 6. Effect of foliar application of boron and zinc on quality traits of broccoli. Vertical bar represents ± SE of means. n = 6. T_0 (control) water spray only T1 = (Zn 0.5%), T2 = (B 0.5%), T3 = (Zn 0.5% + B 0.5%).

improving plant metabolism under foliar application of mineral nutrients (Maghsoudi et al., 2015; Brdar -Jokanović et al., 2020). Zn and B are necessary for the increase of metabolism and translocation of carbohydrates within the plants. Photosynthetic pigments directly influence the productivity of plants. The chlorophyll is considered the principal photoreceptor involved in photosynthesis. The chlorophyll content is important to fix carbon dioxide which further produces carbohydrates and oxygen. Carotenoids are necessary for the photosynthetic process and are known as natural fat-soluble pigments frequently present in plants, algae, and bacteria. So, improved photosynthetic pigments in leaves and heads with Zn and B spray had a positive response to the increase in broccoli yield.

In light of current study results, a significant difference was recorded in N, P, and K percentages found in the leaves and head of broccoli. Foliar spray of Zn and B increased the N content in broccoli leaves and heads. The mixture of Zn and B increased the studied minerals content as proved by different plant research (Rahman et al., 2007; Abedin et al., 2012). Therefore, the current study is in accordance with these researchers because the foliar spray of Zn and B as a mixture more effective for broccoli plants attaining an increased level of minerals which ultimately improved growth and yield. The spray of Zn results in an enhanced level of K in broccoli leaves and heads. Zn and B improve the K content in leaves, while Zn increased the K content in the broccoli head. N is a major component of amino acids in proteins and a part of the chloroplast (Ouda and Mahadeen, 2008). Nitrate is absorbed by plant roots and had a negative charge therefore plants encourage cation absorption. During this process of equilibrium P and K increased in plants (Yildirim et al., 2007; Hewidy et al., 2015). Exogenous application of Zn and B in combination improved the Zn and B content in broccoli heads. So, the application of the Zn and B mixture results in increased nutrient levels in plant leaves and head which improves photosynthesis and ultimately growth and yield improve by improving synthesis and translocation of carbohydrates (El-Nemr et al., 2011; Hassini et al., 2019).

Foliar application of a mixture of micronutrients increased the quality traits of broccoli head. The current study is in line with the earlier study of (Ain et al., 2016) because they also recorded enhanced quality in those broccoli plants treated with a mixture of micronutrients. In another study, El-Mogy et al. (2019) also observed improved quality in broccoli plants under the foliar nutrient application. The mixture of micronutrients i.e. Zn and B is effective for the increased quality of broccoli plants.

5. Conclusion

In conclusion, the results revealed that the micronutrients (Zn and B), when applied exogenously on broccoli plants, increased the yield (head size and fresh weight) plant height, the number of leaves, photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids), nutrient concentration (N, P, K, Zn and B) in leaves, broccoli florets nutrient concentration (N, P, K, Zn and B), photosynthetic pigments and head biochemical quality (TSS, TA, and Vitamin C). Therefore, foliar application of 0.5% Zn + 0.5% B may be used to increase leaf nutrient contents, overall yield, and nutritional quality of broccoli.

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