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Effect of zinc and boron on improved physiological traits, productivity and phytoconstituents of carrot grown at Trans-Himalayan region

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Abstract

Natural growth and development of plants in cold arid regions are affected by drought stress, limited water availability and sandy soils, thereby reducing growth and productivity. This study was aimed at examining the combined effects of boron and zinc supplementation on the physicochemical responses of carrots in high-altitude cold desert environments using different concentrations of these micronutrients. Experiment was carried out in randomized block design (RBD) and treatment means were differentiated using the Tukey's test at a 0.05 level of probability. It was observed that in comparison to control, the foliar application of Borax @ 0.1% + $ZnSO_4$ @ 0.5% significantly improved root diameter, average root weight, yield, sucrose content, total sugar, sweetness index, and total sweetness index in carrots. The maximum chlorophyll content (9.29 CCI) in carrot leaf was observed by foliar application of Borax @ 0.2% + $ZnSO_4$ @ 1.0%, which is statistically at par with foliar application of Borax @ 0.1% + $ZnSO_4$ @ 1.0% (9.27 CCI). However, the highest glucose and fructose content was observed with a foliar application of Borax @ 0.1%. The highest nitrate (351.08 mg/100 g) content was recorded in the combined foliar application of Borax @ 0.1% + $ZnSO_4$ @ 0.5% (T_5). Among the treatments, maximum values of sulphur (210.73 mg/100 g) in carrot root were observed in Borax @ 0.2% + $ZnSO_4$ @ 0.5%.

Keywords

Boron; zinc; carrot; growth; yield; sugars; zero hunger

Introduction

Daucus carota subsp. *sativus* (carrot) is a biennial herbaceous species belonging to the Apiaceae family, typically grown in the winter season (1). Carrots are a rich source of carbohydrates, fiber, vitamins (A, B1, B2, C), antioxidants, and minerals (2). Carrot consumption reduces the risk of diabetes, high cholesterol, cardiovascular disease, hypertension, and xerophthalmia and promotes wound healing (3). Carrots are less perishable crop and stored for longer period. Still, carrot production in the Trans-Himalayan region of Ladakh faces many challenges due to extreme cold and soil characteristics. In such a climate, significant challenges are faced in agriculture in the form of drought stress, low temperature stress, poor fertility, high pH, and extremely coarse-textured sandy soil with an exceptionally low water holding capacity.

Zinc and boron concentrations varied greatly depending on the soil type as well as between the various states. Coarse textured, calcareous, alkaline, or sodic sandy texture, high pH, and low inorganic matter are generally low in available zinc and boron (4). Micronutrients are involved in all aspects of plant metabolism, including cell wall formation, photosynthesis, chlorophyll production, enzyme activity, and nitrogen fixation (5). Micronutrients helps in accelerated nutrient absorption with the help of electron transport by maintaining a balance with other nutrients, therefore small doses of these micronutrients are required by the plants for their normal growth and development (6–8). Nutrient uptake by foliar application occurs substantially more quickly than that by roots. Foliar feeding supports the physiological functions of plants, ensures ideal growth, and significantly contributes to improved quality and increased yields (9).

Zinc is the most important micronutrient for plants since it is essential for various enzyme systems and synthesis of chlorophyll and carbohydrates (10). It helps in nitrogen fixation in soil and makes it available to plants in the form of nitrates (11). Globally, zinc scarcity is primarily present in coarse-textured, sandy, calcareous soils in arid and semi-arid areas (12).

Zinc deficiency can be effectively controlled with 2-4 sprays of 0.5% $ZnSO_4$ salt solution on standing crops (13). Foliar application of $ZnSO_4$ helps maintain the chlorophyll and carbohydrate content of carrots (14, 15). Boron is the seventh fundamental element essential for plant growth, yield, and quality (16). It plays a vital role in various cellular processes such as the development of cell walls, cell elongation, cell division, cell wall strength, protein metabolism, tissue differentiation, sugar transport, and enhanced hormone transportation (17). Insufficient boron hinders plant growth, affecting multiple metabolic processes, including carbohydrate metabolism. Both zinc (Zn) and boron (B) are crucial for basic plant functions such as photosynthesis, carbohydrate metabolism, synthesis of proteins, and chlorophyll. Hence, lack of zinc and boron can reduce sink demand by slowing down growth and sugar transport, which can inhibit photosynthesis and hinder plant growth.

Zinc and boron applications have been reported to increase plants' nitrogen content and nitrate, thereby leading to enhanced growth and development (18). Zinc showed an antagonistic effect on phosphorus, whereas boron positively responded to phosphorus concentration. Anionic form of sulphur (SO_4^{2-}) is the primary source of sulphur for plants, generally in minimal amounts in the soil. It is water-soluble, so readily leaches out of the soil. Sulphur is also a component of several secondary metabolites (SMs) of plants and is required for the plant's physiological functions, growth, and development (19). Nitrate, phosphorus, and sulphur content were significantly influenced by several factorsi.e., genetic factors, soil type, environment factors, fertilizer application and rock weathering in Himalayas area. The application of zinc and boron is correlated with nitrate, phosphorus, and sulphur concentrations.

Due to the increasing demand from consumers for carrot root, higher yield and high-quality root has become a priority (20). Plant metabolic activities are influenced by foliar application of zinc sulphate and boron, significantly impacting the growth development and quality. Hence, foliar spraying of micronutrients is an effective alternative technique that can boost yield while lowering environmental risks. Sufficient study on the role of boron and zinc in enhancing production of vegetables in high-altitude Ladakh region of Trans-Himalayas has not been observed. Since carrot is a major cash crop grown in Ladakh and is an important vegetable that responds well to micronutrient application, this study can provide insights into the foliar spray of boron and zinc at different concentrations in soil to improve the physicochemical traits of crops grown. Hence, this study was conducted in the Trans-Himalayan region to investigate the influence of foliar zinc and boron application at different concentrations on the physical and biochemical constituents of carrots.

Materials and Methods

Experimental location, soil nutrition, and climatic condition

Field experiments were conducted at the Vegetable Research Experiment Station (11526±32.30 ft. amsl), Defence Institute of High Altitude Research-DRDO, Ladakh during the summer season in 2020–2021 and 2021–2022. A total of 32 different shrubs and small herbs, belonging to 25 families, have been recorded as edible, either as vegetables, medicine, or both, used directly or indirectly (21). The soil pH was estimated before the experiment and was determined as the highest value of soil pH having 7.76±0.2. The type of soil was sandy loam having EC-1.36±0.13 ms/cm, organic carbon-0.64±0.01%, and available P-6.03±3.4 ppm and K-132.25±7.4 ppm, Zn-1.41±0.2 ppm, Fe-2.38±0.3 ppm, B-2.04±0.1 ppm, Cu-1.04±1.0 ppm, Mn-0.58±0.2 ppm. However, Zn and boron contents are also based on soil type; for example, sandy soils contain relatively less nutrients, whereas clay soils are enriched.

The soils of the experimental field were sandy, coarse textured and poor water holding capacity (22). High altitude, low humidity, maximum radiation level (6-7 Kwh/mm), longer photoperiod, and one cropping season in a year (May-October) were characteristic of the area. Temperature data was recorded in weather acquisition system of DIHAR-DRDO (Table 1).

Experimental details and crop management

Zinc sulphate ($ZnSO_4$) and borax ($Na_2B_4O_7.10H_2O$) were used as sources for zinc and boron elements, respectively. There were 9 treatments, including the control and its applied foliar application in the field. The treatments were T_0 (Control, i.e. only soil application of a recommended dose of FYM), T_1-Borax @ 0.1%, T_2-Borax @ 0.2%, T_3-ZnSO4 @ 0.5%, T_4-ZnSO4 @ 1.0%, T_5- Borax @ 0.1% + ZnSO4 @

Table 1. Climatic conditions observed during period of trial.

Months	Year, 2020				Year, 2021			
	Temp. (°C)		RH (%)		Temp. (°C)		RH (%)	
	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
January	-2.82	-14.06	76.32	36.11	0.26	-15.72	58.98	27.18
February	3.50	-11.80	75.98	36.24	5.67	-10.07	51.86	19.89
March	7.60	-5.63	37.69	29.82	9.01	-4.51	51.50	15.35
April	13.60	1.98	43.98	11.99	10.65	-2.04	58.17	18.86
May	17.65	3.96	44.45	20.68	18.44	4.88	48.33	14.83
June	20.60	8.38	52.00	24.02	21.23	7.93	52.33	19.97
July	25.52	12.50	38.94	18.09	25.88	12.70	57.63	21.17
August	26.20	13.89	51.05	13.83	25.29	11.61	48.09	17.68
September	23.96	8.59	44.30	12.72	24.16	8.05	49.01	23.71
October	15.52	-2.59	33.42	10.07	14.75	-1.95	52.34	20.09
November	5.78	-8.08	53.78	23.46	7.73	-9.23	46.74	15.96
December	2.63	-12.23	59.52	23.79	2.60	-12.92	57.01	22.49

0.5%, T_6- Borax @ 0.2% + ZnSO4 @ 1.0%, T_7- Borax @ 0.1% + ZnSO4 @ 1.0%, T_8- Borax @ 0.2% + ZnSO4 @ 0.5%. The experimental field was ploughed with a tractor and the removal of stubbles and weeds was done and then a recommended dose of FYM (250 q/ha) was applied to the plots. The present experimental layout was a split plot in randomized block replicated thrice. Carrot var. Early Nantes seeds were sown in the 2nd week of June and harvesting was done in the 1st week of November. First irrigation was applied immediately after sowing of seeds and subsequent irrigation was given at 6-7 day interval. The carrot was cultivated organically and micronutrients were sprayed twice after 45 and 90 days of sowing with the help of hand operated pressure sprayer.

Morphological and physiological attributes

The carrots' leaf length, leaf width, and root length were measured with a scale and the root diameter was measured by vernier calliper. Average root weight and yield were recorded with the help of a digital weighing balance. All the growth and yield parameters were recorded at the time of harvesting. During harvesting, a random sample of 3 carrot plants from each subplot was taken, then immediately carried to the laboratory, where the roots were washed to remove any adhered soil particles. After that, plants were divided into tops and roots to measure root length (cm), root diameter (cm), root fresh weight (g), and yield/ha (q). Leaf anthocyanin and chlorophyll content were measured using a portable anthocyanin and chlorophyll meter at the time of harvesting (CCM-200 plus and ACM-200 plus, ADC Bioscientific, UK) for the 3 youngest completely expanded leaves per plant, and the mean of 3 plants from each subplot was recorded. The result was expressed as anthocyanin content index (ACI) and chlorophyll content index (CCI).

Total soluble solid determination

Total Soluble Solids (TSS) is an index of the concentration of soluble solids in vegetables. Carrot juice was extracted from the juicer-grinder machine and filtered through filter paper Whatman No. 1 and total soluble solids were determined by placing drop of carrot juice on the prism of hand refractometer (ERMA). Results were read and expressed as Brix (°B) (23).

Total acidity determination

Firstly, 10 mL juice was extracted and kept in measuring cylinder and then the volume was made with distilled water up to 100 mL. 10 mL filtrate was taken and 2-3 drops of phenolphthalein indicator were added. The filtrate was titrated with 0.1N NaOH untill the light pink colour appears (24). Total titratable acidity can be calculated as follows:

(Acidity %= (titre value × normality of NaOH × 64 × volume make up × 100) **/** (aliquot taken × weight of sample × 1000) (Eqn. 1)

The total titratable acidity was calculated in terms of citric acid and equivalent weight of citric acid is 64 g. The results were expressed in term of % acidity.

Determination of inorganic anions (nitrate, phosphate, and sulphate) and soluble sugar (glucose, fructose, and sucrose)

Inorganic anions and sugar were extracted in triplicate based on the following method (25, 26). 1 g of carrot sample was taken and homogenized at 15000 rpm in ultrapure (Type-I) water (DQ3, Millipore Waters, USA) for 2 min using a tissue homogenizer (IKA, T10 basic ULTRA-TURRAX, Germany). Homogenized samples were then sonicated using an ultrasonic bath (Ultrasonic cleaner YJ5120-1, India) (40°C at 30 min for sugar profiling and 55°C at 40 min for inorganic anions) and then centrifuged at 15000 rpm for 15 min and filtered through Whatman No. 1 filter paper. Further dilutions were carried out and the final diluted samples were passed through a 0.22 µm microporous membrane filter with a 25 mm diameter. The content of inorganic anions (nitrate, phosphate, sulphate) and sugar profiling (glucose, fructose, and sucrose) was analysed by an ion chromatography (IC) system (930 compact IC Flex, Metrohm, Switzerland). Metro Sep A Supp 5-250/4.0 anions column and RCX-30-7µm-250/4.1mm column were used for anion and sugar analysis, respectively. In the mobile phase, 3.2 mM Na_2CO_3, 1 mM $NaHCO_3$, and 5% acetone were used as eluent for anion analysis with a 0.7 mL/min flow rate. 100 mM H_2SO_4 solution was used as the suppressor solution for anion analysis. For sugar analysis, 0.1 M NaOH was used as an eluent with a flow rate of 1 mL/min. Nitrate, phosphate, and sulphate were detected using a conductivity detector and soluble sugars using an amperometry detector. The conversion of phosphate to phosphorus and sulphate to sulphur is multiplied by 0.436 and 0.333, respectively (25, 26)

Determination of sweetness index and total sweetness

Determination of the sweetness index and total sweetness index was calculated by following formula (27).

Total Sweetness Index (TSI)= (Glucose × 0.76) + (Fructose × 1.50) + (Sucrose × 1.0) (Eqn. 2)

Sweetness Index (SI)= (Glucose × 1.0) + (Fructose × 2.30) + (Sucrose × 1.35) (Eqn. 3)

Statistical analysis

The experiment was designed using a randomized block design (RBD) with 3 replications. Using SPSS 22.0 (SPSS Corporation, Chicago, Illinois, USA), one-way analysis of variance tests (ANOVA) was conducted (28). Tukey's multiple comparison test assessed the statistically significant difference during the harvested carrots at a significance level of $p<0.05$.

Results and Discussion

Growth parameters

The effect of boron and zinc on the number of leaves per plant, leaf length, and leaf width of carrots is shown in Table 2. Pooled data showed that the maximum number of leaves/plants (13.16) was recorded in the foliar application of $ZnSO_4$ @ 1.0% (T_4). But all the treatments were found statistically at par except control (T_0). Whereas, maximum leaf length (29.61 cm) and width (9.33 cm) were recorded in the foliar application of $ZnSO_4$ @ 0.5% (T_3) and $ZnSO_4$ @ 1.0% (T_4), respectively. The lowest values of leaf length and width were found in the control (T_0). The growth, yield and quality of plants are significantly influenced by the application of micronutrients such as boron and zinc. Boron and zinc are vital for several key processes, including protein synthesis, sugar transport, respiration, carbohydrate metabolism, and the regulation of plant hormones.

Yield parameters

The carrot root yield parameters were measured to check if there was an interference of treatment on their growth, development, and yield (Table 2). Evidently, the foliar application of zinc and boron significantly affects the yield and yield attributing character of carrots. Data recorded on root length as compared with control showed that the highest root length (17.25 cm) was recorded with the foliar application of $ZnSO_4$ @ 1.0% (T_4) followed by application of Borax @ 0.1% + $ZnSO_4$ @ 1.0% (T_7) and Borax @ 0.1% + $ZnSO_4$ @ 0.5% (T_5). This significant and positive increment in root diameter (34.59 mm) might be due to the application of Borax @ 0.1% + $ZnSO_4$ @ 0.5% (T_5). The average root weight and yield differed significantly by the application of micronutrients. However, the maximum average root weight (94.95 g) and yield (316.50 q/ha) were recorded with the foliar application of Borax @ 0.1% + $ZnSO_4$ @ 0.5% (T_5), which is statistically at par $ZnSO_4$ @ 1.0% (T_4) and Borax @ 0.1% + $ZnSO_4$ @ 1.0% (T_7). While the lowest yield (205.53 q/ha) was recorded in control (Table 2). These findings align with findings that increasing zinc fertilizer levels improved the root's length and diameter (29).

Leaf anthocyanin and leaf chlorophyll content

It is revealed from the data (Table 3) that all the treatments showed a non-significant effect on anthocyanin content during the field experiment. Thus, the maximum value (4.31 ACI) was found by foliar application of Borax @ 0.1% (T_1). During the experiment, carrots treated with micronutrients were found to have more chlorophyll than untreated carrot leaves. The maximum chlorophyll content was recorded in the foliar application of Borax @ 0.2% + $ZnSO_4$ @ 1.0% (T_6), followed by treatments Borax @ 0.1% + $ZnSO_4$ @ 1.0% (T_7) and Borax @ 0.2% + $ZnSO_4$ @ 0.5% (T_8). While minimum was

Table 2. Effect of preharvest application of zinc and boron on growth and yield parameters of carrot.

Treatments	No. of leaf/ plant	Leaf length (cm)	Leaf width (cm)	Root length (cm)	Root diameter (mm)	Average root wt. (g)	Yield (q/ha)
T_0	9.72±1.3^{a}	20.33±2.8^{a}	7.00±0.3^{a}	12.92±0.3^{a}	24.99±0.4^{a}	61.66±1.9^{a}	205.53±3.9^{a}
T_1	11.28±0.6^{ab}	28.00±2.8^{b}	8.56±0.3^{a}	15.50±1.8^{ab}	31.57±1.2^{bc}	75.11±8.2^{ab}	250.36±30.7^{ab}
T_2	11.89±0.6^{ab}	28.94±0.1^{b}	9.06±1.3^{a}	15.08±1.4^{ab}	31.39±0.9^{bc}	76.65±9.4^{ab}	255.49±31.7^{ab}
T_3	12.39±0.8^{ab}	29.61±1.8^{b}	9.67±1.4^{a}	15.42±0.1^{ab}	30.29±1.7^{b}	78.67±5.8^{abc}	262.21±21.1^{abc}
T_4	13.16±0.3^{ab}	29.17±0.7^{b}	9.33±1.6^{a}	17.25±0.4^{b}	30.69±3.3^{d}	89.93±3.6^{bc}	299.74±8.8^{bc}
T_5	12.34±2.8^{ab}	29.17±0.9^{b}	9.11±0.7^{a}	16.83±0.4^{b}	34.59±0.6^{cd}	94.95±9.3^{c}	316.50±27.4^{c}
T_6	11.00±0.9^{ab}	29.39±1.6^{b}	8.55±0.3^{a}	14.08±1.2^{a}	30.18±1.0^{b}	72.89±2.4^{ab}	242.95±6.3^{ab}
T_7	12.67±0.7^{ab}	27.22±1.9^{b}	8.61±0.8^{a}	17.17±0.3^{b}	29.78±1.5^{b}	86.90±2.7^{bc}	289.65±4.1^{bc}
T_8	11.95±0.6^{ab}	25.33±1.7^{ab}	8.61±0.8^{a}	15.50±0.9^{ab}	32.07±0.2^{bc}	79.33±7.7^{abc}	264.41±26.1^{abc}

[*]Different letters within each column indicate significant differences according to Tukey's test (p= 0.05). All data are expressed as mean ± standard deviation, n= 3. DW- Dry weight, FW- Fresh weight, mm= millimetre, g- gram, q= quintal, cm= centimetre. T_0 (Control, i.e. only soil application of a recommended dose of FYM), T_1- Borax @ 0.1%, T_2- Borax @ 0.2%, T_3- ZnSO4 @ 0.5%, T_4- ZnSO4 @ 1.0%, T_5- Borax @ 0.1% + ZnSO4 @ 0.5%, T_6- Borax @ 0.2% + ZnSO4 @ 1.0%, T_7- Borax @ 0.1% + ZnSO4 @ 1.0%, T_8- Borax @ 0.2% + ZnSO4 @ 0.5%.

found in control.

Titratable acidity

Biochemical analysis of the root revealed that pre-harvest foliar application of boron and zinc fertilizers either alone or in combination positively affected the root juice acidity. The highest acidity (0.37%) was observed in the foliar application of Borax @ 0.1%, which is at par with Borax @ 0.2% + $ZnSO_4$ @ 1.0% (T_6).

Total soluble solid (TSS)

The preharvest foliar application of boron and zinc at different levels was found to have a significant effect on the total soluble solid in carrots (Table 3). Total soluble solid exhibited maximum (9.15°B) in carrots under foliar application of Borax @ 0.2% (T_2), followed by $ZnSO_4$ @ 1.0% (T_4) and Borax @ 0.2% + $ZnSO_4$ @ 1.0% (T_6). Whereas minimum TSS (8.55°B) of carrot was observed in control (T_0).

Nitrate, phosphorus and sulphur

The nitrate content was significantly influenced (p⊠0.05) by the foliar application of zinc and boron. The highest nitrate (351.08 mg/100 g) content was recorded in the combined foliar application of Borax @ 0.1% + $ZnSO_4$ @ 0.5% (T_5), followed by T_6, T_4 and T_3. While the lowest value of nitrate was observed in control. The maximum phosphorus was found in treatment Borax @ 0.2% + ZnSO4 @ 1.0% (T_6) statistically at par with treatments $ZnSO_4$ @ 0.5% (T_3). However, a minimum value of phosphorous was observed in the foliar application of Borax @ 0.1% + $ZnSO_4$ @ 0.5% (T_5), followed by $ZnSO_4$ @ 1.0% (T_4), which was at par with $ZnSO_4$ @ 0.5% (T_3). Among the treatments, maximum values of sulphur (210.73 mg/100 g) were observed in foliar application of Borax @ 0.2% + $ZnSO_4$ @ 0.5% (T_8), followed by $ZnSO_4$ @ 0.5% (T_3). The minimum sulphur content was recorded by applying Borax @ 0.2% (T_2).

Sugars

The soluble sugars such as glucose, fructose, sucrose, total sugar, sweetness index, and total sweetness index are shown in Table 4. The treated carrot root significantly showed higher sugar content ($p<0.05$) when compared to the control. The highest glucose (17.90 g/100 g) and fructose content (7.86 g/100 g) were observed by foliar application of Borax @ 0.1% (T_1). While the lowest glucose and fructose content was found in the control. Maximum sucrose content was recorded in the foliar application of Borax @ 0.1% + $ZnSO_4$ @ 0.5% (T_5), which was on par with Borax @ 0.2% + $ZnSO_4$ @ 0.5% (T_8).

The pooled data showed the highest total sugar (43.51 g/100g) in the foliar application of Borax @ 0.1% + $ZnSO_4$ @ 0.5% (T_5), followed by Borax @ 0.1% (T_1). However, maximum sweetness index (60.02 SI) and total sweetness index (43.27 TSI) were observed in the foliar application of Borax @ 0.1% + $ZnSO_4$ @ 0.5% (T_5).

Discussion

Growth parameters

Micronutrients are necessary for the development and continued existence of plant life, as well as for the nourishment of crops. They showed beneficial effects on the growth, production, and quality of carrots. Zinc and boron may positively influence number of leaves, leaf length and leaf width because they are essential in numerous physiological processes like chlorophyll formation, stomatal regulation, starch utilization and cellular functions. Various studies have shown that boron and zinc, when applied foliarly, increase the vegetative growth of carrots. Zinc is necessary for synthesizing tryptophan, a precursor to Indole acetic acid (IAA), and actively participates in creating auxin, a crucial growth hormone (30). A substantial increase in leaf area with the application of zinc was observed compared to the control (31, 32). The physiological processes of plants, such as cell elongation, cell maturation, meristematic tissue formation, and protein synthesis, essentially require boron (33, 34). The application of boron in carrots accelerates growth and crop productivity. The application of boron encouraged uptake of soil nitrogen in plants, which helped

Table 3. Effect of preharvest application of zinc and boron on biochemical parameter of carrot.

Treatments	Leaf anthocyanin (ACI)	Leaf chlorophyll (CCI)	Titratable acidity (% FW)	TSS (% FW)	Nitrate (mg/100 g DW)	Phosphorus (mg/100 g DW)	Sulphur (mg/100 g DW)
T_0	3.21±0.1^{a}	6.59±0.1^{a}	0.30±0.0^{a}	8.55±0.2^{ab}	281.14±9.8^{a}	383.88±7.1bcd	170.11±15.1b
T_1	4.59±0.5^{a}	7.36±0.2^{b}	0.39±0.0^{f}	8.68±0.0^{ab}	286.15±23.2^{ab}	389.86±9.7cde	173.03±14.7b
T_2	3.93±0.3^{a}	7.56±0.1^{ab}	0.30±0.0^{a}	9.15±0.3^{b}	312.26±14.8^{abc}	401.95±6.1ef	158.58±9.6a
T_3	3.67±0.1^{a}	7.95±0.1^{c}	0.32±0.0^{b}	8.82±0.0^{ab}	325.80±3.0^{cd}	377.93±16.1bc	208.01±12.8d
T_4	4.31±0.2^{a}	8.00±0.2^{c}	0.35±0.0^{d}	9.12±0.2^{b}	326.92±11.2^{cd}	375.71±21.4abc	203.15±2.2d
T_5	3.80±0.5^{a}	8.45±0.3^{d}	0.34±0.0^{cd}	8.42±0.4^{a}	351.08±2.9^{d}	359.79±19.2a	177.76±11.3b
T_6	4.13±0.9^{a}	9.29±0.0^{c}	0.37±0.0^{e}	9.08±0.2^{ab}	327.93±11.5^{cd}	417.21±11.3f	200.29±5.9c
T_7	4.19±0.8^{a}	9.27±0.1^{c}	0.30±0.0^{a}	8.70±0.2^{ab}	312.95±14.9^{abc}	368.17±11.3ab	177.30±8.7b
T_8	3.94±0.5^{a}	8.96±0.2^{c}	0.33±0.0^{bc}	8.90±0.3^{ab}	319.81±10.6^{bcd}	396.31±10.9de	210.73±3.9de

*Different letters within each column indicate significant differences according to Tukey's test (p= 0.05). All data are expressed as mean ± standard deviation, n= 3. DW- Dry weight, FW- Fresh weight, ACI- Anthocyanin content index, CCI- Chlorophyll content index. T_0 (Control, i.e. only soil application of a recommended dose of FYM), T_1- Borax @ 0.1%, T_2- Borax @ 0.2%, T_3- ZnSO4 @ 0.5%, T_4- ZnSO4 @ 1.0%, T_5- Borax @ 0.1% + ZnSO4 @ 0.5%, T_6- Borax @ 0.2% + ZnSO4 @ 1.0%, T_7- Borax @ 0.1% + ZnSO4 @ 1.0%, T_8- Borax @ 0.2% + ZnSO4 @ 0.5%.

Table 4. Effect of preharvest application of zinc and boron on sugar content, sweetness index, and total sweetness index of carrot.

Treatments	Glucose (g/100 g DW)	Fructose (g/100 g DW)	Sucrose (g/100 g DW)	Total sugar (g/100 g DW)	Sweetness index (SI DW)	Total sweetness index (TSI DW)
T_0	14.56±0.3[a]	5.85±0.2[a]	13.37±1.2[a]	33.78±1.3[a]	46.07±2.0[a]	33.21±1.4[a]
T_1	17.90±0.5[e]	7.86±0.8[ef]	15.75±0.1[b]	41.51±0.7[e]	57.24±1.6[ef]	41.14±1.1[e]
T_2	16.66±0.2[cd]	6.92±0.3[cde]	15.82±0.4[b]	39.39±0.3[cd]	53.92±0.5[bcd]	38.85±0.3[bcd]
T_3	15.43±0.4[b]	6.03±0.3[ab]	13.88±0.8[a]	35.34±0.6[a]	48.04±1.1[a]	34.65±0.8[a]
T_4	15.15±0.2[ab]	6.07±0.2[abc]	16.33±0.1[b]	37.55±0.1[b]	51.16±0.3[b]	36.95±0.2[b]
T_5	16.34±0.1[c]	7.37±0.0[def]	19.81±0.2[c]	43.51±0.3[f]	60.02±0.4[f]	43.27±0.3[f]
T_6	16.58±0.2[cd]	6.66±0.1[abcd]	15.88±0.1[b]	39.12±0.3[bc]	53.34±0.4[bc]	38.47±0.3[bc]
T_7	17.15±0.2[d]	7.55±0.0[ef]	15.75±0.3[b]	40.45±0.6[cde]	55.78±0.7[cde]	40.11±0.5[cde]
T_8	15.31±0.1[b]	6.85±0.1[bcde]	18.81±0.2[c]	40.96±0.1[de]	56.45±0.2[de]	40.72±0.2[de]

[*]Different letters within each column indicate significant differences according to Tukey's test (p= 0.05). All data are expressed as mean ± standard deviation, n= 3. DW- Dry weight, T_0 (Control, i.e. only soil application of a recommended dose of FYM), T_1- Borax @ 0.1%, T_2- Borax @ 0.2%, T_3- ZnSO4 @ 0.5%, T_4- ZnSO4 @ 1.0%, T_5- Borax @ 0.1% + ZnSO4 @ 0.5%, T_6- Borax @ 0.2% + ZnSO4 @ 1.0%, T_7- Borax @ 0.1% + ZnSO4 @ 1.0%, T_8- Borax @ 0.2% + ZnSO4 @ 0.5%.

promote plant growth and development (35, 36).

Yield parameters

Root length, diameter, and average root weight influence carrot yield and consumer preference. The data collected in Table 2 showed that using boron and zinc as foliar applications significantly increased carrot yields. Applying low quantity of elements significantly increased the length, diameter, fresh weight and root yield (20). This may be due to increase in chlorophyll pigments, plant's photosynthetic capacity, cell division and elongation (37); additionally, as seen in the current study, improved vegetative development was the cause of the increased root yield. Root development was decreased along with the reduction in the alcohol dehydrogenase enzyme under low zinc levels (38). Therefore, zinc sulphate treatment results in better root development or biomass (29). Using boron also encourages plants' roots to absorb nitrogen, which promotes plant growth (35, 39).

Leaf chlorophyll

Compared with the control, equivalent quantities of zinc and boron were applied to increase the chlorophyll concentration. Zinc and boron do not directly affect the synthesis of chlorophyll but can affect the concentration of different elements like iron and magnesium, which are required in chlorophyll formation. Low zinc or magnesium content may be correlated with a reduction in chlorophyll content (40). The leaves chlorophyll contents and net photosynthetic rate seemed to decrease with reduced zinc contents (41, 42). A different experiment demonstrated that exogenous zinc treatment of tomato plant leaves resulted in the accumulation of leaf chlorophyll content at both low and high concentrations (40). It was observed that the chlorophyll content of hydroponically grown Jatropha seedlings increased when Zn was applied @ 0.1 and 0.5 mM, respectively (43).

Leaf anthocyanin

In the current investigation, no significant change occurs in leaf anthocyanin content of any of the treatments. Anthocyanins are secondary metabolic pigments that can rise in plants in reaction to oxidative stress, which can be brought on by several factors, including exposure to excessive metal concentrations (44, 45). Anthocyanins are generally thought to enhance plant antioxidant defence to maintain the normal physiological status of tissues that have been directly or indirectly affected by biotic or abiotic stresses (46, 47).

Titratable acidity

The improvement in titrable acidity may be attributable to the increased availability of micronutrients, particularly zinc and boron (48, 49).

Total soluble solid

The total soluble solid value in carrot root is greater when boron and zinc are applied in foliar form. The lowest value was observed in control (without micronutrients). Boron and zinc play crucial roles in the photosynthetic activities of the plant, which may explain the increase in qualitative parameters of carrot roots (50-52). This could be attributed to improved metabolic processes in total soluble solids, such as carbohydrates, organic acids, amino acids, and other inorganic compounds (15, 4, 53-55).

Nitrate

The amount of nitrate in carrots was affected by the soil condition, plant's ability to absorb nutrients, number of soluble nutrients added to the soil, amount of light and temperature in the environment, and other factors. In various treatments, nitrogen availability to plants impacted nitrate content. In Ladakh, rock weathering, soil nitrogen content and low temperature at time of harvesting are the main factors influencing the nitrate level in carrot root (25). Vegetables are the major source of dietary nitrate (80%). It is also associated with beneficial health effects since nitrate represents an important alternative pathway to bioactive nitric oxide and its important physiological roles in human vascular and immune function (56).

Phosphorus

Phosphorus (P) is vital to plant growth and is found in every living plant cell. It is involved in several key plant functions, including energy transfer, photosynthesis, transformation of sugars and starches, and nutrient movement within the plant (57). Phosphorus content was significantly influenced by pH level, soil type, environmental factors and application of fertilizer**.**

High concentrations of zinc decreased the phosphorous content, but the overall zinc content either increased or remained the same (58). Phosphorus may cause phosphorus-induced zinc shortage by interfering with zinc absorption, translocation, or use (59). Researchers hypothesized that plant roots contained phosphorus-zinc antagonists. The phosphorus absorption rate of boron-deficient plants rapidly increased upon addition, which may impact phosphate metabolism (60). The distribution of phosphate absorbed between roots and shoots is one of the more easily measurable outcomes of the phosphate metabolic pathway. Whereas, the lower phosphorus content of carrot was observed in application of zinc sulphate @ 1.0%, followed by zinc sulphate @ 0.5%.

Sulphur

The foliar application of zinc sulphate significantly influenced sulphur content in carrot root. It also plays an important role in synthesis of amino acids and proteins (61). It is also important to activate specific enzymes and vitamins, as well as to produce chlorophyll. After foliar treatment with zinc sulphate (15% sulphur), carrots may contain more sulphur. Depending on the availability of sulphur to the plant, different sulphate carriers in plants move sulphur from the rhizosphere to various plant tissues.

Sulphur helps amino acids maintain their shapes so that they are able to perform their roles in the human body. Unless there is severe protein deficiency or protein supplements with sulphur are consumed in large quantities, there is no risk of sulphur deficiency and toxicity (62).

Sugar content

Sugar yield, the most essential aspect of carrot cultivation, is influenced by root weight and sugar percentage. Several factors influence the quantity and quality of carrot root yield, including cultivar, type of weather and climate, planting and harvesting time, soil fertility and plant nutrition, particularly the type of fertilizer and timing of fertilization management and irrigation planning (63). Carbohydrate content in vegetables with roots and tubers typically ranges from 15% to 25% of their fresh weight (64), mainly in sugars. The increased sugar content of vegetables enhances their sweetness, which indicates their quality and market value, as required for further processing and consumption. Foliar application of micronutrients like Zn and Boron significantly increased the amount of sugar (65). Applying zinc considerably increased the recoverable sugar production and morphophysiological responses of sugarbeet. Boron consumption significantly raises sugar levels due to increased glucose levels in root and phloem sap (66). The application of zinc tends to increase the sugar percentage in sugar beet (67, 68). Furthermore, sweetness is dependent on the type of sugar. Sucrose, fructose, glucose, and sorbitol are the primary sugars found in fruits and vegetables. There are variations in the sweetness of each sugar. Compared with glucose and sorbitol, which have only 0.8 and 0.6 sweetness, respectively, fructose has 1.7 times that of sucrose. One widely used indicator of the acceptability of horticultural produce is the sweetness index and total sweetness index, which are based on the proportion of the particular sugar components (69).

Conclusion

Foliar application of micronutrients is most beneficial for plant growth and development. The growth, yield, and quality of plants are enhanced by the direct involvement of zinc and boron in plant metabolism. However, the combined treatment of Borax @ 0.1% + $ZnSO_4$ @ 0.5% considerably improved root diameter, average root weight, and yield. Zinc and boron had a considerable impact on the vegetative parameters. Application of Borax @ 0.1% gave maximum reducing sugar content (glucose and fructose) all over the treatments but statistically similar values for reducing sugar were found in Borax @ 0.1% + $ZnSO_4$ @ 1.0%. whereas, Borax @ 0.1% + $ZnSO_4$ @ 0.5% significantly improved carrots' sucrose, total sugar, sweetness index, and total sweetness index. Results also indicated that the application of Borax @ 0.1% + $ZnSO_4$ @ 0.5% increased the yield and quality of carrots in the Trans -Himalayan region.

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Authors' contributions

VKT carried out the studies of Zn and boron on different characteristics of carrot performed the statistical analysis and drafted the manuscript. KK, NR and MR participated in the design of the study and helped for final manuscript draft. AKP and SKD helped in the checking and removal of plagiarism in file. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

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