Defence Life Science Journal, Vol. 10, No. 1, January 2025, pp. 8-14, DOI : 10.14429/dlsj.19717

Effect of Foliar Micronutrient Application on Phytoconstituents and Mineral Composition of Carrot Grown in Trans-Himalayan Region

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ABSTRACT

The cropping season in trans-Himalayan region of Ladakh is limited to six months. Soil in the region is sandy, coarse textured, pH 7.79±0.2 and deficient in micronutrients. Carrot is one of the major root vegetable crops growing in this region. Therefore, a study investigating the effects of mineral supply on the nutritional quality (phytoconstituents and micronutrients) of carrot roots was undertaken. Maximum values of carotene and total flavonoids were recorded under the foliar application of $ZnSO_4$ @ 0.5 % (T_3), which was at par with application of $ZnSO_4$ @ 1.0 % (T_4). However, the maximum value of total phenol concentration (6.59±0.34 mg GAE/g DW) was recorded under foliar application of $ZnSO_4$ @ 1.0 % (T_4), which was at par with $ZnSO_4$ @ 0.5 % (T_3). Zinc and boron application influenced the mineral content of carrots. During plant growth, adding small amounts of zinc and boron to the feeding solutions affected the Cu, Mn and Zn concentration, in roots. Applying different amounts of minerals nutrients has the potential to improve the nutritional value and morpho-physical quality of carrots. This present study will help to understand the utilisation of optimal quantity of micronutrients to improve carrot cultivation in cold desert of Ladakh. Taking into consideration of variables like soil micronutrient deficiencies, this research opens the door to the biofortification of necessary minerals in crops.

Keywords: Antioxidant; Carrot; Carotenoid; Cold desert region; Foliar application; Phenolics compound

NOMENCLATURE

mMTE : Millimole Trolox Equivalent
GAE : Gallic Acid Equivalent
RE : Rutin Trihydrate Equivalent
rpm : Revolution Per Minute
FW : Fresh Weight
DW : Dry Weight

1. INTRODUCTION

Carrot, botanically named as *Daucus carota* L., is a popular root vegetable in the *Apiaceae* family. A carrot is a crop that grows in cool temperatures and can be planted in temperate climates during spring or in subtropical climates during autumn or winter. Carotene, a precursor to vitamin A, is abundant in orange-coloured carrots, which also have significant amounts of thiamine and riboflavin. Carrots are consumed by many people due to their rich nutritional content and many health benefits. The carrots are considered as a rich source of fibre, vitamins, carbohydrates, and various mineral salts[1]. In Leh district, vegetable production occupies 5.5 % of the total 10,319 hectares of agricultural land, with carrots accounting for only 2.0 % of the total vegetable production (Hill Council of Ladakh, 2018).

Micronutrients are important in agricultural crop production. These are important for plant growth, yield, and quality[2]. The application of micronutrient concentrations at optimum levels alleviates plant biotic and abiotic stress. Micro/macronutrients serve as cofactors of various enzymes and help in the biosynthesis of many biomolecules. Biofortification has been used to increase the nutritional value of crops. Improvement in agronomic qualities can be observed upon the administration of these components in edaphic and/or foliar doses, and it also tends to increase the number of important elements in different edible sections of plants[3].

Zinc is essential for the growth and development of plants, chlorophyll and carbohydrate biosynthesis and serves as a cofactor for enzyme systems. It also helps plant tissues withstand cold temperatures. Zinc deficiency in crops can be found in every part of the world and almost all respond positively to the application of Zn[4]. In this regard, some findings suggest that applying edaphic and foliar zinc fertilisers together can boost crop yield and enhance the concentration of zinc in agricultural commodities[5].

Boron is the seventh major element needed to support plant growth and production and is required

Received : 11 December 2023, Revised : 02 September 2024
Accepted : 23 September 2024, Online published : 24 December 2024

for cell division, cell wall formation, cell elongation, protein metabolism, sugar translocation and improved phytohormone transport[6].

Ladakh is a trans-Himalayan region and geographical condition allows cropping season of only six months during the summer. The agro-climatic region during summer favours the cultivation of European carrots in this region. Soil of Ladakh is sandy, coarse textured and high pH 7.79±0.2 and micronutrient deficiency has been found in the soil[7]. According to previous reports, the soil of Ladakh is deficient in zinc and boron[8]. Enhancing the bioavailability of mineral elements in consumable crops can help overcome mineral deficiencies. Mineral fertilisers and/or increases in mineral element solubilisation and mobilisation in the soil, boost mineral element concentrations in edible tissues. Bio-fortification is the agricultural practice of incorporating the required micronutrients into a plant, seed, or grain. Balanced nutrient content has different roles during various stages of development, growth, and reproduction. Hence, keeping these factors in mind, the present study was investigated to analyse the influence of foliar application of zinc and boron of different concentrations and at different time intervals of the growing stage of carrot to improve the productivity and mineral composition of the carrot. On the other hand, biochemical constituents such as ascorbic acid, carotenes, total flavonoids, total phenols, and total antioxidant activity in the Early Nantes variety of carrots were observed.

2. MATERIAL AND METHODS

2.1 Experimental Details

Carrot var. Early Nantes seeds were procured from the local market and field trials were conducted in randomised block design at the Agriculture Research Unit (11526±32.30 ft. AMSL), DIHAR, Ladakh during the summer season in the years 2020-21and 2021-22. Recommended doses of FYM were applied in the carrot experimental field. Foliar application of zinc sulfate and borax in different treatment combinations *viz., viz.,* (T_0-Control, T_1-Borax @ 0.1 %, T_2-Borax @ 0.2 %, T_3-$ZnSO_4$ @ 0.5 %, T_4-$ZnSO_4$ @ 1.0 %, T_5-Borax @ 0.1 % + $ZnSO_4$ @ 0.5 %, T_6-Borax @ 0.2 % + $ZnSO_4$ @ 1.0 %, T_7-Borax @ 0.1 % + $ZnSO_4$ @ 1.0 %, T_8-Borax @ 0.2 % + $ZnSO_4$ @ 0.5 %) were applied at 45 and 90 days of sowing. All the standard agronomical practices were followed during the growing period of carrots.

Figure 1. Foliar application of zinc and boron on carrot.

2.2 Ascorbic Acid

The method described by Rangana[9] was followed for the determination of ascorbic acid content in carrots. Five grams of root pulp were pulverised with a 3 % metaphosphoric acid buffer, filtered, and diluted to 100 ml. The aliquot of 5 ml was titrated with 2, 6-dichlorophenol indophenol dye till colour changed to light pink. It was expressed as mg/100 g of root pulp.

2.3 Carotene

To analyse the carotene content, the crude pigment was extracted in organic solvent as described by Rangana[10]. A 5 g sample was gently crushed with 10 ml of petroleum ether and diluted with 3 % acetone in water containing 5 % sodium sulfate (Na_2SO_4). A 10 ml aliquot was placed in a separating funnel. Aliquot mixed with distilled water, and left at room temperature for 5 minutes. This extraction was repeated 3 to 4 times to enhance carotenoid recovery. After draining the aqueous layer, the supernatant was collected in a test tube and using UV-Visible Spectra Max i3x Spectrophotometer the absorbance was measured at 452 nm with petroleum ether as the blank. A β-carotene standard curve was used to estimate total carotenoids, reported as μg/100 g.

2.4 Determination of Total Flavonoids Content (TFC) and Total Phenolic Content (TPC)

One-gram pulverized dry sample was homogenized in 20 ml methanol, followed by centrifugation for 10 min at 10,000 rpm. The extraction was done thrice at room temperature in dark conditions. The supernatant was collected using filter paper (WhatmanNo.1 for further quantification.

The extract was used to assess TFC following aluminium chloride method[11], using Rutin Trihydrate as standard. Extracts (300 μl) at various concentrations of Rutin trihydrate were diluted with 1200 μl of distilled water. Then, 90 μl of sodium nitrite reagent (0.724 M) was added and incubated for 5 minutes at room temperature. 90 μl of aluminium chloride (0.749 m) was added and mixture was incubated for 6 minutes. Next, 600 μl of sodium hydroxide (1.0 M) and 720 μl of deionised water were added, to make up total volume to 3000 μl. At 510 nm, absorbance was measured and results were expressed in mg rutin trihydrate equivalent (RE) per g dry weight.

TPC from extracted samples was evaluated by using FC reagent method with minor changes[11]. A mixture of 9 ml of deionized water and 1 ml of extracts at various concentrations was prepared, then 1 ml of FC reagent was added and incubated for 5 minutes at room temperature. Further 2 ml of 20 % sodium carbonate solution and set to incubation at room temperature for 60 minutes in the dark. Absorbance at 750 nm was measured. A standard curve of gallic acid was prepared with different concentrations. Results were expressed in mg (gallic acid equivalent) per g dry weight.

2.5 Total Antioxidant Activity

The total antioxidant activity was determined using Apak[12] method. Trolox was used to create a standard curve and results of total antioxidant activity were expressed as mMTE/L. Carrot Juice was extracted from a 20 g root sample and filtered using filter paper (Whatman No. 1). In a test tube, 1 ml each of copper chloride (1.705 g/L), neocuproine (1.562 g/L ethanol), and ammonium acetate buffer (pH 7, 19.27 g/250 ml) were combined with 0.1 ml of the extracted juice. 6 ml of volume was adjusted with distilled water. 30 minutes incubation at room temperature was done and absorbance at 450 nm was measured.

2.6 Determination of Micronutrient

The mineral content of the carrot samples was determined using the AOAC method[13] with some modifications. 200 mg of samples were digested (microwave digestion, Analytik Jena) with 8 ml of acid mixture (2.0ml HCl and 6.0ml HNO_3). A clear digest was diluted with distilled water up to 50 ml. This prepared sample solution was used to determine sodium, magnesium, manganese, iron, copper, and zinc using AAS (Analytik Jena). Mineral concentrations were calculated using standard calibration curves and expressed as mg/100g.

2.7 Statistical Analysis

All data were tested by Analysis of Variance (ANOVA) and significance of the mean difference test was analysed by using SPSS, Inc. version 22.0 ($p<0.05$). The data were expressed as mean ± standard deviation.

3. RESULT AND DISCUSSION

3.1 Ascorbic Acid

The foliar application of zinc and boron with various concentrations had no significant impact on the ascorbic acid content of carrot roots (Table 1). Ascorbic acid in carrots is less than other vegetables like peas, brassicas, and spinach. Hence are not considered to be a significant source of ascorbic acid[14]. The ascorbic acid in carrots was observed from 7.17 to 9.01 mg/100 g FW among the treatments. But statistically, significant change was not observed in the ascorbic acid content of carrot after foliar application of zinc and boron.

3.2 Carotenes

The concentrations of carotenoids found in the carrot var. Early Nantes grown under the various zinc and boron treatments were presented in Table 1. A significant influence on carotene content was observed by the application of zinc and boron. The maximum value of carotenes (4298.78 µg/100 g FW) was found in the foliar application of $ZnSO_4$ @ 0.5 % (T_3), which was statistically at par with the application of $ZnSO_4$ @ 1.0 % (4294.41 µg/100 g FW). A minimum value of carotenes (3533.04 µg/100 g FW) was also found in the foliar application of Borax @ 0.2 %. The overall phytochemical content in carrots may be affected by genetic and abiotic stresses due to the high-altitude condition. The high or low carotene concentration for given treatments depends on several factors, including morphological and physiological traits of the cultivar, as well as growth factors. In the present study, it was found that boron affected carotenes concentration. More specifically, a larger dose of boron might have decreased the level of carotenoids in plants. Whereas, adequate doses of zinc applied in carrots, might increase carotenes in plants. Zinc treatment increases carotenoid content, stomatal conductance, antioxidant enzyme activities and chlorophyll content, while decreasing electrolyte leakage and water loss in dry conditions[15]. The application of zinc enhances carotenoid contents that have an important role to overcome photo oxidative damage.

Table 1. Combined and individual effects of foliar application of boron and zinc treatments on phenolics compounds and antioxident content in carrot

Treatments	Ascorbic acid (mg/100gFW)	Carotenes (µg/100g FW)	TFC (mgRE/g DW)	TPC (mgGE/g DW)	Antioxidant (mMTE/L FW)
T_0	$7.17±0.43^{a}$	$4105.64±51.64^{d}$	$1.60±0.31^{bc}$	$5.61±0.86^{abc}$	$59.06±1.92^{a}$
T_1	$7.42±0.43^{a}$	$3696.98±12.80^{b}$	$1.28±0.09^{abc}$	$5.33±0.10^{abc}$	$64.37±2.14^{ab}$
T_2	$7.63±0.69^{a}$	$3533.04±42.68^{a}$	$0.90±0.09^{a}$	$4.14±0.81^{a}$	$63.80±0.74^{ab}$
T_3	$9.01±0.6^{9}$a	$4298.78±91.94^{e}$	$1.75±0.22^{c}$	$6.27±0.39^{c}$	$64.17±2.09^{ab}$
T_4	$8.51±1.08^{a}$	$4294.41±24.81^{e}$	$1.73±0.09^{c}$	$6.59±0.34^{c}$	$66.62±1.88^{b}$
T_5	$8.97±0.69^{a}$	$3764.59±61.53^{b}$	$1.41±0.13^{abc}$	$6.00±0.37^{bc}$	$67.81±1.92^{b}$
T_6	$8.51±0.47^{a}$	$3928.86±47.55^{c}$	$0.90±0.36^{a}$	$4.50±1.00^{ab}$	$67.44±1.63^{b}$
T_7	$8.97±0.69^{a}$	$4208.23±48.30^{de}$	$1.05±0.11^{ab}$	$5.32±0.09^{abc}$	$66.42±2.87^{b}$
T_8	$8.97±0.69^{a}$	$3815.10±25.45^{bc}$	$0.87±0.24^{a}$	$4.42±0.52^{ab}$	$65.03±1.32^{b}$

According to Tukey's test, different letters within each column indicate significant differences (P = 0.05). All data are presented as mean ± standard deviation n=3

3.3 Total Flavonoids Content (TFC)

In contrast to ascorbic acid, the flavonoid content was significantly different among the treatments. Individually zinc application produced significantly higher values of TFC in foliar application of $ZnSO_4$ @ 0.5 % compared to boron. The highest TFC (1.75±0.22mg RE/100g DW) was recorded in the foliar application of $ZnSO_4$ @ 0.5 % (T_3), which was statistically at par with $ZnSO_4$ @ 1.0 %. While the lower TFC values 0.87, 0.90, and 0.90 mg RE/100 g DW content were found in foliar application of Borax @ 0.2 % + $ZnSO_4$ @ 0.5 % (T_8), Borax @ 0.2 % + $ZnSO_4$ @ 1.0 % (T_6) and Borax @ 0.2 % (T_2), respectively. Boron concentration seems to affect flavonoid levels. Sarafi[16], reported that boron toxicity considerably boosted flavonoid content in cultivar Odysseo while dramatically decreased it in cultivars Arlequin, Century, Imperial, and Salomon, showing a distinct genotypic response and harvesting time-dependent variation. This could be explained by the increased photosynthesis and sugar accumulation followed by zinc sprays, which might promote the synthesis of phenolic compounds, particularly flavonoids[17].

3.4 Total Phenolic Content (TPC)

Total phenol content in carrot roots increased significantly ($p > 0.05$) by foliar application of different combinations of boron and zinc. It was also observed that total phenol content in carrot roots increased significantly by foliar application of boron and zinc without combination Table 1. Foliar application of zinc showed an increase in Total Phenolic Contents with respect to control. Data shows that total phenolic contents were significantly affected by foliar application of zinc treatments. The highest value of total phenol concentration (6.59±0.34 mg GAE/100 g DW) was recorded under foliar application of $ZnSO_4$ @ 1.0 % (T_3), which was on at par with $ZnSO_4$ @ 0.5 %. There was a reduction in total phenol concentration with an increase in boron application rate compared with the control. Whereas, minimum TPC was observed in foliar application of Borax @ 0.2 % (T_2). This proposes that boron application leads to the reduction of phenols. It indicates that a specific level of boron causes the maximum reduction in the concentration of phenols[18]. However, our result was found similar to Song[19] that noted the accumulation of total phenols upon foliar application of zinc in berry.

3.5 Total Antioxidant Activity

In plants, vitamin C functions as an antioxidant and protects plants from oxidative stress/abiotic stress[20]. Foliar application of zinc and boron significantly affected carrot antioxidant activity compared with the control. The maximum antioxidant (67.81±1.92 mMTE/L DW) was observed in foliar application of Borax @ 0.1 % + $ZnSO_4$ @ 0.5 % (T_5), which is statistically at par with $ZnSO_4$ @ 1.0 % (T_4), Borax @ 0.2 % + $ZnSO_4$ @ 1.0 % (T_6), Borax @ 0.1 % + $ZnSO_4$ @ 1.0 % (T_7),and Borax @ 0.2 %+ $ZnSO_4$ @ 0.5 % (T_8). While the lowest value (59.06±1.92 mMTE/L DW) of antioxidants was found in control (T_0). In Tiwari[21] similar results of antioxidant activity was observed in carrot root. In this study, it was found that the antioxidant activity increases with the foliar spray of zinc and boron. An increase in antioxidant capacity was also reported by Majdoub[22] with the foliar application of zinc.

3.6 Micronutrients

Significant differences with respect to micronutrient content using the foliar application of different concentrations were observed (Table 2). The manganese concentration ranges between 1.30 to 1.84 mg/100 g DW, with the lowest value found with the $ZnSO_4$ @ 0.5 % (T_3), while the Borax @ 0.1 % +$ZnSO_4$ @ 0.5 % (T_5) foliar dose produced the highest value (1.84 mg/100 g DW).

The application of Borax @ 0.2 %+ $ZnSO_4$ @ 0.5 % produced highest zinc concentrations 11.17 mg/100 g DW,

Table 2. Combined and individual effect of foliar application of boron and zinc on accumulation of Mn, Zn, Cu, Na and Fe in carrot

Treatments	Mn (mg/100g)	Zn (mg/100g)	Cu (mg/100g)	Na (mg/100g)	Fe (mg/100g)
T_0	1.36 ± 0.1^{9a}b	5.49 ± 0.66^{a}	0.62 ± 0.06^{b}	310.73 ± 16.88^{a}	8.66 ± 0.47^{a}
T_1	1.39 ± 0.14^{ab}	5.38 ± 0.22^{a}	0.43 ± 0.08^{ab}	341.36 ± 4.69^{a}	9.79 ± 0.45^{a}
T_2	1.69 ± 0.10^{ab}	5.54 ± 0.33^{a}	0.51 ± 0.12^{ab}	373.68 ± 22.34^{a}	10.30 ± 1.01^{a}
T_3	1.30 ± 0.08^{a}	10.16 ± 0.44^{c}	0.46 ± 0.06^{ab}	374.86 ± 58.49^{a}	9.54 ± 0.95^{a}
T_4	1.39 ± 0.04^{ab}	7.14 ± 0.31^{b}	0.41 ± 0.07^{a}	322.94 ± 10.28^{a}	9.82 ± 0.77^{a}
T_5	1.84 ± 0.39^{b}	10.24 ± 0.16^{c}	0.50 ± 0.05^{ab}	406.43 ± 24.31^{a}	10.24 ± 0.51^{a}
T_6	1.74 ± 0.16^{ab}	8.16 ± 0.37^{b}	0.41 ± 0.05^{a}	331.47 ± 5.10^{a}	9.57 ± 1.05^{a}
T_7	1.38 ± 0.18^{ab}	7.97 ± 0.57^{b}	0.44 ± 0.05^{ab}	337.29 ± 5.88^{a}	10.20 ± 0.36^{a}
T_8	1.42 ± 0.13^{ab}	11.17 ± 0.32^{c}	0.49 ± 0.08^{ab}	334.10 ± 12.65^{a}	10.15 ± 0.53^{a}

According to Tukey's test, different letters within each column indicate significant differences (P = 0.05). All data are presented as mean ± standard deviation n=3

which was statistically at par with Borax @ 0.1 % + $ZnSO_4$ (10.24 mg/100 g DW) and $ZnSO_4$ @ 0.5 % (10.16 mg/100 g DW) whereas, foliar dose of Borax @ 0.1 % reported minimum value 5.38 mg/100 g DW. In the present study, zinc was found the best absorbed micronutrient. Gupta[23] reported that due to chelation of Zn^{2+}; Zn is known to be better transported by phloem compared to xylem. Many studies have revealed a negative association of Zn with Cu, Fe, and Mn.

The copper concentration was highest (0.62 mg/100 g DW) in control compared to all the treatments. Minimum copper concentration (0.41 mg/100 g DW) was observed with foliar application of $ZnSO_4$ @ 1.0 % and Borax 0.2 % + $ZnSO_4$ @ 1.0 %. This relationship occurs because of the competition between cations for absorption sites[24]. In this study, it was observed that zinc and boron had a negative correlation with copper but with other cationic micronutrients (Fe and Mn) applications of zinc and boron were not correlated either positive or negative. The absorption of Cu and Mn in carrots was affected by boron individually and by a combination of boron and zinc. The sodium (Na) and iron (Fe) values were presented in table 2, and showed no significant effect of boron and zinc application on carrot roots and the values ranged between 310.73 to 406.43 mg/100 g DW and 8.66 to 10.24 mg/100 g DW, respectively.

Esringü[25] found comparable results in strawberry, where the Fe content in the roots increased and decreased when applied with lesser and higher concentration of boron, respectively. These findings indicate an affinity of boron and iron that might be a synergistic interaction between the two nutrients. Rajaie[26] also observed a positive correlation between the concentrations of iron with an increase in boron concentration in *Citrus aurantifolia.* This study of foliar application of zinc and boron was not influenced by the absorption of Fe content in carrot roots. Our result was similar[27] that micronutrient content in apple leaves and fruit increases after foliar applications of micronutrient. It was reported that an increase in zinc levels in the leaves and fruit of pomegranate spraying with $ZnSO_4$[28]. Minerals like Zn, Cu and B in roots were found higher correlated with the increased concentration of these minerals in leaves that is linked with the foliar spray boron and zinc. These findings suggested the movement of micronutrients from phloem to other parts of the plant[27] that established the source-sink relationship of minerals movement. The higher concentration of Mn in roots is usually considered to be an imperfectly mobile element[29] however foliar application of zinc and boron helps in the movement of Mn from roots to other parts of plants. This enhanced micronutrient concentration in roots after foliar zinc and boron application individually and in combination is highly desired because it can overcome the widespread micronutrient deficiencies in the food chain[30].

4. CONCLUSION

It was found that the supplementation of $ZnSO_4$ in the nutrient application regimes in carrots had positive effect on TFC, TPC and carotene content whereas the opposite effect was observed with the application of boron. There was no significant effect on ascorbic acid content. Uptake of Fe and Na was not influenced by $ZnSO_4$ and boron. The uptake of Zn and Mn was increased by the addition of $ZnSO_4$ and boron in the feeding solution. Therefore, mineral nutrient applications are helpful to manipulate the nutritional value of carrot crops. This will minimize fertiliser use efficiency and reduce deleterious impacts on the environment. Foliar application of micronutrients overcomes the deficiency of micronutrients in crops. This type of agricultural practice helps in the development of biofortification research and improves soil fertility.

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