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EXOGENOUS APPLICATIONS OF BORON AND ZINC INFLUENCE LEAF NUTRIENT STATUS, TREE GROWTH AND FRUIT QUALITY OF FEUTRELL'S EARLY (*Citrus reticulata* Blanco)

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There is wide spread deficiency of micronutrients in the citrus growing areas of Pakistan. To investigate the influence of foliar application of boron (B) and zinc (Zn), on the leaf nutrient status, tree growth, productivity and fruit quality of *Citrus reticulata* Blanco cv. Feutrell's Early, trees were sprayed with boric acid and zinc sulphate either alone or in combination [T1 = control (water spray), T2 = 0.3% boric acid at fruit set stage, T3 = 0.5% zinc sulphate at fruit set stage, T4 = 0.3% boric acid + 0.5% zinc sulphate at fruit set stage, T5 = 0.5% zinc sulphate + 0.3 % boric acid at premature stage)]. Foliar application of B and Zn significantly increased the K, Mn, Fe, B and Zn status of Feutrell's Early leaves. The application of 0.3% boric acid + 0.5% zinc sulphate at the fruit set stage effectively brought the leaf Zn and B level of Feutrell's Early mandarin leaves from deficient to optimum range. The tree height, leaf size, fruit weight, juice weight percentage, SSC and TA were also significantly increased with application of 0.3% boric acid + 0.5% zinc sulphate at the fruit set stage. The application of 0.3% boric acid + 0.5% zinc sulphate at pre-mature stage significantly enhanced the concentration of ascorbic in the Feutrell's Early fruit juice. The total sugars and non-reducing level was also increased with foliar application of B and Zn. In conclusion, the combine application of boric acid (0.3%) and zinc sulphate (0.5%) at fruit set stage effectively improved the B and Zn level in the leaves, vegetative growth, productivity and fruit quality of Feutrell's Early madarin.
Keywords: Boron, *Citrus reticulata*, Feutrell's Early, foliar application, fruit quality, leaf minerals, mandarin, vegetative growth, yield, zinc

INTRODUCTION

Citrus occupies a prominent position in fruit industry of the world and is cultivated on an area of 832.2 thousands ha with 13.9 MT production (FAOSTAT, 2009). Pakistan is among the top thirteen citrus producing countries of the world; having an area of 193.2 thousand ha under citrus cultivation with over 1.47 MT production annually. Punjab province contributes 95% of total citrus production in the country (Anonymous, 2010). At present, among various citrus cultivars being grown in the Pakistan, the Kinnow mandarin is the leading citrus cultivar occupying about 70% share of the total citrus produced (Khan *et al*., 2010). After Kinnow, the Feutrell's Early is the only mandarin cultivar commercially grown in Pakistan. The area under mandarin cultivation in Punjab has been increased during the last five years, but average yield per plant per unit area has not been improved.

Productivity of citrus trees depends on many abiotic (climate, site, soil, nutrition, and irrigation management) and biotic (rootstock, cultivar, insect pest and disease management) factors (Davies and Albrigo, l994; Iglesias *et al*., 2007). Among them adequate supply of micro nutrients is most important to produce good quality fruits (Babu and Yadav, 2005; Ioannis *et al*., 2004). Boron (B) as a micronutrient plays significant role in growth and productivity of citrus. It increases pollen grain germination, pollen tube elongation, consequently fruit set percentage and finally the yield (Abd-Allah, 2006). The B deficiency is mainly found in acidic and sandy soils, and those with low soil organic matter. Plant species differ dramatically in B mobility, and may be classified into species with restricted B mobility and those in which B is highly mobile (Brown and Shelp, 1997).

Zn is another important microelement essential for plants due to its involvement in the synthesis of tryptophan which is a precursor of indole acetic acid synthesis. Zn is required for the activity of various enzymes, such as dehydrogenases, aldolases, isomerases, transphosphorylases, RNA and DNA polymerases (Swietlik, 1999). It has important role in starch metabolism, and acts as co-factor for many enzymes, affects photosynthesis reaction, nucleic acid metabolism and protein biosynthesis (Alloway, 2008). Zn deficiency is probably the most diffused nutritional alteration in all citrus producing areas. It is especially prevalent in sandy soils but can also frequently observed in alkaline soils, and can be aggravated by high level of phosphate or nitrogen fertilization (Boaretto *et al*., 2002; Langthasa and Bhattacharyya, 1995).

Foliar application of micronutrients like Zn, Cu, Mn, B and Fe has advantages over soil application because of high effectiveness, rapid plant response, convenience and elimination of toxicity symptoms brought about by excessive soil accumulation of such nutrients (Obreza *et al*., 2010). Curing micronutrient deficiencies through foliar application is a common practice in getting profitable yield and good quality fruit (Leyden, 1983). Foliar spray of micronutrients has been reported to be more effective than soil application in curing deficiencies in citrus. Keeping in view, the unfavourable physico-chemical conditions of our soils, it is very important to supply micronutrients in proper amount through foliar spray and to increase citrus production. At present, little is known about the effects of combine application of B and Zn on citrus in general and mandarin in particular. Therefore, the present study was conducted to determine the effects of foliar application of B and Zn on the vegetative and reproductive growth, yield and fruit quality of mandarin (*Citrus reticulata* Blanco) cv. Feutrell's Early.

MATERIALS AND METHODS

Fifteen years old Feutrell's Early mandarin trees, budded on the Rough Lemon (*Citrus jambhiri* L.) rootstock, grown under uniform and standard cultivation conditions were selected at Experimental Fruit Garden, Institute of Horticultural Sciences (31°25'N; 73° 09'E), University of Agriculture Faisalabad, Pakistan. The experiment was consisted of five treatments replicated four times and was laid out according to Randomized Complete Block Design (RCBD). Single tree was taken as an experimental unit. The B and Zn in the form of boric acid (H_3BO_3) and zinc sulphate ($ZnSO_4$) were applied through foliar spray according to following treatments; T1 = control (water spray), T2 = 0.3% boric acid at fruit set stage, T3 = 0.5% zinc sulphate at fruit set stage, T4 = 0.3% boric acid + 0.5% zinc sulphate at fruit set stage, T5 = 0.5% zinc sulphate + 0.3 % boric acid at premature stage. Data were collected for leaf nutrient analysis (N, P, K, Ca, Fe, Mn, B and Zn) before and after treatment applications, tree vegetative growth (stem girth, tree height, canopy width, flush length, number of leaves per flush, leaf age), fruit yield, and fruit quality (fruit size, weight, juice weight, pulp weight, peel weight, seed numbers, soluble solids concentrations, titratable acidity, ascorbic acid, reducing, non-reducing and total sugars).

Uniform, healthy and physiologically mature leaves (50 to 70 leaves per tree) of similar age from the experimental trees were collected at random for N, P, K, Ca, Zn, Fe, Mn and B determination. Leaf N, P and K were determined according to the method described by Chapman (1960). The micronutrients (Zn, Fe, and Mn) were determined according to the method outlined by Chapman and Pratt (1978) by using Atomic Absorption Spectrophotometer (PERKIN ELMER Aanalyst 100, Waltham, Massachusetts, USA) with specific lamp for each nutrient element. Whereas, the amount of B in the leaves were determined by the method reported by Han *et al.* (2008). Quantity of each element was estimated in mg L^{-1} by comparing the emission of flame photometer with that of the standard curve.

Four branches of two inch diameter were selected from each side (North, South, East and West) of experimental trees to study the vegetative growth. In April five newly emerged flushes were tagged from each selected branch to record the length of flush (cm), and number of leaves per flush. The vegetative parameters regarding the tree growth were measured at the beginning and at end of the experiment and average increase in the tree height (cm), tree spread (cm) and stem girth (cm) were recorded. Yield per tree was recorded by weighing and counting total number of fruits per tree at the time of harvest. Fruit size was measured by using vernier calliper. Average fruit weight was calculated by weighing fruit on digital electronic balance (UWE–ESP Digital, UK). Juice from experimental fruit was extracted and sieved to eliminate pulp and then peel, juice and pulp weight of each sample was determined and expressed as percentage by using the formula: (Juice/peel/pulp weight per fruit/total fruit weight) x 100.

Digital refractometer (ATAGO, RS-5000, Atago, Japan), was used to measure soluble solids concentration (SSC) of juice, and expressed as ºBrix at room temperature. Titrable acidity (TA) of fruit juice was determined by method given by Hortwitz (1960) and was expressed as % citric acid. SSC:TA ratio ratio was calculated for each sample by dividing the SSC with the corresponding TA value. Ascorbic acid contents of juice were determined following the method described by Ruck (1969), and were expressed as mg 100 ml^{-1} FW. The estimations of sugars in juice were conducted by using the method described by Khan *et al*. (2009), and reducing , non-reducing and total sugars were expressed as percentage.

The experiment was carried out under Randomized Complete Block Design (RCBD). The data were statistically analyzed using the computer software MSTAT- C (Freed, 1994). Least Significant Difference (LSD) test ($P \leq 0.05$) was used to determine the significance differences among treatment means. All the assumptions of the ANOVA were accessed to ensure the validity of statistical analysis.

RESULTS AND DISCUSSION

The levels of N (1.05-1.2%), P (0.01%) and K (0.33-0.39%) of the experimental trees were significantly at par and under deficient range before the foliar application of B and Zn (Table 1). Similarly, the concentrations of Zn and B in the experimental trees were also present within the deficient (15.01-16.5 mg L^{-1} and 24.1-28.5 mg L^{-1}) range, respectively. However, the concentrations of Mn (26.3-39.3 mg L^{-1}) in the leave were in optimum, and Ca (6.17-6.74%) and Fe (210-289 mg L^{-1}) were in the high range (Table 1). The trees did not exhibit significant differences in the level of Zn; however, Mn, Fe and B concentrations were significantly different among the treatments. After foliar application of B and Zn, N and P levels in leaves did not showed any significant change, and remained in the deficient range (1.19-1.25%) and (0.11-0.12%), respectively (Table 1). However, foliar application of B and Zn significantly increased the level of K in the experimental trees. Highest increase in the leaf K was observed in the trees treated with 0.3% boric acid + 0.5% zinc sulphate at fruit set stage (T4) followed by trees treated with 0.3% boric acid + 0.5% zinc sulphate at pre-mature stage (T5), compared to control and all other treatments (Table 1). The level of leaf Ca in the experimental trees did not exhibit any significant difference after foliar application of B and Zn, however, they remained in optimum rage. Foliar application of B and Zn significantly increased the Zn and B contents of experimental trees from deficient to optimum and higher range (Table 1). Trees treated with 0.5% zinc sulphate at fruit set stage (T3) or 0.3% boric acid + 0.5% zinc sulphate at fruit set stage (T4) exhibited 3.4-fold and 3.5-fold increase in the Zn content, compared to control. Foliar application of B and Zn also significantly affected the level of Mn and Fe in the experimental trees; the levels of Mn were within the optimum while Fe in the higher ranges (Table 1). The results of the present study revealed that the experimental trees were deficient in N and P contents. The treatment of B and Zn improved the status of K, B and Zn within the leaves, whilst the level of Ca remained unchanged. The optimum levels of leaf N, P, and K for the production of best fruit quality of citrus have been reported from 2.4 to 2.6%, 0.12 to 0.16%, 0.70 to 1.09% respectively (Fake, 2004). Our results supported the findings of Mann *et al*. (1985) who found that Zn spray is effective in increasing leaf trace elements in the citrus.

Foliar application of B and Zn significantly increased the tree height of Feutrell's Early mandarin trees as compared to control (Table 2). Maximum increase in tree height was obtained when B and Zn was applied with 0.3% boric acid + 0.5% zinc sulphate at fruit set stage (T4). Whereas, foliar application of B and Zn did not affect the number, size and age of leaf, and length of flush (Table 2). However, increase in tree spread and stem girth were higher in all treated trees than control. Similarly, treated trees exhibited significant increase in leaf size compared with control (Table 2).

Table 1. Concentration of macro and micronutrients before and after foliar application of B and Zn in Feutrell's Early mandarin leaves

Treatments	N (%)	P (%)	K (%)	Ca (%)	Zn (mgL^{-1})	Mn (mgL^{-1})	Fe (mgL^{-1})	B (mgL^{-1})
	Before foliar application of B and Zn							
T1	1.15	0.0100	0.34	6.42	15.05	39.3a	232bc	24.1c
T2	1.05	0.0105	0.34	6.17	15.75	27.5c	268ab	26.8b
T3	1.20	0.0105	0.36	6.35	16.25	33.3b	210c	25.0bc
T4	1.07	0.0103	0.39	6.03	16.01	26.3c	256b	28.5a
T5	1.09	0.0100	0.33	6.74	16.50	36.5a	289a	28.3a
	After foliar application of B and Zn							
T1	1.25	0.0113	0.61c	7.29	50.25b	44.75b	286c	24.84d
T2	1.22	0.0115	0.66b	6.62	53.75b	35.25c	285c	108.2b
T3	1.19	0.0115	0.75a	6.85	176.75a	48.75b	331ab	126.7a
T4	1.22	0.0110	0.77a	6.47	148.75a	57.25a	348a	99.8c
T5	1.15	0.0113	0.72a	7.68	51.00b	44.25b	309b	94.8c
LSD ($P \leq 0.05$)								
Before application	NS	NS	NS	NS	NS	2.18*	12.15*	2.59*
After application	NS	NS	0.12*	NS	4.56*	3.97*	12.3*	8.13*

NS = Non-significant; * = significant at ($P \leq 0.05$); Any two means within a column followed by the same letter are not significantly different at $P \leq 0.05$; n = 4 replicates; T1 = control (water spray); T2 = 0.3% boric acid at fruit set stage; T3 = 0.5% zinc sulphate at fruit set stage; T4 = 0.3% boric acid + 0.5% zinc sulphate at fruit set stage; T5 = 0.5% zinc sulphate + 0.3% boric acid at premature stage

Table 2. Effect of foliar application of B and Zn on the vegetative growth of Feutrell's Early mandarin trees

Treatments	Tree height (cm)	Tree spread (cm)	Stem girth (cm)	Leaves (No.)	Leaf size (cm^2)	Leaf age (days)	Flush length (cm)
T1	35.5c	33.8	3.19	24.31	300c	171	24.31
T2	38.0b	36.0	3.85	24.17	310b	166	24.17
T3	40.8a	39.8	4.13	22.75	317a	178	22.75
T4	43.8a	40.0	4.28	24.54	318a	175	24.54
T5	38.7b	38.5	3.65	23.35	307b	177	23.35
LSD ($P \leq 0.05$)	4.13*	NS	NS	NS	9.16*	NS	NS

NS = Non-significant; * = significant at ($P \leq 0.05$); Any two means within a column followed by the same letter are not significantly different at $P \leq 0.05$; n = 4 replicates; T1 = control (water spray); T2 = 0.3% boric acid at fruit set stage; T3 = 0.5% zinc sulphate at fruit set stage; T4 = 0.3% boric acid + 0.5% zinc sulphate at fruit set stage; T5 = 0.5% zinc sulphate + 0.3% boric acid at premature stage

Table 3. Effect of foliar application of B and Zn on the physical quality of Feutrell's arly mandarin fruit

Treatments	Fruit length (mm)	Fruit diameter (mm)	Fruit weight (g)	Peel weight (%)	Pulp weight (%)	Juice weight (%)	Total seeds (No)	Healthy seeds (No)	Aborted seeds (No)
T1	48.32c	58.21b	112.2c	32.9	36.3a	32.1b	11.0	10.1	0.85b
T2	50.19b	59.37b	114.5c	33.8	33.4b	31.2b	13.3	11.8	1.50ab
T3	51.2ab	62.21a	139.1a	30.1	33.0b	36.1a	07.5	06.7	0.85b
T4	53.34a	64.57a	145.3a	30.2	31.1b	37.4a	11.7	09.2	02.5a
T5	52.97a	62.05a	128.4b	30.5	33.0b	34.8ab	10.3	08.8	01.5ab
LSD ($P \leq 0.05$)	4.16*	4.34*	6.29*	NS	3.59*	3.42*	NS	NS	0.13*

NS = Non-significant; * = significant at ($P \leq 0.05$); Any two means within a column followed by the same letter are not significantly different at $P \leq 0.05$; n = 40 (10 fruit x 4 replications); T1 = control (water spray); T2 = 0.3% boric acid at fruit set stage; T3 = 0.5% zinc sulphate at fruit set stage; T4 = 0.3% boric acid + 0.5% zinc sulphate at fruit set stage; T5 = 0.5% zinc sulphate + 0.3% boric acid at premature stage

The leaf age and flush length did not show any change with respect to foliar application of B and Zn. Increase in tree height and leaf size in the treated trees might be due to active involvement of Zn in the synthesis of tryptophan which is a precursor of indole acetic acid synthesis, consequently it increased tissue growth and development (Swietlik, 1999). It has also been reported that sufficient level of Zn is plant promote the photosynthesis, nucleic acid metabolism and protein biosynthesis. Our results are supported by the finding of Dawood *et al.* (2001) who reported that micronutrients especially Zn increases the growth parameters in young trees of Washington Navel oranges. Results also revealed a synergistic effect of B and Zn on the vegetative growth in Feutrell's Early mandarin when applied at fruit set stage.

Observation regarding number of fruit per tree revealed that foliar application of B and Zn significantly increased the numbers of fruit per trees (Fig. 1). Trees treated with 0.3% boric acid + 0.5% zinc sulphate at fruit set stage (T4) and 0.5% zinc sulphate at fruit set stage (T3) exhibited highest increase (20.7% and 16.7%) in the numbers of fruit per tree as compared to control, respectively. This increase in fruit number might be due to reduction in the fruit drop (Data not presented). Earlier, Nijjar (1985) reported that Zn is required for preventing the abscission layer formation and consequently, the reduction in pre-harvest fruit drop. Similarly the present results were supported by the findings obtained by Shawky *et al.* (1990) and Ismail (1994) who found that foliar application of micronutrients increased yield of Navel and Valencia oranges.

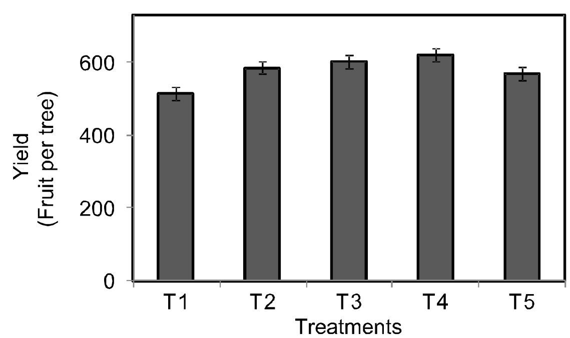

Figure 1. Effect of foliar application of B and Zn on numbers of Feutrell's Early mandarin fruit per tree. Vertical bars ± SE; T1 = control (water spray); T2 = 0.3% boric acid at fruit set stage (FSS); T3 = 0.5% zinc sulphate at FSS; T4 = 0.3% boric acid + 0.5% zinc sulphate at FSS; T5 = 0.5% zinc sulphate + 0.3% boric acid at premature stage

Data regarding effects of foliar application of B and Zn on the physical characteristics of Feutrell's Early mandarin fruit revealed significant differences for fruit length and diameter (Table 3). The maximum increase in the fruit size was exhibited by fruit harvested from the trees treated with 0.3% boric acid + 0.5% zinc sulphate at fruit set stage (T4). The average fruit weight and juice weight percentage increased significantly with foliar application of B and Zn (Table 3). Trees sprayed with combined application of B (0.3% boric acid) and Zn (0.5% zinc sulphate) at fruit set stage (T4) produced fruit with highest fruit weight (145 g) and juice weight (37.4%), and lowest pulp weight (31.1%) as compared to control and all other treatments (Table 3). Effects of foliar application of B and Zn on the peel weight percentage, total seeds and healthy seeds were not significant, whereas, B and Zn applications significantly affected the number of aborted seeds. Trees sprayed with 0.3% boric acid + 0.5% zinc sulphate at fruit set stage (T4) exhibited 3-fold higher number of aborted seeds in contrast to control (Table 3). The Zn is also required for the synthesis of tryptophan, a precursor for the synthesis of indoleacetic acid which is involve in the process of fruit growth and development (Swietlik, 1999). These results are in line with the work of Sahota and Arora (1981) who reported that foliar application of Zn increased the fruit yield by increase in fruit weight in Sweet orange.

The foliar application of B and Zn had a positive effect on the chemical fruit quality of Feutrell's Early mandarin. Fruit harvested from trees treated with combine application of B and Zn at fruit set stage exhibited higher SSC and TA, and lowest SSC:TA ratio as compared to all other treatments (Fig. 2). Increased level of SSC and TA due to Zn sprays may be attributed to their effects on different enzymes which are involved in the formation of different proteins, acids and sugars (Srivastava and Gupta, 1996). Previous study of Sahota and Arora (1981) confirms that SSS and TA ratio was not significantly affected by Zn either alone or in combination with N in Sweet orange. Highest increase in the level of ascorbic acid contents was recorded in the fruit harvested from the trees which were sprayed with 0.3% boric acid + 0.5% zinc sulphate at pre-mature stage (Fig. 3). Zn play an active role in the production of auxin in plant species (Alloway, 2008), as the production of auxin increases ascorbic acid content also increases in Kinnow mandarin as reported by Nawaz *et al*. (2008). Similarly, foliar application of Zn sprays have also been reported to increase ascorbic acid contents in Balady mandarin trees (El-Menshawi *et al*., 1997). Maximum percentage of total sugars was obtained in the fruits where the application of 0.3% boric acid and 0.5% zinc sulphate were sprayed at fruit set stage (T3), whereas, minimum percentage of total sugars was founded in fruit harvested from trees treated with 0.3% boric acid at fruit set stage (T2) (Fig. 4A). Foliar application of B and Zn did not influence the concentration of reducing sugars (Fig. 4B). Fruit harvested from untreated control trees showed maximum amount of reducing sugars as compared to all other treatments. Whereas, concentration of non-reducing sugar was higher in fruit harvested from trees treated with 0.5% Zn than all other treatments (Fig. 4C). The changes in the concentration of total and non-reducing sugars in the treated trees are ascribed to the role Zn starch and nucleic acid and starch metabolism, and activities of various enzymes involved in these biochemical reactions (Alloway, 2008). Results were found to be in agreement with that of Babu and Yadav (2005) who reported that application of Zn with B, Mn and Mg increased the total sugars percentage in Khasi mandarin.

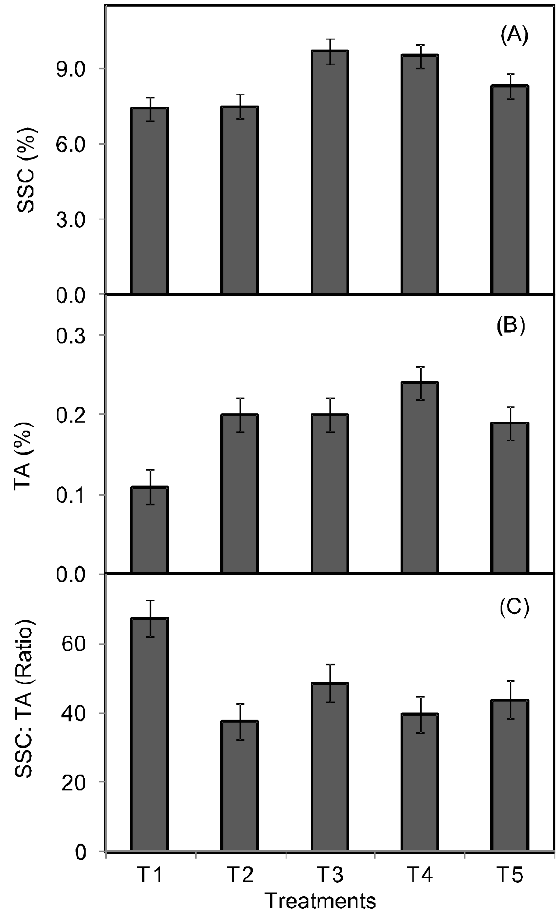

Figure 2. Effect of foliar application of B and Zn on soluble solids concentration (SSC) (A), titratable acidity (TA) (B), and SSC: TA ratio (C) of Feutrell's Early mandarin fruit; Vertical bars ± SE; T1 = control (water spray); T2 = 0.3% boric acid at fruit set stage (FSS); T3 = 0.5% zinc sulphate at FSS; T4 = 0.3% boric acid + 0.5% zinc sulphate at FSS; T5 = 0.5% zinc sulphate + 0.3% boric acid at premature stage

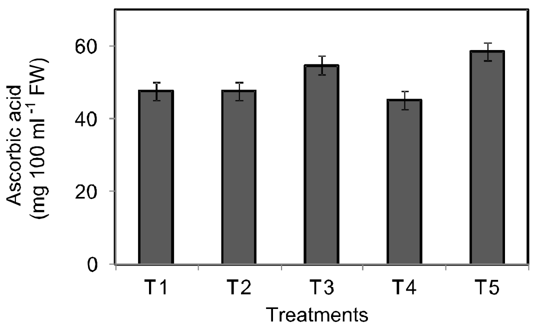

Figure 3. Effect of foliar application of B and Zn on the level of ascorbic acid of Feutrell's Early mandarin fruit; Vertical bars ± SE; T1 = control (water spray); T2 = 0.3% boric acid at fruit set stage (FSS); T3 = 0.5% zinc sulphate at FSS; T4 = 0.3% boric acid + 0.5% zinc sulphate at FSS; T5 = 0.5% zinc sulphate + 0.3% boric acid at premature stage

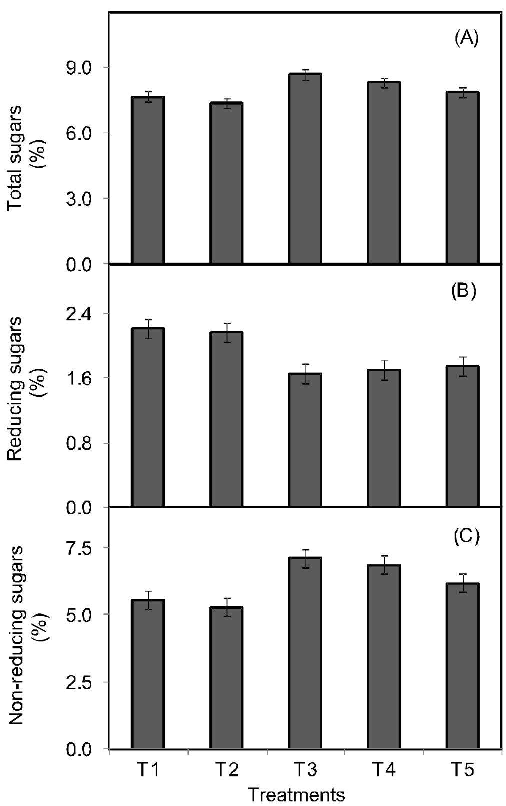

Figure 4. Effect of foliar application of B and Zn on total sugars (A), reducing sugars (B), and non-reducing sugars (C) of Feutrell's Early mandarin fruit; Vertical bars ± SE; T1 = control (water spray); T2 = 0.3% boric acid at fruit set stage (FSS); T3 = 0.5% zinc sulphate at FSS; T4 = 0.3% boric acid + 0.5% zinc sulphate at FSS; T5 = 0.5% zinc sulphate + 0.3% boric acid at premature stage

CONCLUSION

The application of 0.3% boric acid + 0.5% zinc sulphate at fruit set stage effectively increased the leaf Zn and B level of Feutrell's Early mandarin leaves and brought them from deficient to optimum range. The same treatment also significantly increased the fruit height, leaf size, fruit weight, juice weight percentage, SSC and TA. The concentration of ascorbic in the juice increased with application of 0.3% boric acid + 0.5% zinc sulphate at pre-mature stage. The combine application of boric acid (0.3%) and zinc sulphate (0.5%) at fruit set stage effectively improved the B and Zn level in the leaves, growth, productivity and fruit quality of Feutrell's Early fruit.

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