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EFFECT OF MICRONUTRIENTS (Zn, Cu AND B) ON PHOTOSYNTHETIC AND FRUIT YIELD ATTRIBUTES OF *CITRUS RETICULATA* BLANCO VAR. KINNOW

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Abstract

In this investigation, influence of foliar application of micronutrients (Zn, Cu and B) was studied on the improvement in photosynthetic and fruit yield attributes of citrus (Kinnow) plants. Experiments were conducted in two districts of Punjab (Sargodha and Toba Tek Singh), Pakistan varying in soil properties and agro-climatic conditions. Plants at both sites were subjected to foliar spray of three different levels (i.e. 0.1, 0.2 and 0.3%) of each Zn, Cu and B at three different fruit developmental stages while macronutrients (NPK) were applied at recommended rates as soil amendment. Micronutrients (Zn, Cu and B) application caused a significant improvement in net photosynthetic rate (*A*), transpiration rate (*E*), stomatal conductance (*g*s), Chlorophyll "a", "b", "total", and carotenoids in both the citrus orchards. However, effect of micronutrients i.e. Zn, Cu and B was more pronounced at the levels of 0.3, 0.1 and 0.2%, respectively. These levels of nutrients were also effective in improving fruit yield with better fruit quality.

Key words: Micronutrients, Net photosynthetic rate; Stomatal conductance; Transpiration; Photosynthetic pigments; Fruit yield.

Introduction

Nutrition management is one of the most important factor in improving the plant growth and yield through increasing photosynthetic efficiency. Micronutrients deficiency in soil and plants is a worldwide nutritional problem and very severe in many countries (Alloway, 2008; Mousavi *et al*., 2007). Plants vary in their demand for micronutrients, as these are involved in almost all physiological functions. Some of these elements are redox-active and are cofactors in many enzymes. They have enzyme-activating functions and play structural role in stabilizing proteins (Hänsch & Mendel, 2009). In Pakistan, the nutrient deficiencies particularly micronutrients are common due to low organic matter, alkaline pH and calcareous nature of soil (Rashid *et al.,* 1997). By choosing appropriate fertilizer rates, the grower can drive a crop toward earlier and heavier fruit setting (Alva *et al.,* 2006). Micronutrients like zinc (Zn), copper (Cu) and boron (B) are very important for optimal plant growth, physiological and biochemical pathways in citrus cultivation under agro-climatic conditions of Punjab, Pakistan.

Reports (Ashraf *et al.,* 2012; 2013; 2014) indicated that application of Zn improves the citrus fruit yield and its juice quality. Zinc is also involved in photosynthesis, activation of enzyme systems, protein synthesis and carbohydrate translocation (Tsonev & Lidon, 2012). Application of Zn enhances the photochemical reactions occurring in thylakoid membrane, electron transport through PSII and increases photosynthetic rate (Roach & Liszkay, 2014) and chlorophyll content (Alloway, 2004). Foliar or soil supply of Zn increases the biosynthesis of chlorophyll and carotenoid synthesis that are important for proper performance of photosynthetic process (Mousavi, 2011). Foliar application of Zn had positive impact on fruit yield and quality of Kinnow mandarin, sweet orange and grapes (Razzaq *et al*., 2013).

Application of Cu stabilizes chlorophyll by protecting the chlorophyll-protein lipid complex (Tumolo & Marquez, 2012) and restricts in the chloroplasts bound to plastocyanin in plants, and improves electron transport chain because Cu is part of it (Maksymiec, 1997). Stenico *et al*. (2009) concluded that Cu is indispensable for carbohydrate and nitrogen metabolism in citrus. Foliar spray with Cu to orange trees significantly increases fruit yield tree^{-1}, fruit weight, and total soluble solids and fruit size as compared to the untreated trees (Khurshid *et al*., 2008). Similarly, application of Mg, Cu, Zn, Fe and B enhanced the juice content (Ram & Bose, 2000). Application of B increases fruit set and yield by its role in pollen tube germination and elongation (Abd-Allah, 2006). Naz *et al*. (2012) also noted that B increases growth and flowering in tomatoes. The foliar application of Zn and B significantly enhanced fruit yield and juice content, total soluble solids, ascorbic acid and non-reducing sugar (Asad *et al*., 2003).

Keeping in view the active role of Zn, Cu and B in photosynthesis and crop yield and their deficiency in the soils and citrus trees of citrus growing areas of Punjab Pakistan, studies were conducted at two orchards in two major citrus growing districts i.e. Sargodha (75 SB) and Toba Tek Singh (297 GB), Pakistan differing in soil characteristics and agro-climatic conditions with the view to investigate effect of these micronutrients on photosynthetic activities, yield and fruit quality of *Citrus reticulate* (var. Kinnow).

Materials and Methods

Experiments were conducted in two districts of Punjab (Sargodha and Toba Tek Singh), Pakistan varying in soil properties and agro-climatic conditions (Table 2). The experiments were laid out in a factorial design on the base of completely randomized blocks with three replications containing three plants per replication. Before and after experimentation, leaf analysis was carried out to assess changes in nutrient status (Table 1).

Aqueous solutions of $ZnSO_4.7H_2O$ (Merck), $CuSO_4.7H_2O$ (Merck) and H_3BO_3 (Merck) were applied @ 0.1, 0.2 and 0.3% each as Zn, Cu and B. Control trees were sprayed with distilled water. Tween-20 at 0.01% was added in spraying solution as a surfactant. The treatments were applied thrice, first time 15 days before full bloom, second time at fruit setting and third time at fruit enlargement stage. Sprays were applied in the morning (6-9 a.m.) using a hand pressure sprayer.

Gas exchange attributes like net assimilation rate (*A*), transpiration rate (*E*), and stomatal conductance (q_s) were measured using Photosynthesis system (Model CI-340, Inc. USA) portable infrared gas analyzer. Chlorophyll contents were determined by using the method of Arnon (1949) and chlorophyll a, b, total carotenoids contents were calculated as described by Davies (1976).

Fruit samples from control and treated plants were collected and fruit weight was determined by analytical balance and juice volume was measured after mechanical extraction from fruits of equal size selected from both sites. Fruit yield of Kinnow was recorded at harvest time, on an individual tree basis and expressed as total fruit number.

The data pertaining to various parameters were analyzed by ANOVA techniques using STATISTIX 8.1 and significance of means was tested using least significant difference at 5% probability (Steel *et al*., 1997).

Table 1. Leaf Chemical analysis before and after spray of micronutrients.

Nutrients	Sargodha		Toba Tek Singh	
	Before spray	After spray	Before spray	After spray
Zn (mg kg^{-1})	11.45	22.34	8.5	18.23
Cu (mg kg^{-1})	3.7	5.3	4.6	5.7
B (mg kg^{-1})	34.65	47.19	28.37	40.45

Table 2. Soil characteristics (0-60 cm) of both experimental sites (Sargodha and Toba Tek Singh) to study the effect of micronutrients on the yield and quality of citrus.

Soil characteristics	Sargodha (75 SB)	Toba Tek Singh (297 GB)
Soil texture	Clay loam	Sandy clay loam
EC(dS m^{-1})	1.92-3.28	0.96-0.98
pH	7.62-7.77	6.8-7.3
Organic matter (%)	0.6-0.66	0.5-0.6
NO_3-N (mg kg^{-1})	14.1-15.3	17.3-17.4
P (mg kg^{-1})	9.4-11.4	8.6-11.2
K(mg kg^{-1})	82-117	80-115
Ca+Mg (meq L^{-1})	12.6-15.7	7.0-7.5
CO_3 (meq L^{-1})	Nil	Nil
HCO_3 (meq L^{-1})	3.5-4.0	3.0-6.0

Results

Photosynthesis/gas exchange: Foliar application of micronutrients (Zn, Cu, and B) significantly affected the gas exchange attributes such as photosynthetic rate (*A*), transpiration rate (*E*) and stomatal conductance (g_s) which showed increasing trend with increase in levels of micronutrients with few exceptions (Fig. 1). The highest gas exchange attributes were recorded in those plants, where Zn was foliarly applied followed by Cu and B. The differences among different levels of Zn, Cu and B were significant. The highest values were recorded at the highest level of 0.3% Zn followed by 0.2% Zn and 0.1% Zn. In contrast to Zn, Cu influenced these parameters differently and these were maximum at 0.1% followed by 0.3% and 0.2%. In case of B, the highest values for gas exchange attributes were noted under 0.2% B followed by 0.3% and 0.1% B. At flowering stage these attributes (*A*, *E* and *gs*) were significantly higher than other stages. Trees of Toba Tek Singh maintained higher values for *A*, *E* and *gs* as compared to those of Sargodha orchard.

Chlorophyll contents: Foliar application of micronutrients (Zn, Cu and B) significantly affected chlorophyll total chlorophyll (Chl_t), chlorophyll a/b and carotenoids content (Fig. 2). The highest concentration of pigments was recorded in 0.3% Zn treated plants. In case of Cu, values for pigments gradually decreased with increasing its levels. Boron treated plants showed different response, the maximum pigments (Chl_a/Chl_b, Chl_t and carotenoids) concentrations were recorded under 0.2% B followed by 0.1% and 0.3% B, respectively. The maximum pigments contents were observed at fruit setting stage. Trees of Sargodha orchard had higher Chl_a/ Chl_b, Chl_t and carotenoids contents as compared to Toba Tek Singh orchard.

Fruit yield attributes: Foliar application of micronutrients (Zn, Cu and B) significantly influenced yield attributes. It is evident from (Fig. 3) that foliar application of 0.3% Zn, 0.1% Cu and 0.2% B enhanced fruit weight, juice volume and fruit yield/plant at both sites as compared to other levels of nutrients. These yield attributes increased with increasing levels of Zn, these were enhanced by foliar application of Zn at 0.3% followed by Zn 0.2% and Zn 0.1%. In contrast to Zn, Cu influenced it differently and maximum fruit yield was noted in trees sprayed with Cu 0.1% and decreased with its higher doses. In case of B, the maximum yield was noted in trees treated with B 0.2 %.

Discussion

In the present study photosynthetic rate (*A*), transpiration rate (*E*) and stomatal conductance (*gs*) were significantly improved by the foliar application of different levels of Zn, Cu and B. These attributes enhanced with increasing levels of Zn but in contrast to Zn, Cu influenced differently and all these parameters were maximum at 0.1% Cu and decreased by increasing its levels. However, in case of B the highest values for these attributes were recorded in plants sprayed with 0.2% B and decreased at 0.3% level of B. Earlier reports (Wang & Jin, 2005) also showed that micronutrients affect photosynthetic activities or gas exchange characteristics like *A, E* and *gs* which were significantly enhanced by their application. The findings of Ahmed ***et al.*** (2009) indicated that severe reduction in crop photosynthetic activities due to Zn deficiency. However, foliar application of Zn increased gas exchange parameters and maintained membrane integrity (Khan *et al*., 2004).

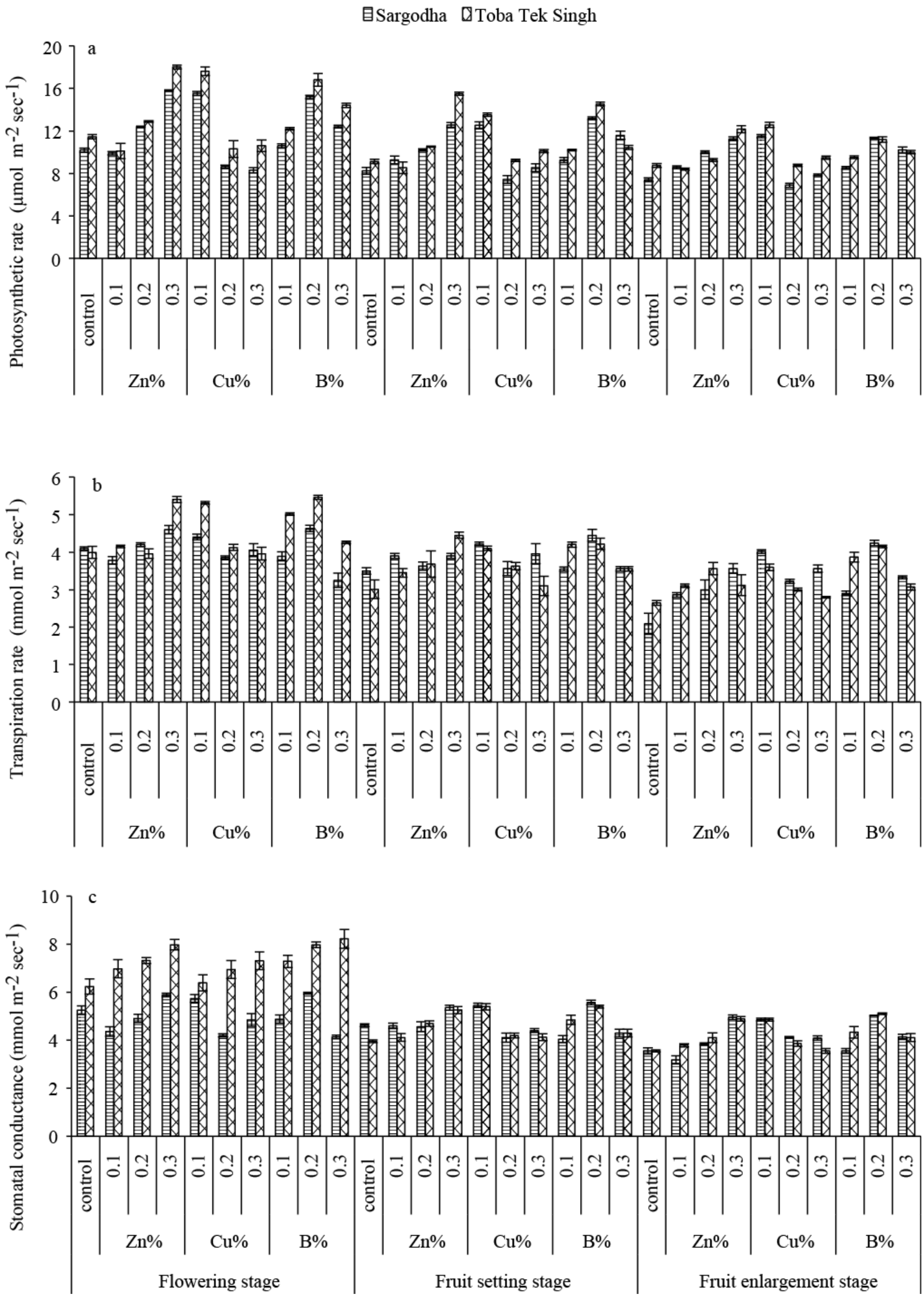

Fig. 1. Effect of micronutrients (Zn, Cu and B) on photosynthetic rate, transpiration rate and stomatal conductance in citrus plants at Sargodha and Toba Tek Singh orchard.

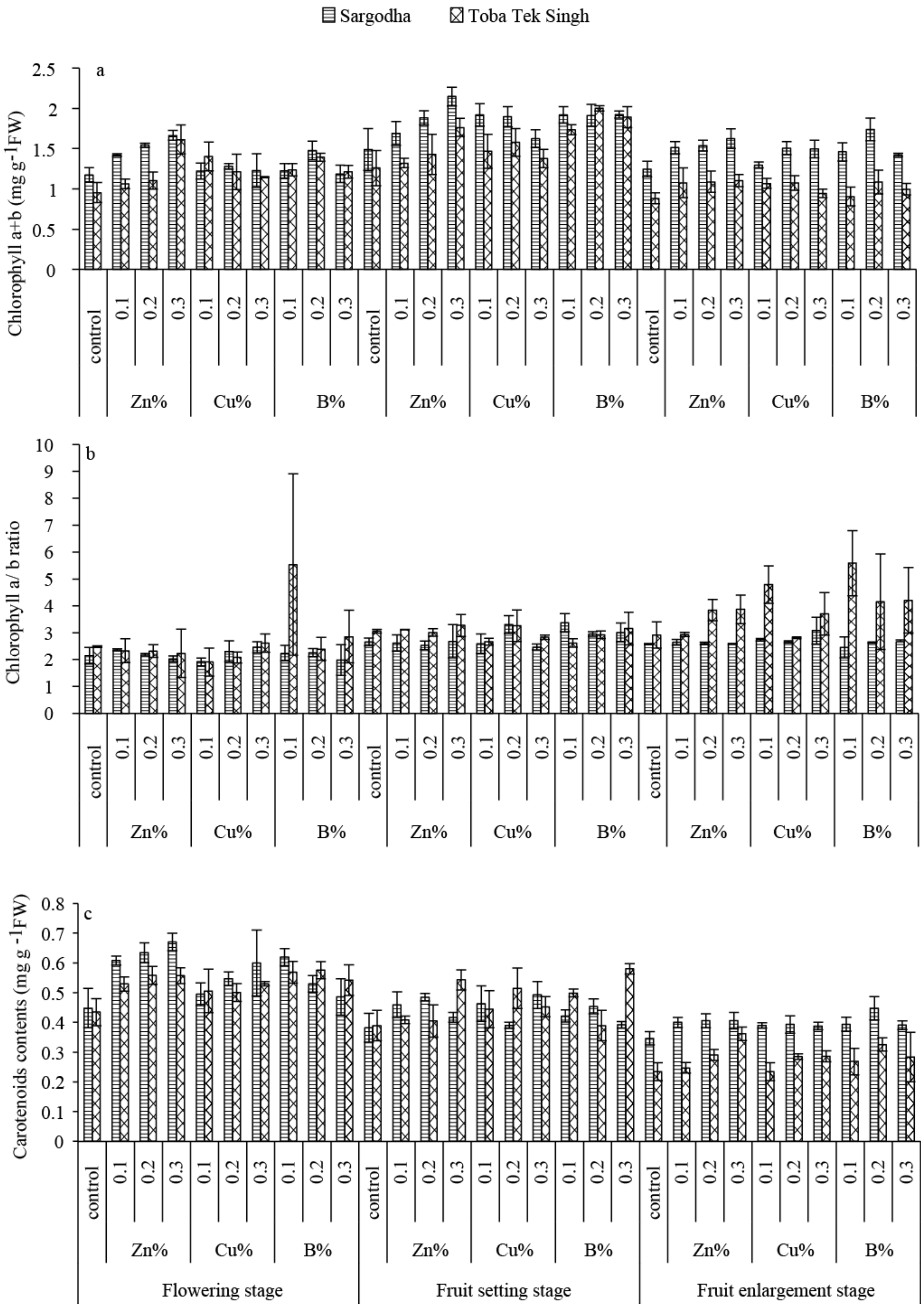

Fig. 2. Effect of micronutrients (Zn, Cu and B) on Chlorophyll a+b, chlorophyll a/b and carotenoids contents in citrus plants at Sargodha and Toba Tek Singh orchard. (FW= Fresh weight).

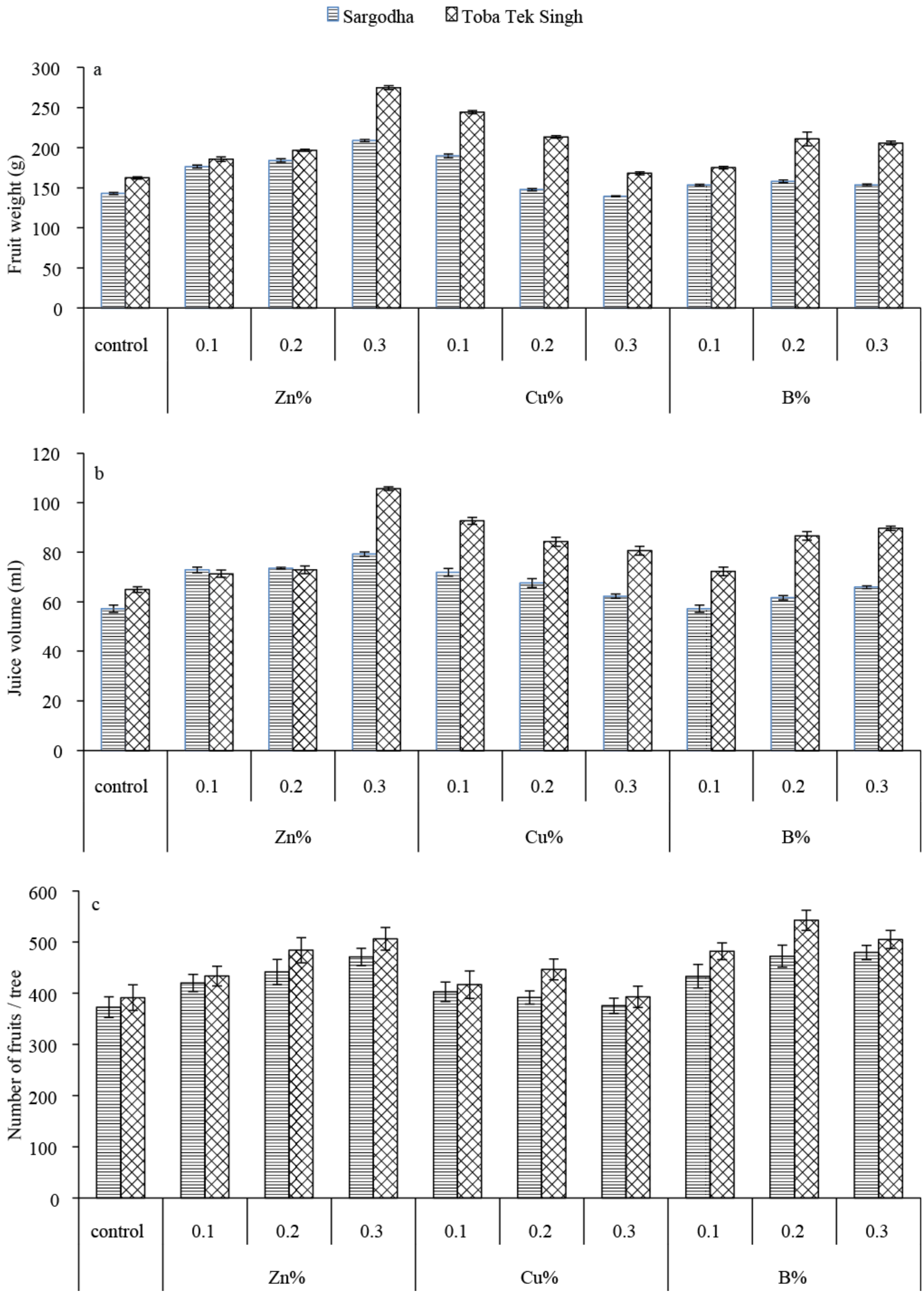

Fig. 3. Effect of micronutrients (Zn, Cu and B) on fruit weight, juice volume and number of fruits/ tree at Sargodha and Toba Tek Singh orchard.

The findings of present study indicated that foliar application of Zn improved photosynthetic activities in citrus plant that may be due its involvement in activation of many enzymes of photosynthesis, cell elongation and cell division (Cakmak, 2008).Yield, crude protein and Zn concentration in alfalfa plants were significantly affected by its supply (Safak *et al*., 2009). Photosynthesis is reduced in Zn-deficient plants as different metabolic pathways are influenced by it. Qiao *et al*. (2014) observed that foliar application of Zn enhanced Carbonic anhydrase activity in rice leaves and hence increased photosynthesis. Carbonic anhydrase is considered as Zn containing enzyme involved in photosynthesis.

The photosynthetic rate is affected by micronutrients through several modes of action. Boron is involved in the carbohydrate metabolism; it directly affects plant growth and indirectly influences the photosynthetic rate (Cakmak & Romheld, 1997). Copper is an important micronutrient and required for normal plant metabolism, and is also involved in the flow of electrons in photochemical systems and is a cofactor of various enzymes involved in different metabolic pathways, including ATP synthesis (Sharma & Agrawal, 2005). At higher level, Cu becomes toxic and influences different metabolic functions such as respiration, photosynthesis, CO_2 fixation and gas exchange parameters (Mocquot *et al*., 1996) as observed in present study where photosynthetic activity adversely affected at 0.2 and 0.3 % Cu. Same was the case with B. The concentration of chlorophyll (Chl) a, b, total and carotenoids contents were significantly increased by foliar application of Zn, Cu and B in both orchards. Similar increase in Chl a, b, total and carotenoids was observed by Nahed *et al*. (2007) in *Salvia farinacea* by the foliar application of Zn. Results are also in line with Massoud *et al*. (2005) for pea plants and Farahat *et al*. (2007) and Wenrong *et al*. (2008) for *Cupressus sempervirens* observed that Zn deficiency resulted decline in leaf Chl content. Zinc application enhances the rate of photochemical reductions and Chl content in cucumber (Kazemi, 2013). In this study, Chl and other biochemical attributes increased at 0.1% Cu but decreased at its higher levels. The reduction in chlorophyll and carotenoids at higher level of Cu may be due to its toxic effect or production of reactive oxygen species (ROS) which hinders the biosynthesis of these pigments or it binds SH group chloroplast and destroys its structure and function and decreases chlorophyll biosynthesis (Hou *et al.,* 2007). Higher concentrations of Cu resulted in a pronounced reduction in the photo-reduction activities of PSII. The physiological analysis of photosynthetic pigments like Chl a, b, proteins and phenols were significantly increased by application of micronutrients due to enhancement in secondary metabolites (Shitole & Dhumal, 2012).

Foliar application of Zn, Cu and B significantly enhanced fruit yield in terms of fruit number and weight. The results are in line with Ashraf *et al.* (2013) and Razzaq *et al.* (2013) reported that foliar application of Zn enhanced productivity with better fruit quality in ‘Kinnow’ mandarin and also noted that trees applied with Zn had a significant effect on ascorbic acid content and pH of the fruits because Zn plays an active role in biosynthesis of auxins (Alloway, 2008; Ashraf *et al*., 2013a). Similarly, Tariq *et al.* (2007) and Ashraf *et al.* (2012) also reported that foliar spray of micronutrients enhanced the number of fruits per tree and juice volume per fruit in sweet orange (*Citrus sinensis* L.) and in Kinnow. Boron application increases fruit set and yield in several fruit and nut trees, including almond, Italian prune, olive, and sour cherry (Slavko *et al*., 2001). The results are similar to Khurshid *et al.* (2008) who noted that when orange trees were foliarly treated with Cu it significantly increased fruit yield $tree^{-1}$, fruit weight, total soluble solids, and fruit size as compared to the untreated trees. Fageria (2002) also observed that application of Cu increased yield of upland rice and common bean. Significant effect of Cu on yield of annual crops has been observed by Galrão (1999).

Conclusion

Foliar application of Zn at 0.3%, Cu at 0.1% and B at 0.2% improved photosynthetic and fruit yield attributes and also effective in enhancing the fruit yield with better fruit quality.

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