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ARTICLE

EFFECT OF FOLIAR SPRAY OF CALCIUM AND ZINC ON YIELD, NUTRIENTS CONCENTRATION AND FRUIT QUALITY OF ORANGE

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ABSTRACT

Balanced nutrients application is essential to increase yield, improve quality and prevent contaminations. Orange orchards in the calcareous soil widely suffer from calcium (Ca) and zinc (Zn) deficiencies because they are immobile in plants while there are abundant of calcium carbonate in the soil. In order to supply Ca and Zn for orange fruits, a study was conducted in the south western of Iran. The foliar application of calcium and zinc on yield, calcium, zinc and boron concentration and some quality Characteristics of orange (cv.valencia) was conducted in two growing seasons as a factorial design based on completely randomized block with nine treatments and three replications. Treatments were spraying of calcium chloride at three levels (0, 0.25%, and 0.5%) and foliar of zinc spraying from zinc sulfate at three levels (0, 0.25%, and 0.5%) at fruitlet and repeated twice every 30 days. Results showed that interaction of calcium and zinc on yield has been significant (p≤0.05) and increased Ca and Zn levels in the foliage. Meanwhile, the boron (B) status decreased by Ca application in 0.25% concentration significantly. Ca and Zn foliar treatments both resulted on B reduction in the orange leaves. Since orange orchards in the region shows visual of high boron symptoms, combined Ca and Zn spraying reduced boron toxicity effect rather than solely application.

KEY WORDS
Foliar application, Nutrients balance, Zinc, Calcium

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INTRODUCTION

Balanced nutrients application is essential to increase yield, improve quality and prevent contaminations. Calcium is vital nutrient that improves quality and yield [1]. Due to high calcium carbonate in the calcareous soils of the southwestern of Iran, it is expected to observe no calcium deficiency in the orchards [2]. Regarding the low soil moisture and light texture exchangeable calcium is not adequate in the soil solution and translocation of calcium in the developing fruits is not properly efficient due to poor evaporation. The concentration of calcium in the soil solution is not sufficient when the soil moisture is drastically low and consequently solubility of calcium decreases. Citrus orchards in the calcareous soil suffer from calcium (Ca) and zinc (Zn) deficiencies because they are immobile in plants while there are abundant of calcium carbonate in the soil [2]. The movement of calcium in the plant is very slow and it is mostly transmitted by xylem vessels [1]. Therefore, providing the necessary amount of calcium is not possible for fruits when they are developing fast. Fruits are faced with calcium deficiency which will rise physiological disorders due to calcium deficiency in the fruit during the growing season and cause low shelf life and decreases marketable yield [2]. Paual and Shaha [3] reported that fruits are not usually a good source of calcium. They also showed that only a few of citrus fruits significantly contain calcium out of which lemon has the most calcium. Sando et al. [4] observed that foliar application of calcium chloride did not change the fruit quality and only led to an increase in the middle skin of fruits. Dong and et al. [5] reported that the use of calcium and boron on the orange caused improvement of fruit tissues and cell membrane and influenced on the activity of enzymes such as polygalacturonase, pectinesterase and b-galactosidase. According to the report of FAO of 30 countries from all over the world, more than 30 percent of the agricultural soils are faced with available zinc deficiency problem for crop and horticultural plants [6]. The results of studies on agricultural soils of Iran and in the region where the study was conducted, confirm that zinc deficiency is common in these soils [7]. According to Malakouti et al. [7] Zinc deficiency is a widespread and common micronutrient deficiency in Iran due to lack of zinc in the soils, calcareous condition and high pH. After nitrogen, zinc deficiency is the most widespread nutritional disorder in citrus [8, 9].

Boron is one of the micronutrients whose sufficiency and toxicity range is very low, unlike other micronutrients [10]. Irrigation water has been a major proof of boron toxicity in the south of Iran [11]. Citrus is very sensitive to the boron deficiency and toxicity and their tolerance has reported as about 0.3 mg per kilogram of soil [12]. Zinc is used as an essential component for the activation of some enzymes in plant whose sufficient level is effective in the absorption and uptake of boron by plants [13]. Mahmoudi et al. [2] reported that foliar spray of calcium chloride increased calcium concentrations and decreased TSS in orange. Tariq et al. [14] reported that zinc sulfate treatment reduced the concentration of boron in the leaves of orange, but this reduction was not significant. In addition, the application of boron decreased the concentration of zinc in the plant, but it was also not significant. A similar result has been found in almonds by others [15, 16]. Contrasting results have been presented by researchers on the interaction of calcium and boron as Rostami et al. [17] reported that the application of boron significantly decreased calcium concentration in olive foliage. Some reports have indicated the positive interaction or lack of interaction of these two elements [18, 19]. In general, by attention to suffering citrus orchards in the calcareous soil from calcium (Ca) and zinc (Zn) deficiency. On the other hand, the concentration of boron is higher than sufficiency range (98± 29 mg per kg). Therefore, investigating the effect of calcium and zinc on boron concentration by aiming to the improvement of the toxicity of this nutrient is necessary.

THE IIOAB JOURNAL

Accordingly, aim of this study is investigating the effect of foliar application of calcium and zinc on the yield and quality, control of boron concentration in the leaves.

MATERIALS AND METHODS

The experiment was conducted in an orchard located at gachsaran region with latitude 30°, 15' N and longitude 50°, 45' E in southwestern of Iran. This area is located in hot and semiarid climate, average rainfall of 425 mm, mean altitude 900 m and a loamy soil texture. At the first, among orange orchards one orchard has been selected in which boron concentration of leaf was higher than the desirable rang (98±29 mg kg-1) that was determined by compositional nutrient diagnosis (CND) method [20]. Soil samples were collected from 0-30 and 31-60 cm depth and compose leaf sample was taken from non-bearing current shoots. The physical and chemical properties of the soil consisted soil texture was measured by the hydrometer method, electrical conductivity in saturated extract by conductimeter, pH with pH meter, calcium carbonate percent by titration, organic carbon by walkley-black [21], available K by ammonium acetate and flame photometer and available P using Olsen method [22]. Extractable Fe, Mn, Zn and Cu were extracted using DTPA solution and measured by atomic absorption spectrometry [23]. Boron was extracted with hot water and measured by Azomethien-H colorimetric method [23]. Total nitrogen content in leaf was measured by micro-kjeldahl, P by spectrophotometer and K by flame photometer. Concentration of Ca, Mg, Zn, Mn, Fe, and Cu in leaf was measured in dry ash extracted with Hcl by atomic absorption spectrophotometer. B concentration was determined by colorimetric Azomethin-H method with spectrophotometer [23]. The physical and chemical properties of soil and nutrient concentration of leaf are presented in Table 1 and 2, respectively.

The field experiment was laid out during 2013-2014 growing seasons as a factorial design on the base of completely randomized block with nine treatments, three replications and two trees per replication. Foliar treatments consisted of three levels of calcium chloride (0, 0.25 and 0.5 percent) and zinc sulfate (0, 0.25 and 0.5 percent), three times on the trees during fruitlet stage and repeated twice every 30 days. Other nutrients were applied according to soil and leaf analysis similarly in all treatments. Concentration of B, Ca and Zn in leaf was measured in the five-month leaves samples. At harvested time, yield and concentration of B, Ca and Zn in fruit were measured. Also, some quality characteristics of fruit juice such as TSS, TA were measured. Statically analysis variance on data was done with SAS and comparison of means was done by Duncan Multiple Rang Test.

Table 1: Physical and chemical properties of the orange orchard soil used in the study

Soil characteristics															
Soil texture									(mg kg^{-1})						
Soil depth (cm)	Sand %	Silt %	Caly (%)	Texture	OC (%)	CCE (%)	pH	EC (dS m^{-1})	P	K	Fe	Zn	Mn	Cu	B
30-0	48	52	20	L	0.86	49.75	7.3	0.55	4.9	224	4.68	0.42	13.4	0.46	0.682
60-31	60	20	20	Sa-Cl-L	0.37	57.57	7.2	0.92	3.3	140	3.82	0.28	7.58	0.34	0.316

Table 2: Concentration of nutrient elements in leaves orange orchard used in this study

N	P	K	Ca	Mg	Fe	Mn	Zn	Cu	B
%					mg kg $^{-1}$				
2.1	0.14	2.1	2.8	0.29	71	34	15	6	141

RESULTS AND DISCUSSION

Analysis of variance for yield, concentration of Calcium (Ca), Zinc (Zn) and Boron (B) in leaf and fruit of orange are shown in Table 3. The results indicated that interaction of foliar spraying of calcium and zinc on yield was significant (P≤0.01), [Table 3].

Table 3: Analysis of variance for yield, Calcium (Ca), Zinc (Zn) and Boron (B) concentration in leaf and fruit of orange

			MS					
			Ca		Zn		B	
Source of variation	Df	Yield	Leaf	Fruit	Leaf	Fruit	Leaf	Fruit
Replication	2	$13.89^{n.s}$	$0.125^{n.s}$	$0.032^{n.s}$	$1549.65^{n.s}$	$0.015^{n.s}$	$346.36^{n.s}$	$0.085^{n.s}$
Calcium(Ca)	2	$10.91^{n.s}$	1.976*.*	$0.012^{n.s}$	14761.27*.*	$0.003^{n.s}$	2283.54*	$0.077^{n.s}$
Zinc(Zn)	2	$37.24^{n.s}$	$0.353^{n.s}$	$0.009^{n.s}$	395159.55*.*	0.213^{*}	$1634.08^{n.s}$	$0.660^{n.s}$
Ca×Zn	4	83.03*.*	$0.138^{n.s}$	$0.018^{n.s}$	2644.729*	$0.006^{n.s}$	$1397.93^{n.s}$	$0.693^{n.s}$
Error	16	13.28	0.212	$0.015^{n.s}$	736.42	$0.048^{n.s}$	603.11	$0.360^{n.s}$
C.V		12.99	11.57	23.45	10.25	22.07	18.62	22.6

ns, * and **: respectively non-significant, significant at (P≤0.05) and (P≤0.01) probability levels

Comparison of mean yield using Duncan test from Table 4 shows that interaction of calcium and zinc has significantly increased yield (kg tree $^{-1}$) compared to control. The highest increasing of yield compared to control, has been with zero level of calcium and 0.25% of zinc sulfate with an average of 36.53 kg per tree which has resulted in an increase of 14 kg in each tree compared to control. Obviously, in various levels of zinc, yield in second and third levels of calcium does not differ significantly.

Table 4: The interaction effect of calcium chloride and zinc sulfate foliar spray on mean yield (kg tree-1) of orange

Treatment	Ca_0	Ca_1	Ca_2	Mean
Zn_0	22.63d*	30.43abc	31.43ab	28.16A
Zn_1	36.52a	26.25bcd	27.37bcd	30.05A
Zn_2	26.72bcd	23.71cd	27.52 bcd	25.98A
Mean	28.62A	26.80A	28.77A	

Means in each row and column with the same letters are not significantly different (P≤0.05) using DMRT
Ca_0= control, ca_1 and Ca_2, foliar spray of calcium chloride with concentration of 0.25 % and 0.5 % respectively
Zn_0= control, Zn_1 and Zn_2, foliar spray of zinc sulfate with concentration of 0.25 %, and 0.5 % respectively

Seyyed Kalayi et al. [9] reported that spraying zinc sulfate with a concentration of 0.3% has increased yield of orange significantly. In addition, they concluded that using zinc sulfate spraying possibly has increased auxin concentration. They mentioned increasing auxin concentration and decreasing fruit fall, is one of the reasons for yield increase. Tryptophan is the substrate of auxin synthesis and has been reported that zinc is important synthesis of this substrate [24, 9]. Asadi Kangar Shahi et al. [8] concluded that using zinc has increased orange yield significantly. Others have reported the positive effect of zinc, especially in the form of being sprayed, on yield of crops such as olive, almond, pomegranate, corn and wheat [9, 24, 25].

Concentration of Calcium, Zinc and Boron in leaf

Investigating the results from analysis variance of data related to calcium concentration from Table 3 shows that only the effect of foliar spraying of calcium on this trait has been significant (P≤0.01). The effect of using zinc and interaction of calcium and zinc on this trait was not significant [Table 3]. Comparison of mean calcium concentration by Dunkan test at (P≤0.05) from Fig 1 shows that by increasing calcium, its concentration in leaf increased significantly in such a way that the highest concentration was related to calcium level of 0.05%. Obviously, no significant difference was observed between two levels of 0.25 and 0.05 % calcium chloride. Regarding that in foliar spraying this element is supplied for leaves directly, this concentration increase seems reasonable.

The results from analysis variance of zinc concentration in leaf from Table 3 show that the main effect and interaction of calcium chloride and zinc sulfate foliar spraying has been significant at (P≤0.01) and (P≤0.05) respectively.

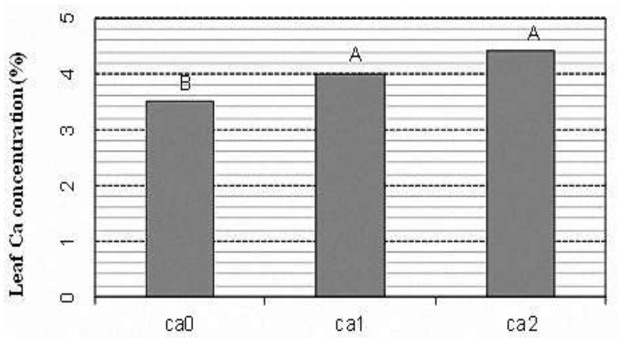


Fig. 1: The effect of different levels of foliar spray of calcium chloride on calcium concentration in orange leaf Ca0= control, ca1 and Ca2, foliar spray of calcium chloride with concentration of 0.25 % and 0.5 % respectively

Comparing mean concentration of zinc from Table 5 shows that combine application of calcium chloride and zinc sulfate has significantly increased zinc concentration in leaf compared to control. The highest mean of zinc concentration has been created by combine foliar spraying of calcium chloride and zinc sulfate with a concentration of 0.25 and 0.05 % respectively. Although there is no significant difference between second and third level of zinc sulfate treatment.

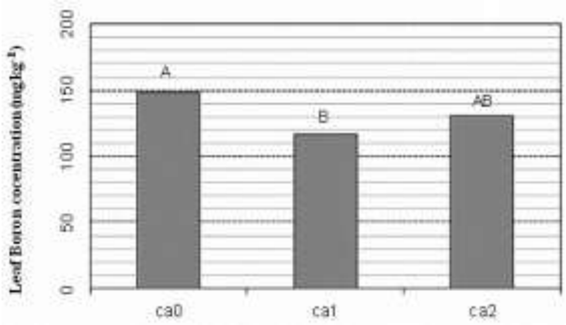
Table 5: The interaction effect of calcium chloride and zinc sulfate foliar spray on the mean zinc concentration (mg kg-1) in leaf of orange

Treatment	Ca_0	Ca_1	Ca_2	Mean
Zn_0	16.27d*	36.45d	19.8d	24.17C
Zn_1	293.37c	395.83a	399.8a	363.00B
Zn_2	344.73b	440.23a	436.53a	407.17A
Mean	218.12B	290.84A	285.38A	

* Means in each row and column with the same letters are not significantly different (P≤0.05) using DMRT
Ca_0= control, ca_1 and Ca_2, foliar spray of calcium chloride with concentration of 0.25 % and 0.5 % respectively
Zn_0= control, Zn_1 and Zn_2, foliar spray of zinc sulfate with concentration of 0.25 %, and 0.5 % respectively

Obviously, in various levels of zinc, there is no significant difference between second and third levels of calcium chloride. Considering the increasing of zinc concentration in leaf by spraying calcium chloride, it will be better to use calcium in low levels of zinc to increase zinc concentration in leaves [Table 5]. Mahmoudi et al. [2] reported that using calcium chloride in orange has increased zinc concentration; however, this increase has not been significant. One of the reasons of increasing yield is the positive effect of calcium and zinc on zinc concentration in orange. Considering the role and importance of zinc in physiologic activities of this plant, increasing yield is expected [2, 24, 8, 9].

Results from statistical analysis of leaf boron concentration from Table 3 show that only the main effect of calcium on this trait is significant (P≤0.05). Comparison of mean for boron concentration from Fig 2 indicates that calcium application levels have decreased leaf boron concentration. The highest decrease has been seen in 0.25 % concentration of calcium chloride.



Calcium application levels

Fig. 2: The effect of different levels of foliar spray of calcium chloride on leaf boron concentration in orange Ca0= control, ca1 and Ca2, foliar spray of calcium chloride with concentration of 0.25 % and 0.5 % respectively

In addition, combined foliar of calcium and zinc in 0.25 % concentration has decreased boron levels in leaf samples to 100.3 mg kg^{-1}. The most decrease was belonging to this concentration. In this regard, Chakerolhosseini et al. (2016), desirable range of boron concentration in orange leaf using Compositional nutrient diagnosis (CND) was determined to be 98±29 mg kg^{-1}. Combined calcium and zinc application in 0.25 % doeses has been able to decrease the concentration of boron to sufficient range.

Rostami et al. [17] concluded that there is a significant negative interaction between boron and calcium in olive. Hosseini et al. [25] also reported this interaction between boron and zinc in corn. They recommended that in areas where boron concentration is higher than optimal level, especially when there is zinc deficiency, a sufficient amount of zinc should be administered for the plant. In addition, in studies conducted on wheat [18] and tomato [19], they were concluded that there is a positive interaction between these two nutrients.

Concentration of Calcium, Zinc and Boron in fruit

Results in Table 3 show that among these three concentrations, only the effect of zinc sulfate foliar spraying on concentration of zinc in fruit is significant (P≤0.05). In Figure 3, foliar application of zinc sulfate has significantly increased zinc concentration in fruit in two applicable levels compared to control. However, no significant difference was observed between two levels of 0.25 and 0.05 % zinc sulfate.

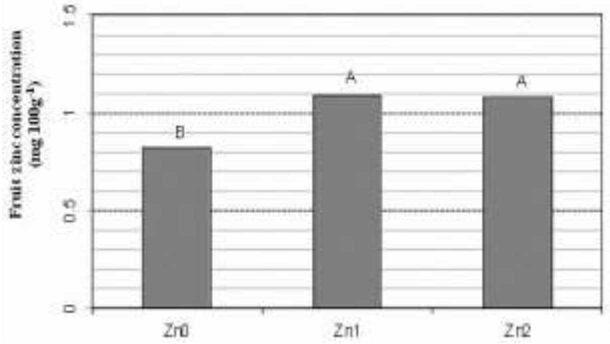


Fig. 3: The effect of different levels of foliar spray of zinc sulfate on zinc concentration in orange fruit Zn0=control, Zn1 and Zn2, foliar spray of zinc sulfate with concentration of 0.25 %, and 0.5 % respectively

The reason for this increase is direct supply of zinc on fruit and indirect Zn uptake by leaf and transferring to fruits [16]. Comparison of mean for calcium and boron concentration in fruits analysis demonstrated that using calcium chloride has increased calcium concentration in orange fruit. This increase is not significant, but considerable. Using calcium and zinc has decreased mean boron concentration of fruit in all applicable levels compared to control; however, this is not significant. In this regard, Rostami et al. [17] reported that using boron has significantly decreased calcium concentration in leaf. However, some reports demonstrate positive interaction or no interaction between calcium and boron [18, 19].

Total Soluble Solids (TSS), pH and Total Acidity (TA) of orange Juice

The results from statistical analysis of these three properties (TSS, pH and TA) revealed that only the effect of calcium chloride spraying on TSS and pH of orange juice is significant ($P \le 0.05$). Comparison mean of TSS and pH in Table 6 shows that calcium application levels decreased and increased these traits respectively.

Table 6: The effect of calcium chloride foliar application on the mean of TSS and pH in orange juice

Calcium application levels	TSS	pH
Ca_0	11.69A	3.1B
Ca_1	10.49B	3.3AB
Ca_2	9.98B	3.4A

Means in each column with the same letters are not significantly different ($P \le 0.05$) using DMRT

Ca_0= control, ca_1 and Ca_2, foliar spray of calcium chloride with concentration of 0.25 % and 0.5 % respectively

Total soluble solids (TSS) indirectly includes the amount of sugars, organic acids, anthocyanin and other soluble materials and by creating alkaline conditions, increase pH [Table 6], calcium can decrease organic acids and consequently the amount of TSS decreases. In addition, by creating bonds with carboxyl groups, calcium stabilizes cell walls and reduces soluble compounds [26]. Another reason is, creating a relatively thick layer on fruit by calcium and postponing change processes including hydrolysis of polysaccharides and finally decreased concentration of soluble minerals [27].

CONCLUSION

The results of this study showed that the combined application of calcium and zinc has significantly increased yield compared to control. The effect of spraying calcium and zinc on calcium and zinc concentration in leaf and fruit was significant. By increasing calcium chloride, Ca concentration in leaf increased significantly, but no significant difference was observed between two levels of 0.25 and 0.05 % calcium chloride. Combined application of calcium chloride and zinc sulfate has significantly increased zinc concentration in leaf compared to control. The highest mean of zinc concentration has been created by combine foliar spraying of calcium chloride and zinc sulfate with a concentration of 0.25 and 0.05 % respectively. Although there is no significant difference between second and third level of zinc sulfate treatment. Considering the increasing of zinc concentration in leaf by spraying calcium chloride, it will be better to use calcium in low levels of zinc to increase zinc concentration in leaves. One of the reasons of increasing yield is the positive effect of calcium and zinc on zinc concentration in orange. The results also showed that calcium application levels have significantly decreased leaf boron concentration. Combined calcium and zinc application in 0.25 % doses has been able to decrease the concentration of boron to sufficient range. Foliar application of zinc sulfate has significantly increased zinc concentration in fruit. According to presented results, the 0.25% $CaCl_2$ + 0.25% $ZnSo_4$ as foliar spray was suitable combination for the most of measured characters in orange during the course of this experiment.

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