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Effects of zinc and manganese as foliar spray on pomegranate yield, fruit quality and leaf minerals

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Abstract

Effects of foliar sprays of zinc and manganese sulfates on the fruit yield and quality as well as leaf nutrients concentration of pomegranate were studied during 2010 growing season in an orchard with a soil pH of 7.5 and EC of 5.2 (dS m^{-1}). Zinc and manganese sulfates were applied two times at the rate of 0, 0.3 and 0.6 percent under a factorial design on the base of completely randomized blocks. Mn sprays had positive significant effects on the fruit yield, the aril/peel ratio, TSS, weight of 100 arils, juice content of arils, anthocyanin index, fruit diameter and leaf area. Zn effects were also significant for TSS, TSS/TA ratio, juice content of arils and leaf area. Foliar spray of Mn significantly increased Mn and N but decreased Zn and Cu concentrations in leaves. Foliar sprays of Zn significantly increased Zn but decreased Mn and P concentrations in the leaves. According to the results, the suitable combination of these two micronutrients for studied characters of pomegranate under prevailing conditions was foliar spray of 0.6% $MnSO_4$ and 0.3% $ZnSO_4$.

Keywords: *Punica granatum*, yield, arils, mineral elements, micronutrients.

1. Introduction

Pomegranate (*Punica granatum* L.) belonging to the Punicaceae family, is one of the favorite table fruits grown in tropical and sub-tropical regions. This plant is native of Iran and is extensively cultivated in the Mediterranean region since ages (Sheikh and Manjula, 2009). The edible part of the fruit is the seeds having a fleshy covering and called arils, which are eaten fresh or used for making juice, jam and paste. In addition, the fruit is also valued for its pharmaceutical properties. The fruit peel, and the tree stem and root bark and leaves are good source of secondary metabolites such as tannins, dyes and alkaloids (Mirdehghan and Rahemi, 2007). The incidence of micronutrient deficiencies in fruit crops has increased markedly in recent years due to intensive cropping, losses of micronutrients through leaching, decreased proportions of farm manure application compared to chemical fertilizers, increased purity of chemical fertilizers, soil erosion and use of marginal lands (with high pH and EC) for crop production (Zia *et al*., 2006). The climate change by weather warming and drying might be another important reason for the disorders.

Zinc (Zn) is an essential trace element for plants, being involved in many enzymatic reactions and is necessary for their good growth and development. Zinc is also involved in regulating the protein and carbohydrate metabolism (Swietlik, 1999). Zinc availability to plants is reduced in high pH soils. Two main theories are offered to account for high Zn deficiency incidence on calcareous soils. First, the solubility of Zn in these soils to be decreased up to 100 fold per unit increase in pH, and the second theory which is based on the adsorption of this element by calcium carbonate ($CaCO_3$); the carbonate found in such soils forms an insoluble complex with Zn added as zinc sulfate (Rasouli-Sadeghiani *et al*., 2002). Zinc deficiency is commonly observed in pomegranate orchards of Iran (Taghavi, 2000; Daryashenas and Dehghani, 2006). Zinc uptake rate was faster in mango trees when zinc sulfate was foliar applied as compared with its soil application (Bahadur *et al*., 1998). However, the foliar or soil application of zinc sulfate showed no effect on fruit yield and quality of mango, except for TSS in the fruit.

Similar to zinc, manganese also is a heavy metal micronutrient, the functions of which are fairly known. It is involved in the oxygen-evolving step of photosynthesis and membrane function, as well as serving as an important activator of numerous enzymes in the cell (Wiedenhoeft, 2006). Soil application of Mn is problematic, since its efficiency depends on many soil factors, including soil pH. A suitable method for the correction and /or prevention of Mn deficiency in plants is the foliar application of ionic or chelated solution forms of this nutrient (Papadakis *et al*., 2007). Silva *et al*. (2009) reported that the application of lime for orange trees for correction of soil pH resulted to reduced manganese concentrations in leaves proportional to increased lime in the soil. In addition, correlation between the time pass after liming with leaf manganese levels was found. Two chemical forms are mostly used for the correction of Mn deficiency of fruit trees, the inorganic ($MnSO_4$) and organic (Mn EDTA) forms (Papadakis *et al*., 2005). According to results of some experiments on apple trees (Thalheimer and Paoli, 2002) and orange trees (Papadakis *et al*., 2005), foliar application of manganese sulfate was more effective than manganese chelate in increasing leaf Mn concentrations. Broschat (1991) also stated that with foliar application of four soluble Mn sources, only manganese sulfate consistently increased Mn concentrations in the pygmy date palm leaves.

The literature on Zn and Mn fertilization of pomegranate is scarce. Balakrishnan *et al*. (1996) reported that foliar application of 0.25% each of zinc sulfate, manganese sulfate and iron sulfate combined with 0.15% boric acid, significantly increased fruit yield and juice content of pomegranate fruit. In addition, foliar application of Zn and Mn alone or in combination with each other showed significant increase in fruit yield of sweet oranges (Tariq *et al*., 2007).

The aim of this study was to assess the effect of foliar spray of zinc and manganese sulfates on some pomegranate fruit traits including yield and number of fruits per tree, average fruit weight, the aril/peel ratio, weight of 100 arils, fruit length and diameter, TSS, TA, TSS/TA ratio, juice content of arils, anthocyanin index and peel thickness. Also, the effects of these elements on concentration of some other nutrients in the leaves were assessed.

Table 1. Physical and chemical properties of the orchard soil used in the study (compound soil samples from depths of 0-30 and 31- 60 cm)

Texture	SL
Saturation Percent (%)	26
EC (dSm^{-1})	5.2
pH	7.5
Total Neutralizing Value (T.N.V %)	28
Organic Carbon (%)	0.19
Total nitrogen (%)	0.023
P ($mg\ kg^{-1}$)	4.5
K ($mg\ kg^{-1}$)	195
Fe ($mg\ kg^{-1}$)	4
Zn ($mg\ kg^{-1}$)	0.7
Cu ($mg\ kg^{-1}$)	0.3
Mn ($mg\ kg^{-1}$)	7

2. Materials and Methods

The experiment was conducted during 2010 in a commercial orchard (Kesht & Sanate Khooshehay e Zarin e Saveh) located at Saveh in Markazi Province of Iran. The area is semiarid, with an average annual rainfall of about 200 mm, which is mostly precipitated in the winter, and average annual temperature is 18 °C and is one of the major pomegranate production centers in Iran. Seventy-two pomegranate (*Punica granatum* cv. Malas e Torsh e Saveh) six years old trees that their 3 years old aerial parts had been developed after the severe winter injury of 2006 and spaced at 2.5×4 m, were selected for experiment. All trees had uniform vigor and were trained to single trunk, as well as being under a drip irrigation system. The orchard was receiving the current applications for nutrition and other horticultural practices. The physical and chemical properties of the soil for the orchard are presented in Table 1.

The experiment was laid out as a factorial design on the base of completely randomized blocks with four replications and two trees per replication. Aqueous solution of $MnSO_4.H_2O$ (Merck) and $ZnSO_4.7H_2O$ (Merck) were applied at the rate of 0, 0.3 and 0.6 percent. The treatments were applied two times on the trees, first, at 15 days before full bloom and the second, one month after the first application. Sprays were applied in the morning (6-9 a.m.) using a hand pressure sprayer. At harvest, the fruits were weighed and counted separately for each tree, and then the yield (Kg $tree^{-1}$) was calculated in each treatment. Average fruit weight was expressed as a fraction of the fruit yield to number of fruit/tree. Four sound fruits per each replicate were selected in 12 October to calculate the other studied characters. Fruit length and diameter measured with digital caliper and averaged for each replicate. To measure the aril/peel ratio, after removing the arils from the fruit peel and placental parts by hand, separated arils and the remaining parts of fruit were weighed, then this character was expressed as a fraction of the total aril weight to total peel weight in each fruit. Total Soluble Solids (TSS) was measured using a hand-held refractometer. Titrable acidity (TA) was determined by titration with an aliquot of juice against 0.1 N NaOH and was expressed as citric acid percentage (Al-Maiman and Ahmad, 2002). Juice content of arils measured for 100 g of arils by extracting the juice by hand and expressed as percentage. To determine anthocyanin content, first, the juice sample was centrifuged (4 minutes at 9500 rpm), then was diluted with distilled water (the ratio of 1 juice to 3 water) and then the absorption by spectrophotometer at 510 nm wavelenght was read and recorded and showed as anthocyanin index (Sarkhosh *et al*., 2009). Peel thickness was measured with digital caliper at the thinnest parts.

In order to determine the leaf nutrients concentration, about 30 leaves were collected from each tree in early August from non-fruiting, spring growth shoots, so that two to three fully expanded mature leaves were collected from the middle of shoots. Leaf samples were washed first with tap water, and next with distilled water and non-ionic detergent. Then they were dried with an air oven at 70°C and subsequently ground manually with mortar and pestle. One gram of powder was burned in muffle oven at 550± 25°C. The resulting white ash was then dissolved in 10 ml of 2 N HCl and adjusted to a volume of 100 ml by distilled water for determination of macro- and micronutrients (Chapman and Pratt, 1961). Zn, Mn, Fe and Cu contents were measured using an atomic absorption spectrophotometer. Potassium was determined by flame photometer and phosphorous content analyzed by the molybdovanadate yellow color method by spectrophotometer. Total nitrogen content was determined using Kjeldhal method. Data were analyzed of variance (ANOVA) and differences among the means were determined for significance using Duncan Multiple Range Test at the 5% level using the SAS software version 9.1.

3. Results and Discussion

3.1 Fruit yield, number of fruit/tree and average fruit weight

Results showed that Mn sprays at both levels significantly increased fruit yield but Zn sprays had no significant effect on this character. The maximum fruit yield of 8.1 kg tree^{-1} was obtained from trees receiving 0.6% $MnSO_4$ + 0.3% $ZnSO_4$ (Table 2). Similar results had been reported that foliar spray of Mn increased fruit yield in ‘Ganesh’ pomegranate (Bambal *et al*., 1991) and ‘Valencia’ orange (Labanauskas *et al*., 1963). Mn sprays increased number of fruit/tree and fruit average weight, although the increase was not statistically significant, therefore the increase in fruit yield caused by Mn could be due to the increase in number of fruit/tree as well as fruit average weight. Zn application had no effect on these traits. Similar results also showed that Zn sprays had no effect on fruit yield in pomegranate (El-Khawaga, 2007; Khorsandi *et al*., 2009) and ‘Valencia’ oranges (Labanauskas and Puffer, 1964).

Table 2. Effect of foliar spray of zinc and manganese sulfates on fruit yield (kg tree^{-1}), number of fruit /tree (Nº of Fruit/tree), fruit average weight (Fruit Av. Wt.), the aril/peel ratio, TSS, TA and TSS/TA ratio of pomegranate fruits.

Treatment		Fruit Yield (kg tree^{-1})	No. of Fruit/ Tree	Fruit Av. Wt (g)^2	The aril/ peel ratio	TSS (°Brix)	TA (%)	TSS/TA ratio
$MnSO_4$ (%)	$ZnSO_4$ (%)							
0	0	6.7c	28.37a	240.7a	1.68c	14.28c	1.78a	8.00b
0	0.3	7.0abc	28.87a	246.9a	1.73bc	15.20b	1.78a	8.54a
0	0.6	6.8bc	28.25a	243.5a	1.69bc	15.00b	1.77a	8.46a
0.3	0	7.3abc	29.50a	250.8a	1.79abc	14.96b	1.81a	8.27ab
0.3	0.3	7.7abc	30.87a	255.7a	1.79abc	15.38ab	1.80a	8.54a
0.3	0.6	7.2abc	29.25a	246.7a	1.74abc	15.13b	1.78a	8.49a
0.6	0	7.8abc	30.87a	260.6a	1.88a	15.11b	1.83a	8.20ab
0.6	0.3	8.1a	31.12a	261.8a	1.84ab	15.73a	1.82a	8.60a
0.6	0.6	7.9ab	30.37a	259.9a	1.79abc	15.22b	1.80a	8.46a
Significance								
Mn		**	NS	NS	**	**	NS	NS
Zn		NS	NS	NS	NS	**	NS	**
Mn×Zn		NS	NS	NS	NS	NS	NS	NS

Mean separation within columns by Duncan Multiple Range Test at $p \leq 0.05$
NS, *,**. Insignificant or significant at $p \leq 0.05$ or 0.01, respectively

3.2 The aril/peel ratio, peel thickness and weight of 100 arils

With application of manganese, the aril/peel ratio followed an increasing trend and foliar spray of 0.6% $MnSO_4$ significantly increased this character. Peel thickness showed decreasing trend with foliar spray of manganese sulfate but was not statistically significant. In addition, decrease of the aril/peel ratio was observed by zinc application compared to manganese spray in this experiment (Table 2). Similar results exist, associated with increasing in fruit peel weight and reducing fruit arils in 'Manfaluty' pomegranate as affected by Zn spray (El-Khawaga, 2007). Manganese had significant positive effect on weight of 100 arils but zinc had no significant effect on it. Among the applied treatments, 0.6% $MnSO_4$ alone and in combination with both levels of $ZnSO_4$ (0.3 and 0.6 percent) and also combination of 0.3% $MnSO_4$ and 0.3% $ZnSO_4$ significantly increased weight of 100 arils (Table 3).

Table 3. Effect of foliar spray of zinc and manganese sulfates on weight of 100 arils, juice content of arils, anthocyanin index, peel thickness, fruit length, fruit diameter and leaf area of pomegranate.

Treatment		Weight of 100 arils (g)	Juice content (%)	An[1]	Peel thickness (mm)	Fruit length (cm)	Fruit diameter (cm)	Leaf area (mm^2)
$MnSO_4$ (%)	$ZnSO_4$ (%)							
0	0	31.2b	65.6c	0.254b	2.12a	7.37a	7.57b	470.7c
0	0.3	32.3ab	67.1abc	0.273ab	2.08a	7.72a	7.91ab	585.2ab
0	0.6	32.0ab	66.0bc	0.261b	2.12a	7.59a	7.87ab	497.5cd
0.3	0	32.6ab	67.6ab	0.290ab	1.98a	7.61a	7.96ab	492.0c
0.3	0.3	33.1a	67.9ab	0.298ab	2.07a	7.71a	8.14ab	614.2a
0.3	0.6	32.8ab	66.5abc	0.265ab	2.13a	7.63a	7.87ab	592.4a
0.6	0	33.1a	68.1a	0.303ab	1.89a	7.73a	8.15ab	584.4ab
0.6	0.3	33.5a	68.2a	0.328a	2.00a	7.82a	8.20a	586.3ab
0.6	0.6	33.0a	67.1abc	0.287ab	2.11a	7.74a	8.10ab	615.6a
Significance								
Mn		**	**	*	NS	NS	*	*
Zn		NS	*	NS	NS	NS	NS	**
Mn×Zn		NS	NS	NS	NS	NS	NS	NS

Mean separation within columns by Duncan Multiple Range Test at $p \leq 0.05$
NS, *,**, Insignificant or significant at $p \leq 0.05$ or 0.01, respectively
[1] Anthocyanin index: Absorption at 510 nm for 1:3 diluted juice

3.3 Fruit diameter, fruit length and leaf area

Mn application increased fruit diameter and fruit length but only the 0.6% rate of manganese was significant on fruit diameter. Zn sprays had no significant effect on fruit length and diameter. There was significant effect of zinc and manganese sprays on leaf area, so that treated trees by combination of Zn and Mn had greater leaf area (Table 3), which is in agreement with data concerning other fruits to foliar spray of Zn (Rasouli-Sadeghiani *et al*., 2002; Arora and Singh, 1970).

3.4 Juice content of arils, anthocyanin index, TSS, TA and TSS/TA ratio

Mn spray had significant effect on juice content of arils and anthocyanin index. Foliar sprays of Mn at both levels (0.3 and 0.6 percent) significantly increased juice content of arils and the 0.6% application of manganese had also significant increase on anthocyanin index. Zn spray had no significant effect on anthocyanin index. The 0.3% application of $ZnSO_4$ significantly increased juice content of arils, while the rate of 0.6% $ZnSO_4$ had negative impact on juice content of arils and anthocyanin index. Combination of manganese sulfate at 0.6% and zinc sulfate at 0.3% was the best treatment on increasing juice content of arils and anthocyanin index (Table 3). By foliar application of zinc, decrease in percentage of juice in 'Valencia' orange fruit had been already reported (Labanauskas *et al*., 1963). El-Khawaga (2007) stated that decreasing in juice percent of pomegranate fruits caused by Zn spray was related to producing smaller fruits, increasing in fruit peel weight and reducing total arils of fruits.

In our experiment each of Mn and Zn sprays had significant positive effects on TSS, and the effect of $ZnSO_4$ was more reasonable than $MnSO_4$ in increasing TSS, but their combination resulted in relatively higher TSS (Table 2). It has been reported that the highest TSS was obtained by foliar application of zinc sulfate (0.4%) combined with boric acid (0.2%) in 'Ganesh' pomegranate (Balakrishnan *et al*., 1996). Titrable acidity increased with application of Mn; however, Zn sprays decreased TA, although they were not significant (Table 2). On the contrary, it has been reported that the foliar application of zinc sulfate (2000 to 4000 ppm) increased titrable acidity of 'Manfaluty' pomegranate fruit (El-Khawaga, 2007). The variation in the results may be attributed to time of application of Zn and variable responses of different cultivars of pomegranate to zinc application or the environment conditions. Effects of $ZnSO_4$ at both levels (0.3 and 0.6%) were significant for TSS/TA ratio but $MnSO_4$ had no significant effect on this character. Maximum juice content of arils and anthocyanin index, TSS and TSS/TA ratio were obtained from the treatment receiving 0.6% $MnSO_4$ + 0.3% $ZnSO_4$ (Tables 2 and 3).

3.5 Macro- and micronutrients in pomegranate leaves

According to Table 4, the effects of all foliar treatments on the concentration of P, K, Fe and Cu were statistically insignificant. Results showed that the foliar spray of zinc and manganese sulfates significantly increased the Zn and Mn concentrations in pomegranate leaves, respectively. The highest Zn and Mn concentrations (139.5 mg kg^{-1} and 163 mg kg^{-1}dry weight of leaf, respectively) obtained each in the 0.6% treatment which the Zn and Mn were applied alone (Table 4). Khorsandi *et al*. (2009) reported similar increase in the Zn concentration of pomegranate leaves. Results also showed that the applied Mn insignificantly decreased the concentrations of Zn and Cu and significantly increased concentration of N in pomegranate leaves. In other plants, it has been stated that Mn could be antagonistic on concentration of Zn (Tariq *et al*., 2007) and Cu (Labanauskas *et al*., 1963). According to the results, foliar-applied Zn decreased the concentration of both Mn and P and increased concentration of Cu in pomegranate leaves. Therefore, it can be stated that there was an antagonistic effect between Zn and these nutrients (Mn and P) and a synergism relation between Zn and Cu. Similarly, reports are available that with Zn foliar sprays, the concentration of Mn decreased in orange leaves (Labanauskas and Puffer, 1964; Tariq *et al*., 2007) and maize leaves (Aref, 2011). According to a previous report, the concentration of P was low in the samples from Zn sprays in apple leaves (Rasouli-Sadeghiani *et al*., 2002). In addition, Aref (2011) stated that foliar sprays of Zn increased concentration of Cu in maize leaves. Fruit size (both length and diameter) increased with increasing the concentration of $MnSO_4$ and the increase in fruit size was associated with Mn, N and K concentrations in pomegranate leaves (Table 4). Fallahi *et al*. (1997) stated that levels of leaf N had positive effect on fruit size in apple, so that heavier fruits obtained from trees that had high levels of N in leaves. It should be mentioned that in present experiment, foliar spray of 0.6% $ZnSO_4$ in each of two times, alone and combined with both levels of $MnSO_4$, caused necrotic spots on leaves.

Table 4. Effect of foliar spray of zinc and manganese sulfates on macro- and micronutrients concentrations (percent or mgkg^{-1} dry weight) in pomegranate leaves.

Treatment		**N**	**P**	**K**	**Fe**	**Zn**	**Mn**	**Cu**
$MnSO_4$ (%)	**$ZnSO_4$ (%)**	**(%)**	**(%)**	**(%)**	**(mg kg^{-1})**	**(mg kg^{-1})**	**(mg kg^{-1})**	**(mg kg^{-1})**
0	0	1.94ab	0.199a	0.85a	129.5a	37.40b	92.20b	6.50a
0	0.3	1.81b	0.190a	0.90a	110.5a	93.50ab	86.50b	8.30a
0	0.6	1.81b	0.182a	0.86a	126.5a	139.5a	74.50b	8.35a
0.3	0	1.92ab	0.260a	0.99a	136.5a	38.40b	162.5a	5.15a
0.3	0.3	2.09a	0.230a	1.00a	91.00a	64.50ab	89.80b	5.15a
0.3	0.6	2.03ab	0.228a	0.98a	91.50a	74.20ab	89.70b	6.85a
0.6	0	1.97ab	0.224a	0.93a	112.5a	26.50b	163.0a	5.10a
0.6	0.3	2.04ab	0.192a	1.04a	122.5a	103.8ab	154.0a	5.35a
0.6	0.6	2.12a	0.186a	1.00a	96.50a	92.20ab	122.0ab	5.20a
Significance								
Mn		*	NS	NS	NS	NS	**	NS
Zn		NS	NS	NS	NS	*	*	NS
Mn×Zn		NS	NS	NS	NS	NS	NS	NS

Mean separation within columns by Duncan Multiple Range Test at $p \leq 0.05$
NS, *,**, Insignificant or significant at $p \leq 0.05$ or 0.01, respectively

According to soil and nutritional conditions of pomegranate trees cv. Malas e Torsh e Saveh, we can say that Mn is an important element in enhancing yield and improving the qualitative properties of this fruit. On the other hand, under conditions of present experiment, Zn had no significant effect on most measured characters and we can state that this nutrient element was not a limiting factor for reproductive processes of this fruit.

4. Conclusion

Manganese and zinc sulfates each had significant effects on some measured characters on pomegranate, but their interaction was not significant. Application of Mn at both levels (0.3 and 0.6%) increased some characters like fruit yield of trees, weight of 100 arils, fruit diameter, TSS, juice content of arils, the aril/peel ratio, anthocyanin index and leaf area. Zn effects were also significant for TSS, TSS/TA ratio, juice content of arils and leaf area. The treatments had no statistically significant effects on number of fruit/tree, average fruit weight, fruit length, titrable acidity and peel thickness. Foliar sprays of zinc sulfate significantly increased the Zn concentration but insignificantly decreased the concentrations of Mn and P in pomegranate leaves. Manganese sprays significantly increased concentration of Mn and N and it seems that Mn had antagonistic effects on Zn and Cu concentrations in leaves. According to presented results , the 0.6% $MnSO_4$ + 0.3% $ZnSO_4$ as foliar spray was suitable combination for the most of measured characters in pomegranate fruits and leaves during the course of this experiment.

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