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Changes in quantitative and qualitative traits of miagava tangerine (*Citrus reticulata* L.) as affected by Fe, Zn and Mn micronutrients foliar application

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Abstract

Miagava tangerine (*Citrus reticulata*) is an early citrus cultivar that is extensively planted worldwide including Iran. The present study was carried out to determine the best source of Mn, Zn and Fe spray of Miagava tangerine trees for realizing the maximum yield per unit area with the highest quality. This trial was operated based on a Randomized Complete Block Design (RCBD) with eight treatments including different ratios of three micronutrient sources (iron chelate, zinc chelate and Manganese chelate). The trees were sprayed at two stages with three different concentrations. The measured traits included fruit and fruit skin fresh and dry weight, acidity, vitamin C, sugar and fruit Zn, Fe and Mn content. Significant differences were found among different fertilizer sources in terms of their effect on quantitative and qualitative factors. Different treatments significantly increased fruit fresh and dry weight, Fe content, Mn content, sugar and vitamin C of fruits, so that the treatment of Zn + Fe + Mn had the highest indices of these traits in the fruits. The simple effects and interactions between fertilizer treatments were not statistically significant for skin fresh and dry weight, acidity and fruit Zn content.

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Introduction

Foliar application of mineral nutrients is a method for quick supply of the elements for the higher plants. This technique allows the plants to consume nutrients much faster than their uptake from soil by their roots. Despite some shortcomings of nutrients foliar application, it is regarded as the best method under certain conditions (Marschner and Marschner, 2012). Growing price of chemical fertilizers, the requirement for economical production, the contamination of underground waters and the demolition of soil structure due to anomalous and ignorant use of fertilizers are problems that need to be solved. Foliar application is method for reducing the use of chemical fertilizers (Malakouti and Tabatabaei, 1999). Uncontrolled and unbalanced application of fertilizers mainly phosphorous fertilizers has interrupted the nutrients, especially micronutrients, balances in soil and has reduced the uptake of Fe, Mn and Zn by plants. In addition, the limy and alkaline conditions of soils in Iran are other factors limiting the uptake of micronutrients. Given the constraints of soil application of micronutrients including fixation, foliar application is an effective method for meeting the demand of plants for micronutrients (Swiader, 2000). Eliminating micronutrients requirements through foliar application is a common practice to obtain profitable yield and producing marketable fruits (Leyden, 1983).

In citrus orchards, the deficiency of micronutrients especially Fe, Zn and Mn can be observed due to calcareous soil, low solubility of micronutrients, high pH, low organic matter content of soil and the presence of bicarbonate ions in irrigation water (Malakouti and Mashayekhi, 1997). Foliar application of some nutrients can be 10-20 times more effective than their soil application (Alva *et al.*, 2006). The spray of B, Cu, Mg, Mn and Zn is more appropriate than their soil application for rapid remedy of their deficiencies, reducing the toxicity in soil and avoiding their fixation (Camberato, 2004). To grow well, plants needs some elements among which the micronutrients like Fe, Mn, Zn, B and Mo are required although in a very small amount (Dezfuli, 1998). Since some citrus orchards of Iran suffer from lack of micronutrients, so the study on foliar application of these elements is necessary. The aim of present study was evaluation of foliar application effect of Zn, Fe and Mn on fruit and its peel fresh and dry weight, acidity, vitamin C content, sugar content and Zn, Fe and Mn content in Miagava tangerine (*Citrus reticulata*).

Materials and methods

Trial location and materials

The study was carried out in a commercial orchard in central part of Sari City, Iran. To study, trees were selected at the same age (3 years old), uniform, productive and free of pests and diseases with 5 × 6 m^2 spacing. The fertilizers iron chelate, zinc chelate and manganese chelate were sprayed in February and July with the ratios mentioned in Table 1.

Experimental design and measured characteristics

The measured traits included fruit and its peel fresh and dry weight and fruit acidity, vitamin C content, sugar content and Zn, Fe and Mn content of fruits. The study conducted based on Randomized Complete Block Design (RCBD) with eight treatments in three replications (each plot including three trees) with 72 trees.

Statistical analysis

After normalizing, all data were statistically analyzed by SAS statistical software. The data of fruit Zn content and fruit dry weight were normalized by logarithmic and reverse method, respectively. Then, the means of the measured traits were compared by LSD test and the graphs were drawn by MS-Excel software.

Results

Analysis of variance of the studied traits (Table 2) revealed the effect of treatments was significant on fruit fresh and dry weight, vitamin C, sugar, Fe and Mn ($p<0.01$). But, no significant difference was observed in fruit peel fresh and dry weight and fruit Zn content and pH.

Fruit flesh and peel fresh and dry weight

According to analysis of variance of the data, the treatments significantly influenced fresh and dry weight (Table 2). In addition, means comparison of the data (Fig. 1) revealed that trees sprayed with "Zn + Fe + Mn" had the highest fresh fruit weight (138.16 g) and fruits of control trees had the lowest weights (88.33 g). The effect of different treatments significantly affected fruit dry weight ($p<0.01$) (Table 2). The means comparison showed the trees sprayed with different fertilizer compositions had higher yield than control. Furthermore, spraying the trees with "Zn +Fe + Mn" resulted in the highest fruit dry weight (22.5 g) (Fig. 2).

Table 1. Types and concentration of trial treatments.

Treatments	Type of treatments	Concentration of nutrients in chelate
T_1	Control (water spraying)	-
T_2	Mn chelate	32%
T_3	Fe chelate	13%
T_4	Zn chelate	25%
T_5	Mn chelate + Zn chelate	32% + 25%
T_6	Zn chelate + Fe chelate	25% +13%
T_7	Fe chelate + Mn chelate	13% + 32%
T_8	Fe chelate + Mn chelate + Zn chelate	13% + 32% + 25%

According to analysis of variance table, foliar application did not significantly affect fruit peel fresh and dry weight (Table 2). Based on data mean comparison, the treatments had no significant different among treatments but, the highest fruit peel fresh and dry weight was obtained by the compound treatment "Zn + Fe + Mn".

Table 2. Analysis of variance of treatments effect on the traits of Miagava tangerine fruits.

Variables	df	Vitamin C	Fruit Sugar	Fruit pH	Fruit fresh weight	Fruit dry weight	Peel fresh weight	Peel dry weight	Fruit Zn	Fruit Fe	Fruit Mn
Replication	2	0.328^{ns}	0.033^{ns}	0.038^{ns}	274.87^{ns}	0.001^{ns}	2.19^{ns}	0.32^{ns}	0.12^{ns}	0.17^{ns}	0.006^{*}
Treatments	7	2.91^{**}	0.23^{**}	0.011^{ns}	812.87^{**}	0.002^{**}	8.91^{ns}	0.78^{ns}	1.054^{ns}	0.65^{**}	0.099^{**}
Error	14	0.25	0.021	0.011	122.16	0.0001	3.81	0.31	0.59	0.14	0.001
CV (%)	-	7	18	5	9	10	10	10	38	28	8

*,** and ^{ns}: Significant at the 5% and 1% and non-significant, respectively.

Fruit acidity and vitamin C

Results of the effect of different treatments of spray on the acidity of fruit juice of Miagava tangerine showed that the increase in the quantity of the elements did not cause statistically significant differences in total acidity (Table 2). However, the highest acidity (2.36) was obtained under "Zn + Fe" treatment and the lowest one (2.18) was obtained under the treatment "Fe + Mn". According to analysis of variance (Table 2), there were significant differences in vitamin C among the treatments and control at $p<0.01$. Data mean comparison revealed the highest vitamin C (9.25 mg) was obtained under the treatment of "Zn + Mn" and the lowest one (5.75 mg) was observed in control (Fig. 3).

Fruit sugar

The results for the effect of different spray treatments on the sugar content of Miagava tangerine fruit showed statistically significant differences among the treatments at $p<0.01$ (Table 2). According to the results of means comparison, the highest sugar content (1.2 mg) was observed in the compound treatment "Zn + Fe + Mn" and "Zn + Mn", while the lowest one (0.48 mg) was obtained under simple treatment of Fe (Fig. 4).

Fruit Zn, Fe and Mn content

Based on analysis of variance of data (Table 2), the treatments did not cause significant differences in Zn content, but means comparison revealed that the fruits of trees sprayed with triple compound treatment "Zn + Fe + Mn" had the highest Zn content (0.49 mg) and those treated with simple Mn treatment had the lowest Zn content (0.06 mg). Given the results of analysis of variance (Table 2), the studied treatments resulted in significant differences in Fe content at $p<0.01$, so that the treatment of "Zn + Fe + Mn" produced the highest Fe content in fruits and the treatment of Mn produced the lowest content (0.89 mg) (Fig. 5).

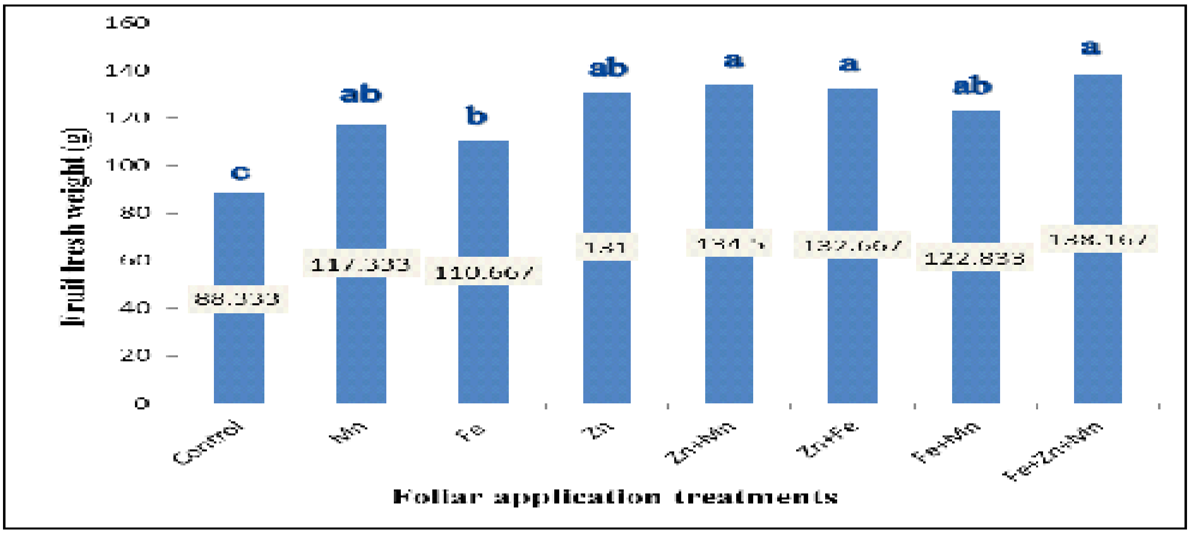

Fig. 1. Effect of foliar application treatments on Fruit fresh weight of Miagava tangerine Means with similar letters are not significantly different at 5% probability level, (LSD).

According to the results of analysis of variance (Table 2), the studied treatments caused significant difference in Mn content at $p<0.01$. Means comparison revealed that the highest Mn content was observed under the treatment of "Zn + Fe + Mn" and the lowest one was observed in control (Fig. 6).

Discussion

In a study on *Citrus inshiu*, Asadi Kangarshahi and Akhlagi Amiri (2005) observed that spraying of trees with $ZnSO_4$ significantly influenced mean fruit weight. The higher mean fruit weight of citrus under the application of Fe is reported in other studies, too (Horesh *et al.*, 1991; Gandomkar *et al.*, 2001). Dixit *et al.* (1977) reported that the spray of orange trees with micronutrients in April increased fruit size, soluble solids and fruit juice. In another study on lemon (Lisbon variety), it was observed that the heavier and larger fruits were observed under treatments with higher Fe concentration (and sometimes, other essential nutrients) which is in agreement with the results of the present study. It is revealed that the availability of Fe and other essential nutrients increases photosynthesis and dry matter production as well as fruit size and weight (Khoyi, 1992; Malakouti, 1999). In addition, Khayyat *et al.* (2007) reported that the foliar spray of date palm with $ZnSO_4$ significantly increased fruit yield, fruit length and flesh weight. Bacha *et al.* (1995) studied the effect of foliar application timing of Zn, Fe and Mn on the quantitative and qualitative characteristics of grape fruits and concluded that in addition to crop yield, the weight, size and other traits of the fruits were increased. El-Masry (1995) stated that the soil application of $ZnSO_4$ increased fruit yield of pomegranate, which his results correspond with our findings.

Elabdeen and Metwally (1982) investigated the effect of Fe and Mn on the quantity and quality of tomatoes and observed that Fe and Mn spray improved fruit weight and its early ripening and yield. Bose and Tripathi (1996) revealed that Mn, Zn and Fe foliar application enhanced mean fruit weight and yield which could be related to the effect of Zn on increasing tryptophan (auxin precursor), which in turn, increased auxin leading to higher fruit growth. Therefore, the results of the present study were agreed with these studies.

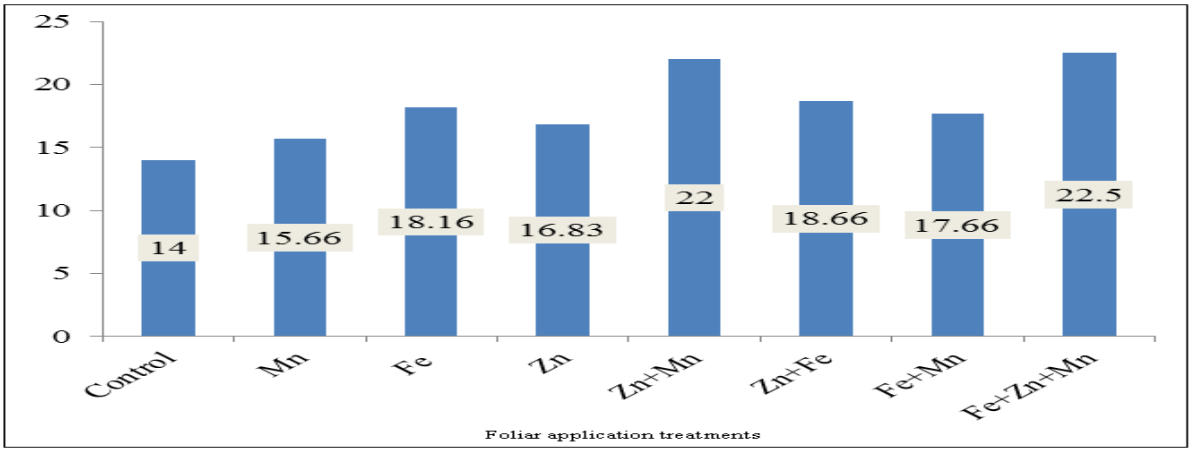

Fig. 2. Effect of foliar application treatments on Fruit dry weight of Miagava tangerine.

Hesami *et al.* (2012) found that treatment of "Zn + Mn" treatment resulted in the highest fruit weight, fruit length and flesh weight of date. In a study on pomegranate, Sobhi Rostami and Golchin (2010) observed that the highest flesh weight/fruit juice ratio was obtained under the treatment of $MnSO_4$ + $ZnSO_4$. The increase in total dry weight can be related to the increase in photosynthesis the resulting increase in chlorophyll concentration, especially chlorophyll a as well as the increase in the activity of phosphoenolpyruvate carboxylase and ribulose bisphosphate carboxylase and the increase in Fe and Mn and their positive role in photosystem I and II which as a result, more photosynthates were built (Junus and Cox, 1987). Soleimani *et al.* (2010) found that the higher corn yield and total dry weight were obtained under the treatments of Fe and Fe + Mn. The higher dry matter yield under the application of three types of micronutrients can be related to different reasons including higher biosynthesis of auxin in the presence of Zn and higher concentration of chlorophyll, greater activity of phosphoenolpyruvate carboxylase and ribulose bisphosphate carboxylase, lower accumulation of Na in plant tissues, and greater efficiency of N and P uptake in the presence of Zn. Zn is important because of the role it plays in the production of auxin which, in turn, increases leaf area and finally, fruit yield of the trees. In a study on olive, it was observed that flesh fresh and dry weights were significantly increased under the treatment of "B + Zn" (Sa'adati *et al.*, 2011). Fruit fresh and dry weight increased under all simple and compound treatments of Zn in our study that concurs with the most authors' results.

Shahabian *et al.* (2006) examined the effect of various source of Fe fertilizer on the orange and observed no difference between treatments in fruit skin thickness, acidity, vitamin C concentration and soluble solids percentage. Zn deficiency causes the fruits to be small, smooth and pale with thin skin and causes the fruit flesh to tend to be woody, waterless and bland (Nagy, 1980). According to the results on a study on the effect of micronutrients spray on qualitative and quantitative traits of apples conducted by Akhlagh Roshan *et al.* (2011), different concentrations of treatments did not result in significant differences in fruit acidity and fruit juice pH which is in agreement with the results of the present study. In a study on the effect of Fe foliar spray and soil application for solving Fe chlorosis in kiwi fruits, Mahmoudi *et al.* (2009) observed that Fe spray did not result in significant differences in total acidity and pH of fruit juice. The trial results revealed that micronutrient foliar application did not affect fruit pH.

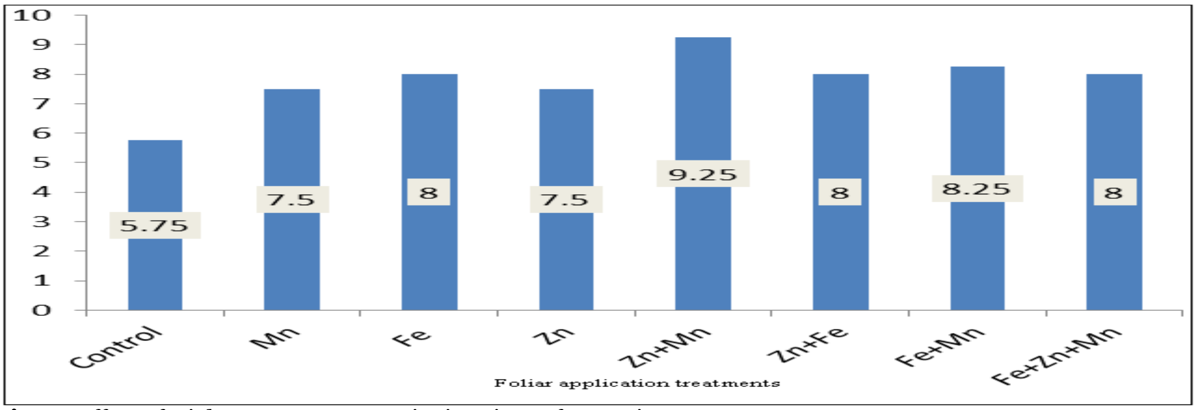

Fig. 3. Effect of trial treatments on Fruit vitamin C of tangerine.

It is reported that Mn spray of Mn-deficient trees significantly increased TSS of the fruit juice of citrus. Other qualitative traits including fruit juice percentage, total acidity and ascorbic acid did not change (Nijjar, 1990). In a study on the effect of Fe, Zn and Cu spray on improving the quality and yield of pomegranate, Moslehi *et al.* (2011) observed that the application of "Zn + Fe", "Cu + Fe" and "Cu + Zn + Fe" significantly decreased pH as compared to control. Perovic (1988) showed that the foliar spray of B, Mn, Zn and Cu in spring increased the yield of grape and its sugar content and total acidity. Based on studies on citrus, Zn application increases fruit acidity and its vitamin C content (Langthasa and Bhattacharyya, 1991; Davies and Albrigo, 1994; Qin, 1996). The results showed that fruit sugar of tangerine increased under compound treatments "Zn + Mn" and "Fe + Zn + Mn".

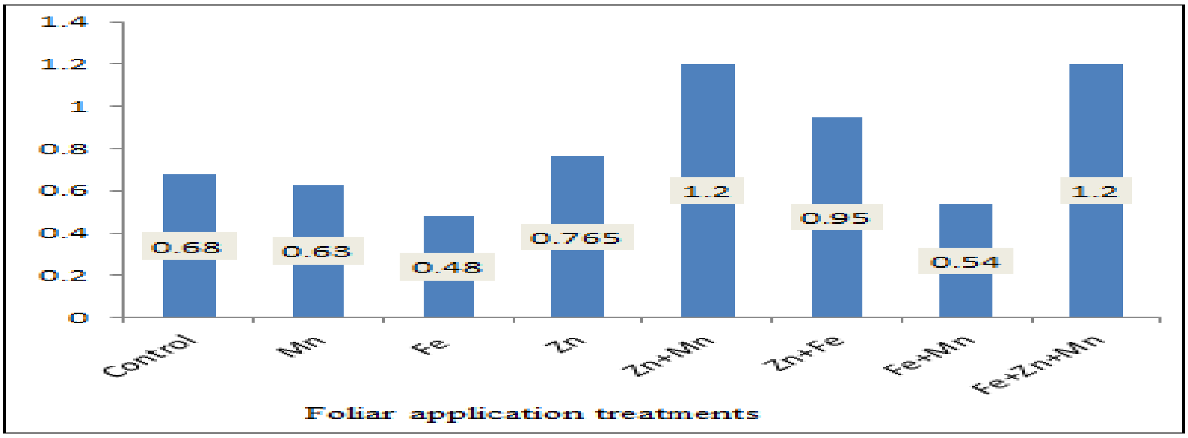

Fig. 4. Effect of foliar application of some nutrients on fruit sugar of tangerine.

Taddayon and Rastegar (2004) reported that the interaction between Zn and Mn spray increased vitamin C concentration and TSS in fruits that is consistent with our results which can be related to the important role of Zn in the activity of plant enzymes that facilitates and increases the buildup of organic compounds. Dixit *et al.* (1978) increased citrus fruit size, fruit juice percentage, TSS and vitamin C by Zn spray. Nijjar (1990) reported that the spray of Zn-containing compounds increased ascorbic acid content and fruit juice percentage of oranges. Therefore, the vitamin C content of the fruits is deeply affected by spray, which is in agreement with our results. In a study on lemon, Jahanshahi (2008) observed that the highest ascorbic acid content of fruits was obtained by foliar spray of 500 g $FeSO_4$. Singh and Riwari (1996) observed that foliar spray of Fe, Zn and Mn significantly increased ascorbic acid of onions which is consistent with our results.

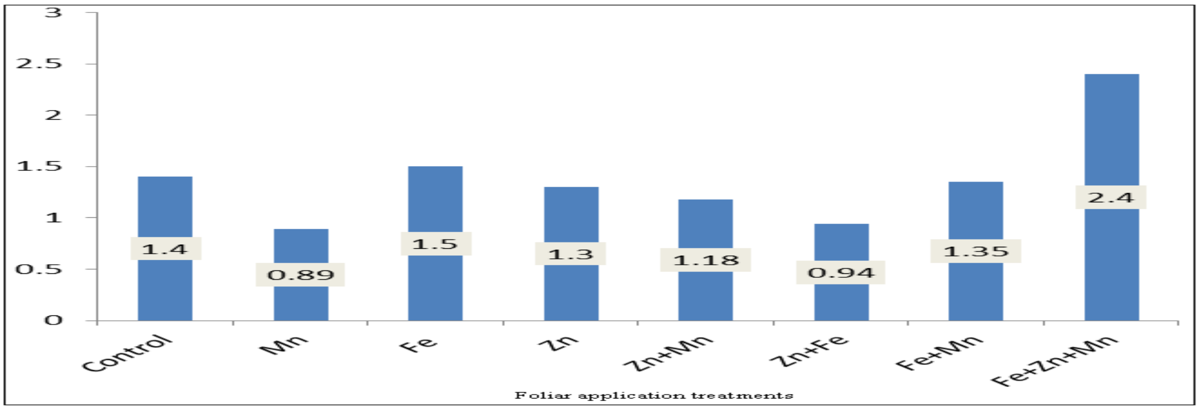

Fig. 5. Effect of foliar application of some nutrients on the Fe content of tangerine fruit.

As seen in Fig. 4, treatments with Zn produced more sugars. Yamdagni *et al.* (1979) also found that foliar application of Zn increased yield and sugar content of grape. It can be due to the effect of Zn spray on photosynthesis improvement and sugars producing. In a study on B and Zn spray of orange, Qin (1996) observed that the growth of pollen tube, fruit set, crop yield and sugar content of fruits were significantly increased. Ahmad *et al.* (1995) reported that B, N and Zn foliar application on orange enhanced crop yield, fruits weight and diameter, TSS and total sugars.

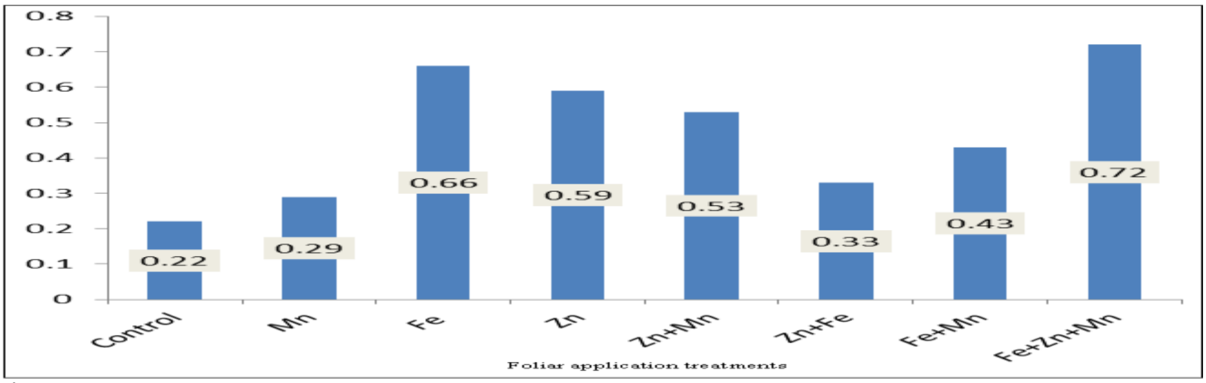

Fig. 6. Effect of foliar application of some nutrients on the Mn content of tangerine fruit.

Horesh *et al.* (1991) found that the application of $FeSO_4$ in a small volume of soil solve Fe chlorosis of citrus. The significant increase in yield with the application of Fe by local placement method has been reported in citrus (Gandomkar *et al.*, 2001), too. Samar and Malakouti (2000) observed that $FeSO_4$ application solved the symptoms of Fe chlorosis in apple trees. Different methods of the application of Fe fertilizers alleviate Fe chlorosis (Obreza *et al.*, 1993) and increases Fe concentration of plants (Malakouti, 1999; Malakouti and Shahabian, 1998).

In a study on tomato, Abedi Qeshlagi and Tafazoli (2004) concluded that Fe, Zn and Mn application increased their concentration in fruits indicating their deficiencies in nutrient solution. This finding is in agreement with the reports of our study. Balakrishnan *et al.* (1996) stated that $MnSO_4$, $ZnSO_4$ and $FeSO_4$ spray with 0.25% concentration + boric acid (0.15%) increased pomegranate fruit yield. Also, it was shown that the application of micronutrients increased their concentration in plant. Finally, based on the results, foliar application of all trial micronutrients (Mn, Zn and Fe), especially if combined, can improve Fruit weight, sugar content, Fe and Mn content of orange fruits.

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