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Responses of 'Belidi' olives to foliar and soil applied iron, manganese and zinc fertilizers

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Abstract

Present study was carried out to evaluate impact of micronutrients application (manganese, iron and zinc) with different methods (foliar or soil application) on quality parameters of 'Belidi' olive in Fars province. Chelated iron sequestrene 138, zinc and manganese sulfates were soil applied once a year and iron, zinc and manganese sulfate were foliar applied on plants, three times a year, alone or in combination. Results indicated that fertilizer application method did not have significant impact on tree productivity and fruit quality parameters in bearing or non-bearing years and alternate bearing in 'Belidi' olive trees was not correlated significantly with manganese, iron and zinc deficiency. It seems that foliar application of micronutrients fertilizers in regions with alkaline soils is a better strategy for improvement of olive production.

Key words: alternate bearing, fruit quality, micronutrients, *Olea europaea*, soil application

Introduction

Olive (*Olea europae*a L.) is an evergreen tree and an important horticultural crop. The adaptability of this fruit tree (because of its special leaf structure and ramified root system) led to its wide distribution in different parts of Iran with various climates. Traditionally, north of Iran was the hub of olive orchards. However, olive growing in southern regions such as Fars province has increased during the last decade. Fars province has more than 13000 hectares of olive orchards and now is one of the main olive producing regions in Iran (Statistic database of Ministry of Agriculture of Iran, 2016). 'Belidi' is a olive cultivar suitable for canning with high adaptability characteristics to different climates and is wide distributed and grown in Fars province.

Presence of essential macro and micronutrients at sufficient concentrations within plant organs is prerequisite of successful orchard management. Manganese (Mn), iron (Fe) and zinc (Zn) are essential micronutrients and play important roles in plant growth and development. Manganese is indirectly related to chlorophyll formation and is considered to be a constituent of some respiratory enzymes, Mn activates several important metabolic reactions in the plants and is involved in the evolution of O_2 in photosynthesis (the hill reaction), and, also, it is involved in the oxidation-reduction reactions and electron transport systems (Marschner, 1995; Millaleo *et al.*, 2010). Since, divalent manganese ions (Mn^{2+}) convert to Mn^{3+} or Mn^{4+} readily, this element plays an important role in oxidation-reduction processes within plant (Amao and Ohashi, 2008). Zinc is required for the synthesis of tryptophan which is the precursor of indoleacetic acid (IAA). Zinc is necessary for producing chlorophyll and forming carbohydrate and aids plant growth substances and enzyme systems and along with Cu, has been shown to be a constituent of the enzyme superoxide dismutase and is closely involved in the nitrogen metabolism of the plant. The reduction of photosynthesis observed in Zn-deficient plants can also be due, in part, to a major decrease in chlorophyll content and the abnormal structure of chloroplasts (Marschner, 1995). Flowering and fruiting are much reduced under conditions of severe Zn deficiency (Sparrow & Graham, 1988; Marschner, 1995). Iron is essential for the synthesis of chlorophyll. It is involved in nitrogen fixation, photosynthesis, and electron transfer. As an electron carrier, it is involved in oxidation-reduction reactions. Iron is required in protein synthesis and is a constituent of hemoprotein. It is also a component of many enzymes and involved in respiratory enzyme systems as a part of cytochrome and hemoglobin (Marscner, 1995; Expert, 2007). Chlorophyll and thylakoid synthesis, and photosynthesis are dependent on the integrity of many Fe-containing proteins, including heme and iron sulfur proteins (Imsande, 1998). The activity of ribulose-1, 5-biphosphate carboxylase/oxidase (rubisco) is also reduced under Fe deficiency (Expert, 2007).

Previous studies show beneficial influence of these three micronutrients on olive and also other crops. Ramezani and Shekafandeh (2009) reported significant increase in fruit weight, length and diameter when 'Shengeh' olive trees were foliar applied with 0.75 % zinc sulfate solution. Canasveras *et al.* (2014) reported that Fe chlorosis was alleviated in 'Manzanilla' and 'Picual' olive trees by foliar application of iron sulfate since leaf chlorophyll concentration as estimated by the soil plant analysis development (SPAD) value in the trees treated with Fe sulfate was increased by 4-7% relative to the control trees, and leaf weight was increased by 6-9%. Sayyad-amin *et al.* (2015) indicated that spray of zinc sulfate at 2000 mg L^{-1} concentration increased total yield and oil contents of 'Shengeh' olive fruits. Similarly, a foliar application of a mixture of chelated Fe, Zn and Mn significantly improved vegetative growth (shoot length and diameter, number of leaves/shoot and leaf area) and also the treatments significantly increased fruit set, yield and quality characteristics such as average fruit weight, diameter and length in 'Picual' olive (Sourour *et al.,* 2011) . Spraying a combination of Fe, Zn and Mn fertilizers solution on 'Manzanillo' olive was effective in stimulating shoot length, number of leaves/shoot, leaf area, yield, mean fruit weight, pulp weight and oil content in the pulp as compared with the control treatment (El-Khawaga, 2007). Fageria (1992) reported that iron supply at an adequate level improved the root system of rice in nutrient solution. Obareze *et al.* (2009) found that Fe deficiency in citrus plants grown in soils with high calcium carbonate content which could be mitigated by foliar application of Fe fertilizers.

Because of existing of differences in physiochemical soil characteristics in southern and northern parts of the country and availability of micro and macronutrients; fertilizers recommendations cannot be carried out without precise investigations. Soils belong to southern regions of Iran possess alkaline characteristic which induce reduction in availability of micronutrients such as Fe, Zn and Mn (Marschner, 1995). The present study was carried out in order to evaluate the effects of Fe, Zn and Mn fertilizers and different methods (soil and foliar) of application of these fertilizers on some quality characteristics of 'Belidi' olive.

Materials and methods

Plant material and field conditions: The present study was carried out in Kazeroun Agricultural Research Station, Fars province, Iran for six consecutive years (2003-2009). Uniform ten years old 'Roghani' olive trees were selected. The selected trees were checked for previous seasons bearing, non-bearing status and all of them were in the same fruiting status [year one (2003): on, year two (2004) off…]. The soil of the orchard was sampled at two depths and analyzed for soil texture, mineral content, organic matter, pH and EC (Table 1). Drip irrigation system was used in the orchard during the experimental period. Average annual climate parameters in the experimental region were; precipitation (474 mm), relative humidity (Max: 63%; Min: 23%), temperature (Max: 45; Min: 16; average: 23 °C).

Treatments: The treatments included foliar and soil application of Fe, Zn and Mn fertilizers (each fertilizer alone, or combination of two or all of them). 150 g of chelated iron sequestrene 138, 500 g zinc sulfate ($ZnSO_4.7H_2O$) and 250 g manganese sulfate ($MnSO_4.H_2O$) were soil applied per tree in the first week of March (once) during the experimental period. Iron sulfate ($FeSO_4.7H_2O$), zinc sulfate ($ZnSO_4.7H_2O$) and manganese sulfate ($MnSO_4.H_2O$) were foliar applied on plants three times; just before full bloom, two weeks after petal fall and five weeks after petal fall. In order to avoid leaf scorch, the final concentration of spray solutions was kept at 6 g L^{-1} (Table 2).

Measurements: Total fruits of trees (productivity) in each replication unit were harvested and weighed with digital weighing scale. Fruits were harvested in November when fruit surface color was violet.

The average weight of 10 fruits was measured with digital weighing scale and reported as gram. Seed weight was measured by separating 10 seeds from fruits and weighed with digital weighing scale. Fruit length and width of 10 fruits were measured with digital caliper. Pulp to seed weight ratio was measured by separating seed and pulp of 10 fruits carefully (by cutting the fruits, removing the pits that cover the seeds), and weighing them (the pulp and seeds) using a digital weighing scale then ratio of pulp to seed was determined. Fruit volume was determined by water replacement according to change in water volume in a graduated cylinder after adding 10 fruits. Oil content was measured using soxhlet extractor (AOAC, 1984).

Table 2. Treatment categories and concentrations of different fertilizers

Treatments	
Soil application	Concentration (g per tree)
Chelated iron sequestrene 138 (Fe)	150
$ZnSO_4.7H_2O$ (Zn)	500
$MnSO_4.H_2O$ (Mn)	250
Fe-Mn	150-250
Fe-Zn	150-500
Mn-Zn	250-500
Fe-Zn-Mn	150-500-250
Foliar application	Concentration (g L^{-1})
$FeSO_4.7H_2O$ (Fe)	6
$ZnSO_4.7H_2O$ (Zn)	6
$MnSO_4.H_2O$ (Mn)	6
Fe-Mn	3-3
Fe-Zn	3-3
Mn-Zn	3-3
Fe-Zn-Mn	2-2-2

Leaf Fe, Zn and Mn concentration: Oven-dried leaf samples (0.5 g) were ground and ashed at 550°C in a porcelain crucible for 6 h, separately. The white ash was taken up in 2 M hot HCl, filtered into a 50 mL volumetric flask, and finally made up to 50 mL with distilled water. Atomic absorption spectrophotometer (Elmer perkin) was utilized to determine Zn, Fe and Mn (Kalra, 1998).

Statistical analysis and experimental design: The experimental design was factorial in a completely randomized block design with 3 replications (two trees in each replication). Data were analyzed by MSTAT-C software and the means were compared using Duncan's multiple range test at 5% probability level.

Results and discussion

The effect of fertilizer application method on quality parameters is shown in Table 3. The highest fruit weight was obtained from plants foliar applied with Fe-Mn solution which was significantly higher in comparison to plants received soil applied Fe-Zn, Fe-Mn and Fe-Zn-Mn, other treatments caused no statistically different response in plants compared to control. Length of fruits (2.43 cm) was significantly higher in plants with soil applied Zn, than those treated with soil applied Fe-Zn-Mn (2.32cm). The maximum

Table 1. Analysis of soil samples in the experimental region

Soil texture	Cu	Zn	Mn	Fe	K	P	Organic carbon	pH	EC (dSm^{-1})	Soil depth
			(mg.kg^{-1})				(%)			(cm)
Loamy clay	0.76	0.84	11.4	8.6	260	12.4	0.65	7.7	0.82	0-30
Loamy sand	0.62	0.66	8.5	7.5	295	8.2	0.5	7.8	0.76	31-60

Table 3. Effect of fertilizer micronutrient and application method on fruit quality parameters in 'Belidi' olive

Treatment	Fruit weight (g)	Fruit length (cm)	Fruit width (cm)	Seed weight (g)	Fruit volume (cm^3)	Pulp to seed weight ratio	Oil (%)	Productivity (kg/tree)
Control	2.04ab	2.37ab	1.51ab	0.59b	2.08bc	2.50a	16.09a	7.3a
Fe Soil application	2.07ab	2.35ab	1.50ab	0.61ab	2.03c	2.50a	16.26a	7.3a
Zn Soil application	2.04ab	2.43a	1.52ab	0.61ab	2.16abc	2.34a	15.89a	7.7a
Mn Soil application	2.09ab	2.42ab	1.49ab	0.59b	2.07abc	2.49a	15.92a	7.8a
Fe-Mn Soil application	2.03b	2.39ab	1.49ab	0.59b	2.13abc	2.46a	15.95a	7.2a
Fe-Zn Soil application	2.03b	2.37ab	1.49ab	0.61ab	2.16abc	2.27a	15.99a	7.8a
Mn-Zn Soil application	2.06ab	2.40ab	1.49ab	0.62ab	2.16abc	2.40a	16.41a	7.3a
Fe-Zn-Mn Soil application	2.03b	2.32b	1.43b	0.60ab	2.05c	2.26a	16.01a	7.2a
Fe Foliar application	2.05ab	2.41ab	1.47ab	0.63a	2.12abc	2.34a	16.17a	7.9a
Zn Foliar application	2.07ab	2.39ab	1.50ab	0.60ab	2.15abc	2.39a	16.27a	7.9a
Mn Foliar application	2.07ab	2.39ab	1.55a	0.62ab	2.12abc	2.33a	16.15a	7.5a
Fe-Zn Foliar application	2.08ab	2.39ab	1.43b	0.60ab	2.12abc	2.43a	16.49a	7.4a
Fe-Mn Foliar application	2.13a	2.40ab	1.52ab	0.62ab	2.39a	2.46a	15.82a	8.0a
Mn-Zn Foliar application	2.05ab	2.40ab	1.51ab	0.61ab	2.22abc	2.50a	16.10a	9.7a
Fe-Zn-Mn Foliar application	2.07ab	2.36ab	1.44b	0.60ab	2.06c	2.50a	16.00a	7.5a

In each column, the means with the same letters are not significantly different at 5% probability using DMRT test.

fruit width (1.55 cm) was observed in plants sprayed with Mn fertilizer solution which was significantly higher than samples foliar applied with Fe-Zn solution or soil applied with Fe-Zn-Mn fertilizers. In control samples and trees received soil applied Mn or Fe-Zn fertilizer treatments, seed weight was statistically lower compared to plants received foliar application of Fe fertilizer. Trees sprayed with Fe-Mn fertilizers solution had the highest fruit volume (2.39 cm^3) which was significantly higher than control. Other treatments caused non-significant response in comparison to samples of control. Pulp to seed ratio, oil content of fruits and plant productivity were not statistically different.

According to results of soil analysis (Table 1), deficiency of Fe, Zn and Mn existed in the soil of the region where the experiment was conducted, but by comparing the effects of treated plants and control, it seems that this deficiency did not significantly influence some of the fruit quality parameters. The non-significant effects of application of micronutrient fertilizers might be due to low requirements of plant (Hassan *et al.*, 2010). Fernandez-Escobar *et al.* (1999) concluded that olive trees have low requirement for micronutrients as they observed no effect of crop load on micronutrient accumulation in leaves during 'on' or 'off' years. However, application of fertilizers in this study had beneficial and elevating impacts on some other quality parameters of fruits, *e.g.* fruit volume increased after foliar application of Fe-Mn fertilizer. This result was in accordance with findings of Zipori *et al.* (2011). They investigated response of irrigated oil-olive orchards, planted in high pH soils, to springtime application of iron chelate (Sequestrene-G100). They reported sequestrene application resulted in higher SPAD values, indicating higher chlorophyll content in the treated trees. However, the improvement in Fe concentration and SPAD values did not result in a significant increase in fruit or oil production. Fernandez-Escobar *et al.* (1993) found that direct Fe injection into highly chlorotic olive trees increases olive yield in the year following the treatment. Olive trees with symptoms of Fe chlorosis increased their oil yields by 18 to 26% following application of Fe-EDDHA (Campillo *et al.* 2000). These effects might be due to essentiality of Fe for plant growth and development.

The average 6-year effects of treatments on leaf Fe, Zn and Mn concentrations are shown in Table 4. When trees were sprayed with Fe, Zn or Mn solution, each micronutrient alone, the concentration of the same applied micronutrient increased significantly and reached its highest content. This result was in agreement with findings of Basar and Gurel (2015). They reported soil application of iron sulfate did not increase leaf and fruit Fe concentrations of 'Gelmik' olive but foliar applications of iron sulfate considerably elevated leaf total and active Fe concentrations. Similar results were found by Zipori *et al.* (2011), they indicated a general trend of higher leaf Fe concentration with increasing level of sequestrene application. Hasani *et al.* (2012) reported elevated level of leaf Zn and Mn following foliar application of Mn or Zn fertilizer solution in pomegranate.

Changes in quality parameters during the experimental period are indicated in Table 5. Fruit weight was significantly higher in years 2, 4 and 6 (non-bearing or "off" years) compared to "on" years; 1, 3 and 5. Different fertilizers (alone or in combination) and their method of application did not influence the fluctuations in "on"

Table 4. Effects of fertilizer micronutrient and application method on leaf Fe, Zn and Mn concentrations in 'Belidi' Olive

Treatment	Fe	Zn	Mn
Control	120.40ef	27.67f	60.78de
Fe Soil application	133.30d	26.44f	61.22d
Zn Soil application	118.60f	34.22d	58.22def
Mn Soil application	113.10f	26.28f	71.89bc
Fe-Mn Soil application	132.20d	32.17de	57.89ef
Fe-Zn Soil application	127.20de	29.11ef	68.67c
Mn-Zn Soil application	118.60f	33.17d	71.33bc
Fe-Zn-Mn Soil application	134.00d	35.44d	71.56bc
Fe Foliar application	199.00a	27.44f	56.78f
Zn Foliar application	116.80f	91.67a	58.00def
Mn Foliar application	118.90ef	29.11ef	84.89a
Fe-Zn Foliar application	169.20b	28.28f	74.00d
Fe-Mn Foliar application	120.90ef	53.06bc	73.00de
Mn-Zn Foliar application	165.60b	55.77b	55.33f
Fe-Zn-Mn Foliar application	149.30c	52.61c	70.89bc

In each column, the means with the same letters are not significantly different at 5% probability using DMRT test.

Table 5. Change of some quality parameters of ‘Belidi’ olive fruits during the experimental period

Year	Fruit weight (g)	Fruit length (cm)	Fruit width (cm)	Seed weight (g)	Fruit volume (cm^3)	Pulp to seed weight ratio	Oil (%)	Productivity (kg/tree)
1	1.78b	2.18b	1.22b	0.61ab	2.14bc	1.92c	16.21a	9.88a
2	2.41a	2.55a	1.79a	0.59b	2.10c	2.85a	16.27a	5.14b
3	1.67c	2.19b	1.24b	0.62a	1.85d	1.74d	16.33a	9.75a
4	2.38a	2.69a	1.71a	0.60ab	2.17bc	2.80a	15.72b	5.70b
5	1.69c	2.14b	1.24b	0.61ab	2.19b	2.27b	16.00ab	9.52a
6	2.43a	2.56a	1.73a	0.62a	2.30a	2.89a	16.18a	5.77b

In each column, the means with the same letters are not significantly different at 5% probability using DMRT test.

Table 6. Change of leaf Fe, Zn and Mn concentrations (µg. g fresh weight^{-1}) in ‘Belidi’ olive during the experimental period

Year	Fe	Zn	Mn
1	135.8a	38.33a	63.07b
2	134.4a	38.22a	63.11b
3	137.3a	37.82a	67.78a
4	135.3a	38.76a	67.38a
5	134.6a	39.84a	67.47a
6	137.2a	39.89a	69.02a

In each column, the means with the same letters are not significantly different at 5% probability using DMRT test.

and “off” years. Similar pattern was recorded for fruit length and width; in “off” years fruits were significantly longer and wider. Seed weight was significantly lower in third, fifth and sixth year. The largest fruit volume (2.30 cm^3) was obtained in the sixth year of experimental period. Pulp to seed weight ratio was significantly higher in year 2, 4 and 6 in comparison to on years (1, 3 and 5). Oil content decreased significantly in the fourth year but was not statistically different in other years of the experimental period. The tree productivity was significantly higher in “on” years (1, 3 and 5) in comparison to “off” years.

Changes of leaf Fe, Zn and Mn concentrations during the experimental period are presented in Table 6. Leaf Fe and Zn concentration was not significantly different during the experimental period. Leaf Mn concentration was significantly higher in third to sixth year.

Application of Fe, Zn and Mn fertilizers had non-significant impact on most of fruit quality parameters during the experimental period. The method of application of different fertilizers (soil or foliar application) had little influence on fruit quality parameters. Furthermore, alternate bearing in olive trees was not correlated significantly with Fe, Zn and Mn deficiency or sufficiency.

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