Iron and zinc interaction on leaf nutrients and the essential oil of *Pimpinella anisum* L.

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Abstract

Foliar spraying of a small amount of micronutrients (iron and zinc) may increase the yield of anise (*Pimpinella anisum* L.), an anethole-rich important medicinal plant. In order to evaluate the effect of iron (Fe) and zinc (Zn) spraying (0, 2, 4, and 6 g l^{-1}) on the nutrient contents and the essential oils of anise, a factorial experiment was conducted based on a randomized complete block design in Urmia University, Iran. Means comparison showed that the highest yield of biomass (2652 kg ha^{-1}) and essential oils (49.83 kg ha^{-1}) were observed in foliar application of 6 g l^{-1} of iron and 4 g l^{-1} of zinc. The highest leaf iron (4.89 mg g^{-1} dry matter) was observed at 6 g l^{-1} of Fe and Zn. On the other hand, there was a sharp rise of leaf zinc with higher amounts of Fe and Zn foliar sprays. The highest leaf nitrogen (35.7 mg g^{-1} dry matter) was obtained from 2 Fe + 6 Zn (g l^{-1}). The highest leaf phosphorus (10.15 mg g^{-1} dry matter) and potassium (7.9 mg g^{-1} dry matter) concentrations were obtained from 4 g l^{-1} of Zn. In conclusion, Fe and Zn foliar applications enhanced the yield of anise through nutrient accumulation due to application of 4 g l^{-1} and higher. So, the highest yield was obtained from 4 g l^{-1} Zn with 6 g l^{-1} Fe for biological yield, and from 2 to 4 g l^{-1} Zn with 6 g l^{-1} Fe for essential oils.

Keywords: *Pimpinella anisum*; harvest index; nitrogen; phosphorus; potassium

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Introduction

Anise (*Pimpinella anisum* L.) also called aniseed, belongs to the family Apiaceae, is native to the Eastern Mediterranean region and Southwest Asia, and is an annual herb indigenous to Iran, India, Turkey, and many other warm regions in the world. Anise was cultivated and well appreciated by the ancients. The Greek physician Pedanius Dioscorides mentioned the use of aniseed in medicine and that he preferred aniseed from Crete, with the Egyptian produce as his second preference (Jodral, 2004). The volatile oil of anise contains anethole (85%) as an active ingredient, in addition to eugenol, methyl chavicol, anisaldehyde, and estragole. Anise has been used as a carminative, antiseptic, antispasmodic, expectorant, stimulant, and stomachic. The oil of anise is used as an ingredient in cough medicine and lozenges and is reported to have diuretic and diaphoretic properties (Ciftçi et al., 2005; Simon et al. 1984). Antibody levels were increased by aniseed positively (Bayram et al., 2007).

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A small amount of micronutrients, particularly zinc (Zn) and iron (Fe), applied by foliar spraying increases the yield of crops significantly (Sarkar et al. 2007; Wissuwa et al. 2008), especially in environmental stress conditions (Sultana et al. 2001; Khan et al. 2003; Cakmak 2008). Iron is observed in the structure of cytochromes, ferredoxin, and also in the structure of some enzymes, e.g. superoxide dismutase, catalase, peroxidase, and nitrate reductase. It also plays a dominant role in the oxidation-reduction reactions of photosynthesis and respiration. The deficiency of Fe in plants causes significant changes in the plant metabolism and induces chlorosis, especially in young leaves and leads to very low reutilization (Kabata-Pendias, and Pendias, 1999).

Zinc is an essential trace element for every living organism so that about 200 enzymes and transcription factors require zinc as a functional component (Grotz and Guerinot, 2006). Zinc plays an important role in protein and carbohydrate synthesis and takes part in the metabolism regulation of saccharides, nucleic acid, and lipid metabolism. Zinc deficiency causes an inhibition of cell growth and proliferation. The growth symptoms of Zn toxicity in plants are similar to those of Zinc deficiency. Toxic concentrations of Zn negatively affect photosynthetic electron transport and photophosphorylation. One of the primary mechanisms of Zn toxicity may be an increased permeability of the root membranes, which will cause nutrients to leak out from the roots (Kabata-Pendias, and Pendias, 1999; Auld 2001; Genc et al. 2006).

In *P. anisum*, 80 to 100 kg K_2O/ha and 50 to 70 kg P_2O_5/ha have proven to be favorable. About 20 to 30 kg N/ha applied some weeks after emergence has proven to be successful (Jodral, 2004). But, there was no report on the effect of Fe and Zn on the growth of anise. The results of studies by Ghasemian et al. (2010) showed that 40 kg ha^{-1} zinc and 50 kg ha^{-1} iron soil application led to the highest seed yield, biological yield, and yield components in soybean such as grain number and seed weight per plant, as well as pod number, in comparison with the control treatment. Previous studies (Brar and Sekhon, 1976; Ghasemi-Fasaei and Ronaghi, 2008; Novais et al., 2016) showed that zinc absorption increased with zinc application but decreased with increasing concentrations of iron in the nutrient medium. The total zinc uptake decreased up to 85% by increasing the concentration of iron. The inhibitory action of iron on zinc absorption was "non-competitive.

The main aim of this research was to assay the nutrient uptake (important for growth and yield of plants) and the essential oils of anise by the foliar application of Zn and Fe. The interaction of Fe and Zn may show synergistic and/or antagonistic effects on plant productivity. Thus, the study also attempted to evaluate the effect of different concentrations of Fe and Zn on the yield (essential oils and biological yield) through the nutrients accumulation.

Materials and Methods

Experimental site

This experiment was set up at the Research Farm of Urmia University, Urmia, Iran (latitude 37°, 46' N, 45°, 20' E, altitude 1320 m). The soil characters are presented for two separate depth of soil (Table 1).

Table 1
Soil analysis of experiment site

Soil Depth (cm)	Soil Texture	Silt	Clay	Sand	Field capacity	ρ (g cm^{-3})	pH	EC (dS m^{-1})	Organic Carbon	N	P	K	Fe	Zn
		(%)							(%)		(mg kg^{-1})			
0-30	Clay-Loam	28	33	40	22.5	1.51	7.6	0.46	0.88	0.20	21	450	2.1	0.54
30-60	Clay-Loam	29	36	36	22.5	1.57	7.6	0.63	0.60	0.18	6.5	336	1.9	0.51

Plant materials

Seeds of *Pimpinella anisum* L. were planted by hand in 130×230 cm plots on May 2. The experimental units in each replication comprised of five lines of 230 cm long. The row to row and plant to plant spacing was 30 and 5 cm, respectively. Weed control was manually done for a clear farm. Plants were irrigated when the soil moisture received up to 70% field capacity.

Treatments

Treatments were foliar application of iron (Fe in Sequestrene) and zinc (Zn in sulfate) at four concentrations (0, 2, 4, and 6 g l^{-1}) at the beginning of flowering stage.

Yield (biological and seed)

The plant material (whole aerial parts) were harvested (September 22) for biological yield, and the seeds were separated from straw. The seeds were prepared for essential oil extraction.

Isolation of essential oils

Ripe anise fruit (100 g) was subjected to steam distillation in a clevenger-type apparatus for 3 h. The essential oil percentage (W/W) and yield were calculated and reported based on kg ha^{-1} (Pirzad et al., 2011). The oil was dried over anhydrous sodium sulphate (Fluka, Germany) (Kosalec et al. 2005).

Harvest index (HI)

The harvest index was calculated as essential oil divided by total above-ground plant dry weight for the harvest index of essential oil (Pirzad et al., 2011).

Table 2
Analysis of variance of the content of leaf nutrients, yields, and harvest index of essential oil in *Pimpinella anisum* L. affected by Iron and Zinc spraying

Source of Variation	df	Percentage of Essential Oil	Essential Oil Yield	Biological Yield	Harvest Index of Essential Oil
Block	2	0.38^{ns}	0.26^{ns}	45690.2^{ns}	0.0011^{ns}
Iron	3	3.31^{**}	41.87^{**}	29384.2^{**}	0.016^{ns}
Zinc	3	1.16^{**}	23.45^{**}	15755.8^{**}	0.029^{*}
Iron×Zinc	9	1.06^{**}	7.02^{**}	12012.6^{**}	0.011^{ns}
error	30	0.14	0.73	14520	0.0079
Coefficient of Variation (%)		9.54	6.78	16.58	24.51

Leaf Nutrients (N, P, K, Fe and Zn)

To measure leaf nitrogen, phosphorus, potassium, iron, and zinc content, the youngest fully expanded leaves were harvested from 10 plants. The leaves were carefully washed with distilled water in order to remove any surface soil or dust deposits and then oven-dried at 70 ˚C for 72 h. The determination of zinc, and iron concentration was done by atomic absorption spectrometry (Shimadzu AA-6300, Japan) (Fakayode et al., 2013). A flame photometer (Jenway pfp 7, UK) was used to determine the potassium concentration (Stephan et al. 2013), and the phosphorus concentration was determined by a spectrophotometer (PD 303, Japan) (Stephan et al. 2013). Total nitrogen was determined by the Kjeldhal (Gerhardt Bonn, Germany) method (Tandon, 1993).

Statistical analysis

This study was conducted as a factorial experiment based on a randomized complete block design with two factors (Fe and Zn) at four levels and triplicates. Data were analyzed using SAS 9.1 statistical package. Comparisons of means were carried out through the Student-Newman Keul's test (SNK) with a significance level of 95% ($P \leq 0.05$).

Results

Table 2 (Continued)

Source of Variation	df	Leaf Iron	Leaf Zinc	Leaf Nitrogen	Leaf Phosphorus	Leaf Potassium
Block	2	0.40^{ns}	0.0006^{ns}	0.001^{ns}	0.68^{ns}	0.0038^{ns}
Iron	3	4.28^{**}	0.04^{**}	0.18^{**}	11.27^{**}	0.047^{**}
Zinc	3	2.54^{ns}	0.044^{**}	0.05^{**}	17.95^{**}	0.082^{**}
Iron × Zinc	9	2.46^{*}	0.015^{**}	0.10^{**}	38.83^{**}	0.033^{**}
error	30	0.87	0.0036	0.0006	0.046	0.0076
Coefficient of Variation (%)		6.59	7.33	0.8	10.96	13.53

ns, * and ** ; non-significant, significant at 5 and 1% ; df, degree of freedom

Analysis of variance showed the significant effect of zinc on the harvest index of the essential oil (P≤0.05). The interaction effect of iron × zinc was significant on the percentage of essential oil, the yield of essential oil, biological yield, leaf zinc, leaf nitrogen, leaf phosphorus, leaf potassium (P≤0.01), and leaf iron (P≤0.05) (Table 2).

Means comparison exhibited that the highest yield of biomass (2652 kg ha^{-1}) was obtained from the foliar application of 6 g l^{-1} iron + 4 g l^{-1} zinc followed by spraying of 6 g l^{-1} iron. The lowest biological yield (biomass) (716 kg ha^{-1}) belonged to interaction of 6 g l^{-1} zinc spraying without iron application. In the control treatment (0 g l^{-1} of Fe spraying), 2 and 4 g l^{-1} Zn spraying increased significantly the biomass yield, although 6 g l^{-1} zinc application led to a notable reduction of biological yield. In 2 and 4 g l^{-1} iron treatment, the significant increase in biomass yield was observed at 4 and 6 g l^{-1} zinc (Figure I.1).

The highest percentage of essential oil (5.21%) was obtained from 6 g l^{-1} iron + 2 g l^{-1} zinc. The lowest essential oil percent (2.55%) belonged to 2 g l^{-1} zinc without iron foliar application. In the foliar application of 6 g l^{-1} iron, the essential oil percentage increased by 2 g l^{-1} zinc. In spite of the same essential oil percent, it was significantly reduced in 2 g l^{-1} Zn spraying for lower concentrations of Fe (0, 2, and 4 g l^{-1}) applications (Figure I.2).

The highest yield of essential oil (49.83 kg ha^{-1}) was obtained from 6 and 4 g l^{-1} iron and zinc spraying. The lowest (18.35 kg ha^{-1}) yield of essential oil was obtained from 2 g l^{-1} iron foliar application without Zn usage. Foliar application of 6 g l^{-1} Fe resulted in the maximum yield of essential oil for 0 - 4 g l^{-1} Zn, while it was maximum in 2 (Fe) + 6 (Zn) g l^{-1} (Figure I.3).

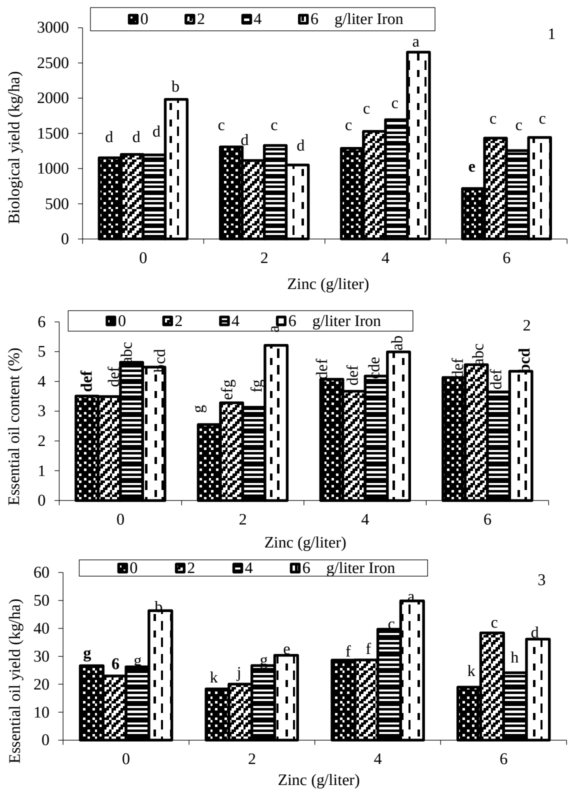

Fig. I. Means comparisons of biomass yield (1), essential oil content (2) and essential oil yield (3) of *Pimpinella anisum* L. at varying concentrations of iron and zinc foliar sprays; the same letters show non-significant differences.

Changes in the yield of biomass (Figure I.1) and essential oil (Figure I.3) led to the lowest harvest index of essential oils in the control treatment (0 g l^{-1} zinc foliar spray) which was the same in value with 2 g l^{-1} zinc. While, the higher concentrations of zinc caused a rising ratio of essential oil harvest index, the highest HI (2.71%) belonged to 6 g l^{-1} zinc application. The binomial regression ($y = 0.04x^2 + 0.044x + 1.91$; $R^2 = 0.9834$) showed an increasing trend in the harvest index (Fig. II).

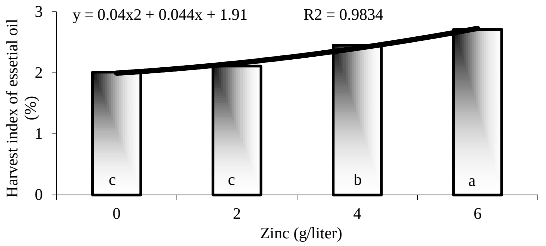

Fig. II. Means comparisons of harvest index of essential oil of *Pimpinella anisum* L. at different concentrations of zinc foliar sprays; the same letters show non-significant differences. There is a binomial regression between zinc concentration and harvest index.

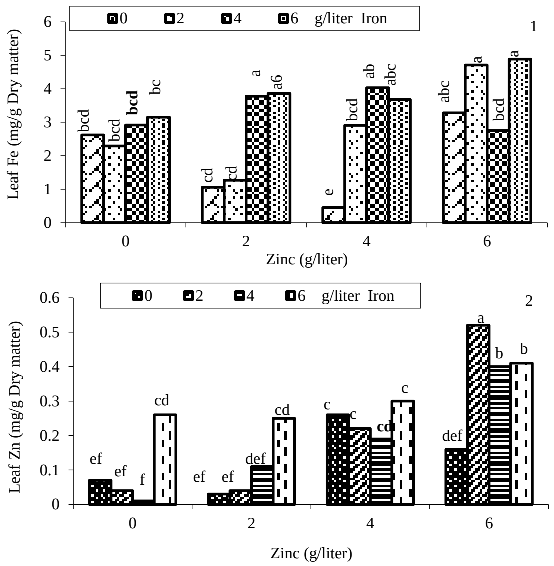

Fig. III. Means comparisons of leaf iron (I) and zinc content (II) of *Pimpinella anisum* L. at varying concentrations of iron and zinc foliar sprays. The same letters show non-significant differences.

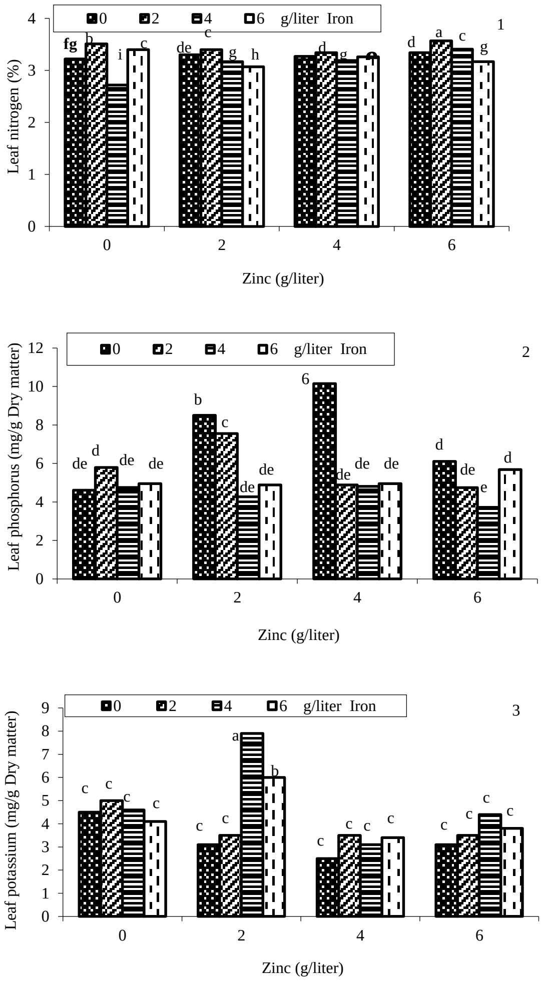

Fig. IV. Means comparisons of nitrogen (I), phosphorus (II) and potassium (III) content of *Pimpinella anisum* L. leaves at varying concentrations of iron and zinc foliar sprays. The same letters show non-significant differences.

The highest leaf iron concentration (4.89 mg g^{-1} dry matter) was obtained at 6 g l^{-1} iron and zinc foliar application while the lowest leaf iron concentration (0.45 mg g^{-1} dry matter) was obtained at 0 g l^{-1} iron and 4 g l^{-1} zinc sprays. There was a significant reduction in leaf iron obtained from 2 and 4 mg g^{-1} zinc sprays compared with the control treatment (Figure III.1).

Pimpinella anisum was more sensitive to zinc accumulation than iron, so that there was a sharp rise in leaf zinc by higher levels of iron and zinc foliar sprays. Consequently, the highest (0.52 mg g^{-1} dry matter) leaf zinc was observed in 2 Fe + 6 Zn (g l^{-1}) treatment. Any deviation from this optimal point caused a significant reduction in leaf zinc; the minimum zinc in leaf (0.01 mg g^{-1} dry matter) was observed at 0 and 4 g l^{-1} iron and zinc application. At all levels of zinc application, the highest leaf zinc concentration belonged to 6 g l^{-1} iron foliar spray (Figure III.2).

The highest leaf nitrogen concentration (35.7 mg g^{-1} dry matter) was obtained from 2 Fe + 6 Zn (g l^{-1}) iron and zinc applications while the lowest leaf nitrogen (27.2 35.7 mg g^{-1} dry matter) was obtained from 4 g l^{-1} iron without zinc spray. In 0, 2, and 4 g l^{-1} iron, the highest leaf nitrogen content belonged to 6 g l^{-1} zinc application. While 6 g l^{-1} iron led to higher leaf nitrogen obtained from the 0 g l^{-1} of zinc spray (Figure IV.1).

The highest leaf phosphorus concentration (10.15 mg g^{-1} dry matter) was obtained from 4 g l^{-1} zinc without iron application and the lowest phosphorus (3.72 mg g^{-1} dry matter) belonged to 4 Fe + 6 Zn (g l^{-1}) treatment.

In the 0 (untreated) and 6 g l^{-1} zinc, the leaf phosphorus showed a lower percentage, because of deficit and excess zinc, respectively (Figure IV-2).

The highest leaf potassium (7.9 mg g^{-1} dry matter) was obtained from 0 and 4 g l^{-1} iron. All other treatments produced leaves with the minimum levels of potassium (Figure IV.3).

Discussion

As expected, Zn and Fe supply generally increased Zn and Fe concentrations in anise. Our findings exhibited that the lower Fe belonged to 0 Fe + 4 Zn (g l^{-1}) and the lowest Zn was observed at 0 Zn + 4 Fe (g l^{-1}) treatments. Antagonism effects between Fe and Zn is well known. Previous studies have shown that Zn interfered with Fe concentration (indication of uptake and translocation), whereas Fe interfered with Zn translocation only when Zn concentrations were high (Alloway, 2008). There are three possible mechanisms for this antagonism. First, there could be competition between Zn^{2+} and Fe^{2+} during the uptake, second, there could be interference in the chelation process during Fe uptake and translocation (Kabata-Pendias, 2010). Third, there could be competitive inhibition between Zn and Fe during unloading in the xylem (Alloway, 2008).

The highest leaf iron concentration was obtained at the highest level of iron and zinc foliar application, and the lowest leaf iron concentration was obtained at 4 g l^{-1} zinc without iron sprays. These results show the increasing trend in iron accumulation in leaves by higher concentrations of iron and zinc, so that the lower leaf iron was observed at 0 g l^{-1} of all zinc application treatments. There was a reduction of leaf iron obtained from 2 and 4 mg g^{-1} of zinc sprays compared with the control treatment. This reduction could be because of higher biomass than the control treatment (dilution effect).

The external application of Fe increases photosynthesis, net assimilation, and relative growth in seawater-stressed rice, because of the important role in the metabolism of chlorophylls (Sultana et al. 2001). Application of Zn or Fe has been reported to have significant positive effects in most cases on the growth measurements and chemical composition of safflower (Lewis and Mc Farlane, 1986), lupine (Brennan, et al. 2001), cumin (El-Sawi and Mohamed, 2002), soybean (Gadallah, 2000; Heitholt et al. 2002), barley (Hebbern et al., 2005), sunflower (Mirzapour and Khoshgotar, 2006), mustard (Chatterjee and Khurana, 2007), common bean (Fernandes et al., 2007), and rice (Wissuwa, Ismail, and Graham, 2008).

Plants subjected to iron deficiency respond in different ways (Abadia, 1992). Even though iron is one of the most abundant elements in soils, the low solubility of iron minerals makes the inorganic form of iron unavailable to plants and forms the most widespread nutritional disorder the world over.

Understanding the physiological effects and functions of zinc in plant systems is needed to find effective ways to increase the available levels of zinc in soils to improve crop productivity and to increase the bioavailable levels of zinc in edible portions of food crops that feed the world's poor.

Jirali (2001) found that the use of micronutrients such as $FeSO_4$ (0.5%) was more effective in increasing biochemical parameters, such as chlorophyll content and nitrate reductase activity, in turmeric. Furthermore, he noticed that the use of plant growth regulators such as cytozyme (2000 ppm) and miraculan (2000 ppm) increased chlorophyll content and nitrate reductase activity in comparison to the control.

Finally, the interaction of Zn and Fe with other elements decreases the availability and influences their adsorption, distribution, and utilization in plants (Sadeghzadeh, 2013). These interactions are mainly due to the influences of other cations on the rate of absorption by the plant roots, rather than their effects on the availability of Zn and Fe or its forms (Loneragan and Webb, 1993). Zn interactions with Fe, P, and N are the most important and widespread in soils with limiting supplies of Zn and P or N. A high level of applied N in the absence of Zn can cause Zn deficiency through a dilution effect, thus changing the pH of the root environment (Loneragan and Webb, 1993). In the lowest and the highest levels of zinc, the leaf phosphorus was lower in percentage, because of deficit and excess zinc, respectively. Zn application in the soil may cause the formation of insoluble ZnO_3 $(PO_4)_2$, which decreases the P concentration of the soil solution and thus lowers the P availability. The results showing an increase in the biological yield but a reduction in the observed concentration of certain elements may be attributed to the dilution effect in plants (Sadeghzadeh, 2013).

The significant increases in essential oil in relation with nitrogen, phosphorus, and potassium of leaf (Rahimzadeh et al., 2016), and however resulted in higher concentrations of iron and zinc (Rahimzadeh et al., 2017) were previously reported. These essential elements can improve growth in whole plants especially roots and aerial parts to enhance photosynthesis, increasing seed yield and synthesis of secondary metabolites, which increases the percentage of essential oils (Nasruddin et al., 2018; Singh et al., 2016). It seems that application of zinc at low concentrations increases plant growth parameters while the higher concentrations might have toxicity for plants (Nasiri Savadkoohi et al., 2017; Rezaei and Abbasi, 2014).

Conclusion

Findings suggest that iron and zinc foliar application enhanced the yields (biological and essential oil) and improved growth of anise through absorption of micronutrients (Fe and Zn) from leaves. Also the best results (biological yield and essential oil concentration) were obtained from interaction of 4 g l^{-1} Zn and 6 g l^{-1} Fe through foliar application.

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