### EFFECT OF SOME TRACE ELEMENTS ON GROWTH, YIELD AND CHEMICAL CONSTITUENTS OF *OCIMUM BASILICUM* PLANTS

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> his research was conducted at El-Qantara Sharq Station, North Sinai, Egypt of Desert Research Center during the two successive seasons of 2014 and 2015. The main objective was to study the effect of microelements [Fe, Zn, Mn, Fe+Zn, Fe+Mn, Zn+Mn and Fe+Zn+Mn, at 50 ppm each], on growth, yield and essential oil content of sweet basil (*Ocimum basilicum* L.) plant. Treatment of Fe+Zn+Mn, at 50 ppm each, produced the highest values of plant growth characters, oil content, N, P, K, Fe, Zn and Mn in plant tissues. Also, this treatment increased the concentrations of l-linalool to 52.69% and 1,8 cineole to 25.66%.

## Keywords: basil, Ocimum basilicum, Fe, Zn, Mn, l-linalool, cineole, iron, zinc, manganese, microelements

Basil or sweet basil (Ocimum basilicum L.) is an annual plant belongs to family Lamiacea, native to the tropical regions of southeastern Asia and Africa, that includes around 30 species, which are much differentiated in respect to morphological and chemical features (Vina and Murillo, 2003 and Telci et al., 2006). Sweet basil is the smell of the plant aromatic good savoring, added to food to impart delicious taste and fine odor and basil oil uses are diet benefactor and enters in scented soaps and aromatherapy industry. Sweet basil contains some antibiotics for some types of cancer, antioxidant vitamins, beta-carotene and metal salts; such as Mn, Cu, Mg and K (Marotti et al., 1996). Sweet basil medical antiseptic properties are including the gut, repelling gas and anthelmintic, assistant digestion, lowers cholesterol, blood sugar and tonic for the immune system. It also inserts in the treatment of some diseases; such as intestinal colic, dysentery, boiled chronic diarrhea, vomiting in children, dental pain, kidney stones, headaches, colds, coughs and asthma (Ozcan and Chalchat, 2002 and Sajjadi, 2006).

Considered nutritious micronutrients; such as iron, zinc and manganese on Egyptian territory continues to drop, as a result of the cultivation of more than one crop on the same area of land during the year, or cropping high productivity and short-lived, leading to the depletion of a large amount of nutrients, lack of interest by adding organic matter that will restore vitality to the soil and make up for the lack of them and increase fertility. High fertilization rates of major elements lead to an increase in yield and thereby increase the amount missing from the micro-earth elements and the expansion in the cultivation of a few desert land fertility. Micronutrients are essential for plant and lack of them reduces the productivity of the crop.

Zinc is an important mineral element to activate many enzymes; such as carbonic anhydrase and alcohol dehydrogenase. It is also necessary for the synthesis of the amino acid tryptophane, which turns into auxin (IAA) that helps to increase the growth of the plant. It was found that in plants suffering from zinc deficiency, auxin concentration in the roots and shoots are very few and has a role in the synthesis of nucleic acids and proteins (Amberger, 1974).

Iron plays an essential and necessary role in many enzymes, especially enzymes intervention or help in the process of respiration, which include system catalase, peroxidase and cytochrome oxidase. The iron participate in the processes of oxidation of these compounds, which is one of the important roles in cell metabolism operations. It is important in the synthesis and maintenance of chlorophyll and the lack of iron at least lead to the appearance of yellowing on the plant. It has also an important role in the representation of nucleic acids and chloroplasts (Nikolic and Kastori, 2000).

Manganese has an important role in the representation of nitrogen within the plant. It also activates a lot of enzymes; such as dehydrogenase and carboxselase and plays a direct role in the processes of oxidation and reduction. Manganese is at the center of the composition of chlorophyll, where the chloroplasts are affected by a lack of manganese, because of its essential role in the fusion of water molecule during the process of photosynthesis (Hill's reaction) (Piagentini et al., 2002 and El-Fouly et al., 2002).

The purpose of this research was to study the effect of microelements on growth, yield and active ingredients of sweet basil (*Ocimum basilicum* L.) plant to compensate for the lack of microelements in Egyptian land and special desert lands of sandy soil.

#### **MATERIALS AND METHODS**

This work was conducted in the farm of El-Qantara Sharq Station (Desert Research Center), North Sinai, Egypt, during the two successive

seasons of 2014 and 2015 to study the effect of some microelements on growth, yield and active ingredients of sweet basil.

The seeds of sweet basil plant were obtained from the Medicinal and Aromatic Plants Research Department, Horticulture Institute, Agricultural Research Center, Dokki. Seeds of sweet basil plant were transplanted in the nursery on  $1^{st}$  of March in both seasons of 2014 and 2015. The seedlings were sown in plots  $4x4 \text{ m}^2$  on  $15^{th}$  of April for both seasons. Each plot contained 66.66 plants and the distance between rows was 60 cm, while the distance between plants was 30 cm. All experimental plots were treated with recommended agriculture practices. The physical and chemical analyses of the experimental farm soil in El-Qantara Sharq Station are shown in table (1).

Maakaniaal	Chemical analysis									
analysis	Value	Soluble anions (meq/l)	Value	Soluble cations (meq/l)	Value	Available (mg/l)	Value			
Fine sand %	43.28	CO <sub>3</sub>	-	Ca <sup>++</sup>	8.92	Ň	0.16			
Coarse sand	<b>1</b> 42.26	Cľ	9.00	$Mg^{++}$	7.95	Р	13.21			
Silt %	13.28	$SO_4$	25.35	$\mathbf{Na}^+$	20.42	K	69.67			
Clay %	1.18	pH	8.29	<b>K</b> <sup>+</sup>	1.21	CaCO <sub>3</sub>	6.20			
Soil texture		Sandy		E.C mmhos/ cm		3 85				

 Table (1). Physical and chemical analysis of the used soil in of El-Qantara Sharq- North Sinai Research Station.

The experiment consisted of 8 treatments, the used microelements in this research were:

—	Control (distilled water)	_	Fe + Mn (50 ppm + 50 ppm)
_	Fe (50 ppm)	_	Fe + Zn (50 ppm + 50 ppm)
—	Mn (50 ppm)	_	Mn + Zn (50 ppm + 50 ppm)
—	Zn (50 ppm)	_	Fe+ Mn + Zn (50 ppm + 50

The sources of Fe, Mn and Zn were commercial products contains 12% chelated iron, 12% manganese and 14% zinc as Fe, Mn and Zn EDTA, respectively, which were obtained from Tabarak Company for Fertilizers and Chemicals (www.tabarakfert.com). Plants were treated with microelements as foliar spray with aqueous solutions two times, the first one was done 45 days after transplanting and the second one 45 days after first cut.

The treatments of the experiment were arranged in complete randomized block design with three replicates. The obtained data were subjected to the statistical analysis according to Snedecor and Cochran

(1980) and the differences between the means of the treatments were considered by Duncan using Statistix<sup>9</sup> Analytical Software (1985).

The recorded data included: (i) Vegetative growth parameters (plant height, number of branches per plant and fresh and dry weights of herb per plant and plot). (ii) Essential oil parameters (essential oil percentage, yield and composition). Oil percentage was determined in two cuts according to British Pharmacopoeia (1936), essential oil yield (ml) per plant and per plot were calcutated and oil composition was analyzed by using GC-Mass according to Bunzen et al. (1969) and Hoftman (1967) methods. (iii) Chemical constituents included macroelements (nitrogen, phosphorus and potassium) percentage were determined according Koch and Mc-Meekin (1924), Troug and Mayer (1939) and Brown and Lilleland (1946), respectively. The microelements (Zn + Mn and Fe) were determined in the digested samples by atomic absorption by Chaman and Pratt (1961).

The plants were harvested two times through the growing season. The first and second cuts took place on  $15^{\text{th}}$  of June and  $15^{\text{th}}$  of September in both seasons; respectively.

#### **RESULTS AND DISCUSSION**

#### 1. Vegetative Growth

#### 1.1. Plant height

Data in table (2) cleared that, all treatments gave a progressive increase in the height of *Ocimum basilicum* plants, this increasing is significant. Spraying with Zn, Mn and Fe at 50 ppm singly or with each other increased plant height. The best results were achieved by the plants treated with Zn + Mn + Fe together, comparing with the effect of each one. This treatment gave the highest values of 104 and 111 cm for the first and second cuts, respectively, in first season. Whereas they were 108 and 113.17 cm at the first and second cuts, in the second season, respectively. These results hold true in the two cuts in two seasons. It is apparent that Fe was the most effective element, when the elements were sprayed separately, followed by data recorded for Zn then Mn.

Consistent with the these results, many investigators found that Zn spraying caused on increase in plant height such as Hendawy and Khalid (2005) on *Salvia officinalis* plant, Abd El-Hady (2007) on barley plant, Pande et al. (2007) on mint plant and Said-Al-Ahl and Omer (2009) on *Coriandrum sativum*. They concluded that spraying the plants with Zn significantly increased the height of studied plants. The positive effect of zinc on plant height may be due to that, Zn accelerated the cell division; it is also controls auxin metabolism (Amberger, 1974).

The present results of manganese are in harmony with those of Gohain and Barbora (2000) on *Camellia sinensis* and Dewidar (2002) on digitalis plant. They concluded that, spraying the plants with Mn

significantly increased the height of studied plants. The role of Mn of increasing the plant height may be due to that, Mn has important functions in plant metabolism (Amberger, 1974). Therefore, Mn application as foliar spray on plant in this work stimulated the plant height. The results dealing with the effect of Fe on the plant weight are in agreement with those obtained by Said-Al-Ahl and Omer (2009) on *Coriandrum sativum*, Said-Al-Ahl and Mahmoud (2010) on (*Ocimum basilicum*) and Osińska et al. (2012) on parsley (*Petroselinum sativum* ssp. *crispum*). They concluded that, spraying the plants with Fe significantly increased the height of studied plants. The positive influence at Fe on plant height, might be due to that, iron had an important role in the biosynthesis of chlorophyll (Amberger, 1974)

Characters	Plant height							
	1 <sup>st</sup> Se	eason						
Treatments	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	1 <sup>st</sup> cut	2 <sup>nd</sup> cut				
Control (distilled water)	41.00 G	43.53 G	43.00 G	53.00 H				
Fe (50 ppm)	83.50 D	87.67 D	85.33 D	90.67 E				
Mn (50 ppm)	55.83 F	61.50 F	57.83 F	63.83 G				
Zn (50 ppm)	74.50 E	79.00 E	76.33 E	82.67 F				
Fe+Mn (50 ppm)	94.60 BC	97.27 B	96.33 B	104.50 C				
Fe+Zn (50 ppm)	97.27 B	100.17 B	98.83 B	109.67 B				
Mn+Zn (50 ppm)	88.83 CD	92.50 C	90.67 C	100.83 D				
Fe+Mn+Zn (50 ppm)	104.00 A	111.00 A	108.00 A	113.17 A				

**Table (2).** Effect of some microelements on the plant height of Ocimumbasilium during two seasons of 2014 and 2015.

Means having the same letter within the same column are not significantly different according to LSD for all-pairwise comparisons test at 5% level of probability.

The results of the mixture of elements are in agreement with these reported by Aziz and El-Sherbeny (2004) on *Sideritis montana* and Abd El-Wahab (2008) on *Trachyspermum ammi*. They concluded that, spraying the plants with Zn, Mn and Fe separated or mixed increased the height of the studied plants. The increased in plant height of sweet basil plants as a result of spraying with mixture of microelements might be due to the effect of microelements on cell division and elongation (Malakouti and Tehrani, 1999).

#### 1.2. Number of branches

Data dealing with the effect of microelements on the number of branches per plant are presented in table (3). Spraying of *Ocimum basilicum* plants with Zn, Mn and Fe at 50 ppm insignificantly increased number of branches per plant when the three elements were sprayed alone or in mixture, compared with the control. Moreover, a significant increase was observed when the treatment of Zn+Mn+Fe was applied on plants in the first

season, they were 10.67 and 11.33 in the first and second cuts, respectively, and also in the second season they were 11.67 and 12.67 in the two cuts, respectively.

Similar results with Zn treatments were stated by Abd El-Aziz and Balbaa (2007) on *Salvia farinacea* plant and Said-Al-Ahl and Mahmoud (2010) on *Ocimum basilicum*. They reported that spraying plants with Zn significantly increased number of branches per plant. The positive effect of Zn on number of branches may be explained that, Zn accelerated the cell division; it is also controls auxin metabolism (Amberger, 1974).

The increase in number of branches per plant due to spraying Mn are in accordance with the finding of El-Sawahly (2000) on *Borago officinalis* and Hendawy and Khalid (2005) on *Salvia officinalis*. It concluded that spraying plants with Mn increased number of branches per plant due to the that, Mn has important function in plant metabolism (Amberger, 1974).

The positive effect of Fe was in harmony with those obtained by Said-Al-Ahl and Omer (2009) on *Coriandrum sativum* and Said-Al-Ahl and Mahmoud (2010) on *Ocimum basilicum*. They concluded that foliar spraying with Fe increased the number of branches per plant. The positive influence of Fe on plant height, might be explained by its vital role in enzymes activity as nitrogenase, catalase and peroxidase (Marschner, 1998).

These remarkable findings of the effect of the mixture of the trace elements are in agreement with those obtained by Aziz and El-Sherbeny (2004) on *Sideritis montana* and Pande et al. (2007) on mint plant. They cleared that, the mixture of any two of the elements; Zn + Mn, Mn + Fe and Zn+Fe or the mixture of the three elements Zn + Mn + Fe, increased number of branches per plant.

Characters	Number of branches						
-	1 <sup>st</sup> Se	eason	2 <sup>nd</sup> S	eason			
Treatments	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	1 <sup>st</sup> cut	2 <sup>nd</sup> cut			
Control (distilled water)	4.00G	5.00G	4.67E	5.00F			
Fe (50 ppm)	7.33DE	8.00D	8.33CD	8.00D			
Mn (50 ppm)	5.67F	6.00F	5.33E	6.00E			
Zn (50 ppm)	6.33EF	7.33E	7.33D	7.33D			
Fe+Mn (50 ppm)	9.00BC	9.00C	9.00C	9.33C			
Fe+Zn (50 ppm)	9.67AB	10.00B	10.33B	10.33B			
Mn+Zn (50 ppm)	8.33CD	9.00C	8.67C	9.33C			
Fe+Mn+Zn (50 ppm)	10.67A	11.33A	11.67 A	12.67A			

**Table (3):** Effect of some microelements on the number of branches per<br/>plant of *Ocimum basilicum* during two seasons of 2014 and<br/>2015.

Means having the same letter within the same column are not significantly different according to LSD for all-pairwise comparisons test at 5% level of probability.

#### 1.3. Fresh and dry weights

Data presented the plant herb yield are shown in table (4). Results showed that, spraying sweet basil with trace elements produced heaviest fresh and dry weights of herb per plant and plot compared with the untreated plants. Spraying sweet basil with microelements (Zn, Mn or Fe at 50 ppm), separately or in mixture, significantly increased the fresh and dry weights of herb per plant and plot, in both cuts and seasons, compared with the untreated plants. Also, results showed that, Fe alone significantly increased the fresh and dry weights of plant compared with Zn or Mn alone. The mixture of Fe + Zn gave higher values of fresh and dry weights per plant and feddan compared to the other two mixtures. The treatment of Zn + Mn +Fe produced the highest values the fresh and dry weights of herb per plant and plot, the least value was obtained from control plants. This was obvious in both cuts and seasons of the experiment. The differences between treatments and control were significant, when the experiment was repeated in the two cuts and two seasons, similar data were observed.

The remarkable findings of the effect of Zn on fresh and dry weights of plants are in agreement with those obtained by Grejtovsky et al. (2006) on *Matricaria chamomilla*, Abd El-Aziz and Balbaa (2007) on *Salvia farinacea* and Akhtar et al. (2009) on *Mentha piperita*. They concluded that foliar sprays of Zn significantly increased the fresh and dry weights of the studied plants. The simulative effect of Zn application on the dry weight may be due to its indirect influence on the biosynthesis of oxidation and reduction enzymes of photosynthesis. Amberger (1974) reported that, Zn plays a role in metabolic pathway from tryptophan to IAA.

As for the effect of Mn on increasing the fresh and dry weights of herb per plant, in this respect, El-Sawahly (2000) on *Borago officinalis* and Abd El-Wahab (2008) on Ajowan plant concluded that foliar sprays of Mn significantly increased the fresh and dry weights of the studied plants. The increment in leaf dry weight may be due to that Mn application has important functions in plant metabolism (Amberger, 1974). Also, Mengel and Kirkby (1978) concluded that, Mn in some way involved in the oxidation/reduction processes in photosynthetic electron transport system.

Fe stimulated the fresh and dry weights of plants. In this concern, Reffat and Balbaa (2001) on lemongass, Kassem (2002) on rosemary, Hashem (2007) on thyme and Abd El-Wahab (2008) on *Trachyspermum ammi*. They concluded that spraying with Fe significantly increased the fresh and dry weights per plant and per plot. This increase in weight by Fe spraying could be explained in the light of its role in the oxidation-reduction reactions in respiration and photosynthesis and due to that iron has a number of important function roles in plant, and hence it causes a marked effect on photosynthetic efficiency (Gauch, 1957).

Characters	Fresh w herb per	eight of plant (g)	Fresh w herb p (k	eight of er plot g)	Dry weight of herb per plant (g)		Dry weight of herb per plot (kg)	
				1 <sup>st</sup> 9	Season			
Treatments	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	1 <sup>st</sup> cut	2 <sup>nd</sup> cut
Control (distilled water)	123.93G	132.10G	6.20G	6.61G	42.33G	42.50F	2.12G	2.13F
Fe (50 ppm)	213.67D	228.29D	10.68D	11.41D	72.33D	78.50CD	3.62D	3.93CD
Mn (50 ppm)	152.00F	160.80F	7.60F	8.04F	52.67F	56.83E	2.63F	2.84E
Zn (50 ppm)	183.67E	207.09E	9.18E	10.35E	62.17E	73.17D	3.11E	3.66D
Fe+Mn (50 ppm)	252.00C	266.81C	12.60C	13.34C	85.67B	89.20B	4.28B	4.46B
Fe+Zn (50 ppm)	268.83B	279.02B	13.44B	13.95B	87.00B	92.67B	4.35B	4.63B
Mn+Zn (50 ppm)	240.50C	256.62C	12.03C	12.83C	78.83C	79.50C	3.94C	3.98C
Fe+Mn+Zn (50	288 834	203 284	1/ // Δ	14 66 4	97.004	101 174	1 85 4	5.064
ppm)	200.05/1	275.2011	17,77/1	14.00/1	J7.00/1	101.177	4.0011	5.0011
				2 <sup>nd</sup>	Season			
	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	1 <sup>st</sup> cut	2 <sup>nd</sup> cut
Control (distilled water)	165.60E	177.11G	8.28E	8.86G	58.77G	60.83G	2.94G	3.04 G
Fe (50 ppm)	293.67C	302.82D	14.68C	15.14D	100.17D	104.00D	5.01D	5.20D
Mn (50 ppm)	192.00E	210.66F	9.60E	10.53F	69.10F	69.67F	3.46F	3.48F
Zn (50 ppm)	245.50D	274.70E	12.28D	13.74E	89.17E	93.83E	4.46E	4.69E
Fe+Mn (50 ppm)	342.00B	352.18B	17.10B	17.61B	116.33B	119.00BC	5.82B	5.95BC
Fe+Zn (50 ppm)	355.17B	364.64B	17.76B	18.23B	119.67B	123.33B	5.98B	6.17B
Mn+Zn (50 ppm)	328.17B	335.68C	16.41B	16.78C	110.20C	114.00C	5.51C	5.70C
Fe+Mn+Zn (50 ppm)	386.67A	387.13A	19.33A	19.36A	126.67A	131.17A	6.33A	6.56A

**Table (4).** Effect of some microelements on the fresh and dry weights of herb per plant (g) and per plot (kg) of *Ocimum basilicum* during two seasons of 2014 and 2015.

Means having the same letter within the same column are not significantly different according to LSD for all-pairwise comparisons test at 5% level of probability.

As the mixture of microelements was studied, the stimulatory effect was observed by many investigators, such as El-Sawahly (2000) on *Borago officinalis* and Gharib (2001) on *Tagetes minuta*. They concluded that the mixture of any two elements (Zn+Mn or Mn+Fe or Zn+Fe) resulted in significantly increase in the day matter content compared with the effect of any separate element or control. They added that the mixture of the three elements (Zn+Mn+Fe) resulted in a significant increase in the dry matter content compared with the effect of any separate or two elements.

#### 2. Active Ingredients

#### 2.1. Volatile oil

Data in table (5) show that, there is a progressive increase in the volatile oil percentage of *Ocimum basilicum* plant during both seasons. Spraying with Zn, Mn and Fe at 50 ppm individually or in combination increased the volatile oil percentage in most cases. The best results were obtained by spraying plants with Zn + Mn + Fe jointly, comparing with the effect of the three elements alone. It is obvious that Fe was the most effective element, when the elements were sprayed separately, followed by Zn then Mn. This was recorded in the two cuts and two seasons.

 Table (5). Effect of some microelements on the oil percentage based on dry weight basis of *Ocimumbasilicum* during two seasons of 2014 and 2015.

Characters	Oil percentage						
	1st S	eason	2 <sup>nd</sup> S	eason			
Treatments	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	1 <sup>st</sup> cut	2 <sup>nd</sup> cut			
Control (distilled water )	0.067E	0.110H	0.080H	0.087G			
Fe (50 ppm)	0.127D	0.213E	0.167E	0.183E			
Mn (50 ppm)	0.083E	0.133G	0.103G	0.127F			
Zn (50 ppm)	0.110D	0.170F	0.130F	0.147F			
Fe+Mn (50 ppm)	0.187B	0.273C	0.217C	0.237C			
Fe+Zn (50 ppm)	0.210A	0.307B	0.240B	0.260B			
Mn+Zn (50 ppm)	0.160C	0.243D	0.190D	0.210D			
Fe+Mn+Zn (50 ppm)	0.223A	0.327A	0.260A	0.310A			

Means having the same letter within the same column are not significantly different according to LSD for all-pairwise comparisons test at 5% level of probability.

Data dealing with effect of micronutrients on the volatile oil content of herb per plant and per plot in both seasons are shown in table (6). It is evident that, spraying sweet basil plants with microelements (Zn, Mn or Fe at 50 ppm) alone or in mixture significantly increased the volatile oil content of herb per plant and per plot, in the two cuts and two seasons, compared with the untreated plants. Spraying Fe alone obtained the greatest values in comparison with Zn or Mn alone. The mixture of Zn+Fe gave higher values of volatile oil content in comparison to the other two mixtures. Spraying sweet basil plants with Zn+Mn+Fe together produced the highest values of volatile oil percentage. This was obvious in the two cuts and two seasons.

The increase in the volatile oil content of leaves by spraying Zn are in accordance with the findings of Akhtar et al. (2009) on *Mentha piperita*, Said-Al-Ahl and Omer (2009) on *Coriandrum sativum* plant and Nasiri et al. (2010) on *Matricaria chamomilla*. They reported that, the application of Zn

(50 ppm) to plants caused an increase in volatile oil content if compared with the untreated ones.

The obtained results of the effect of Mn on the volatile oil yield are in accordance with those of Said-Al-Ahl and Mahmoud (2010) on *Ocimum basilicum* plant. They reported that spraying plant with Mn (50 ppm) caused a significant increase in the volatile oil content. The present data of Fe agreed with those of Abd El-Wahab (2008) on *Trachspermum ammi* and Nasiri et al. (2010) on *Matricaria chamomilla*, they found that iron application on plant caused an increment in the volatile oil content.

As the mixture of microelements was studied, this activation effect was observed by many investigators, such as Abd El-Wahab (2008) on *Trachspermum ammi* and Kalidasu et al. (2008) on *Coriandrum sativum*. They indicated that spraying with Fe+Mn, Fe+Zn and Zn+Mn+Fe produced the highest volatile oil content per plant and plot compared with the other treatments.

#### 2.2. Volatile oil yield

Data of the volatile oil yield productivity are shown in table (6). Results show that, spraying sweet basil plants with some trace elements produced the heaviest volatile oil yield per plant as compared with untreated plants. Statistical analysis showed a highly significant increase in both seasons. Fe alone had significantly increased the volatile oil yield per plant compared with Zn or Mn alone. These results are true in the two cuts and two seasons.

All mixtures resulted in highly significant increases, compared with the control plants. The best results were obtained from the use of the three elements together on sweet basil plants followed by Fe with Zn or Mn treatments and to a less extent the mixture of Zn+Mn. The increase in the volatile oil yield per plant in treatments of Fe alone or Zn+Mn+Fe together may be attributed to the increase in the number of branches and leaves per plant resulted from these treatments.

Data dealing with the effect of micronutrients on the volatile oil yield per plot in both seasons are shown in table (6). The results show that, spraying sweet basil plants with trace elements produced the highest volatile oil yield per plant and per plot compared with the untreated ones. Statistical analysis showed that there is a highly significant increase in both seasons. Fe alone significantly increased the volatile oil yield per plant and per plot compared with Zn or Mn alone. These results are true in the both seasons.

All mixtures resulted in highly significant increase, compared with the control. The best results were obtained from using of three elements together on plant; followed by Zn+ Fe treatment and to a less extent the mixture of Zn+ Mn. The increase in the volatile oil yield per plant and per plot in treatments of Fe alone or Zn + Mn + Fe together may be due to the increase in the number of branches and leaves per plant and plot resulted from these treatments.

These remarkable findings of the effect of Zn on herb yield are in agreement with El-Sawi and Mohamed (2002) on cumin plant and Said-Al-Ahl and Mahmoud (2010) on *Ocimum basilicum*. They reported that, the application of Zn at 50 ppm to the plants caused an increased in the volatile oil yield if compared with the untreated plants.

The obtained results for the effect of Mn on volatile oil yield are in accordance with those of Abd El-Aziz (2000) on basil plant and Eisa (2000) on fennel plant. They reported that spraying plants with Mn caused a significant increase in the volatile oil yield.

The present data of Fe agreed with those of Zehtab-Salmasi et al. (2008) on *Mentha piperita* and Said-Al-Ahland Mahmoud (2010) on *Ocimum basilicum*. They found that iron application on plant caused an increment in the volatile oil yield.

Table (6). Effect of some microelements on the Volatile oil yield per plant and per plot (ml) of *Ocimum basilicum* during two seasons of 2014 and 2015.

Characters	Volatile oi plant	l yield per	Volatile per plo	oil yield ot (ml)				
Treatments	1 <sup>st</sup> Season							
	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	1 <sup>st</sup> cut	2 <sup>nd</sup> cut				
Control (distilled water )	0.030 G	0.047 H	1.50 G	2.35 H				
Fe (50 ppm)	0.093 E	0.167 E	4.65 E	8.35 E				
Mn (50 ppm)	0.043 G	0.073 G	2.15 G	3.65 G				
Zn (50 ppm)	0.070 F	0.127 F	3.50 F	6.35 F				
Fe+Mn (50 ppm)	0.157 C	0.243 C	7.85 C	12.15C				
Fe+Zn (50 ppm)	0.183 B	0.287 B	9.15 B	14.35 B				
Mn+Zn (50 ppm)	0.123 D	0.193 D	6.15 D	9.65 D				
Fe+Mn+Zn (50 ppm)	0.217 A	0.333 A	10.85 A	16.65 A				
		2 <sup>nd</sup> Sea	ason					
	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	1 <sup>st</sup> cut	2 <sup>nd</sup> cut				
Control (distilled water )	0.047 H	0.053 G	2.35 H	2.65 G				
Fe (50 ppm)	0.167 E	0.190 E	8.35 E	9.50 E				
Mn (50 ppm)	0.073 G	0.087 G	3.65 G	4.35 G				
Zn (50 ppm)	0.117 F	0.137 F	5.85 F	6.85 F				
Fe+Mn (50 ppm)	0.253 C	0.283 C	12.65 C	14.15 C				
Fe+Zn (50 ppm)	0.290 B	0.323 B	14.50 B	16.15 B				
Mn+Zn (50 ppm)	0.210 D	0.240 D	10.50 D	12.00 D				
Fe+Mn+Zn (50 ppm)	0.330 A	0.407 A	16.50 A	20.35 A				

Means having the same letter within the same column are not significantly different according to LSD for all-pairwise comparisons test at 5% level of probability.

As the mixture of microelements was studied, the stimulatory effect was observed by many investigators, such as Abd El-Wahab (2008) on

*Trachyspemum ammi*, Zehtab-Salmasi et al. (2008) on *Mentha piperita* and Younis et al. (2013) on *Rosa* hybrid, cvs. They indicated that spraying with Fe + Mn, Fe + Zn and Zn + Mn+ Fe produced the highest volatile oil yield per plant and plot compared with the other treatments.

#### 2.3. Volatile oil components

Chromatographic analysis of the volatile samples taken from the second cut in the second season are shown in table (7). It is clear that l-linalool is the main component of sweet basil oil followed by 1,8 cineole (the second component) and estragole (the third component), while other components such as  $\alpha$  –phellandrene, camphene, trans sabinenehydrate,  $\alpha$  – terpineol .... etc. constitute relatively small percentages of volatile oil in the second season.

Concerning the effect of some microelements on the different volatile oil components in both seasons are shown in table (7) and fig. (1, 2, 3, 4, 5, 6, 7, 8). It could be observed that for L-linalool is; the main component of sweet basil., when spraying plants with Zn, Mn and Fe at 50 ppm singly or in mixture, increased its percentage in all treatments. The best results were obtained by the plants with Zn + Mn + Fe. Comparing the effect of the three elements, it is obvious that Fe was the most effective element, when the elements were sprayed separately, followed by Zn then Mn. Data recorded in the second season were confirmed by those of the first one, where microelements improved the quality of the volatile oil at all treatments.

1,8 cineole; the second important volatile oil component, percentages in sweet basil oil increased by treating the plants with the micronutrients separately or in mixture. The best results were obtained by the plants treated with Zn + Mn + Fe together. Comparing the effect of the three elements, it is obvious that Fe was the most effective element, when the elements were sprayed separately, followed by Zn then Mn.

Estragole is the third important volatile oil component, percentages in sweet basil oil decreased by treating the plants by Mn or Fe at 50 ppm. The treatment of Zn + Mn + Fe together gave the lowest value of estragole. Data recorded in the second season were confirmed by the improved quality of the volatile oil at all treatments.



Fig (1): chromatogram of Ocimum basilicum, L. distilled from control plants during the second cut in the second season 2015.





Fig (4): chromatogram of Ocimum basilicum, L. distilled from plants treated by Zn (50 ppm) during the second cut in the second season 2015.



Fig (3): chromatogram of *Ocimum basilicum*, L distilled from plants treated by Mn (50 ppm) during the second cut in the second season 2015.



Fig (5): chromatogram of Ocimum basilicum, L. distilled from plants treated by Fe + Zn (50 ppm) during the second cut in the second season 2015.



Fig (7): chromatogram of Ocimum basilicum, L. distilled from plants treated by Mn + Zn (50 ppm) during the second cut in the second season 2015.







Fig (8): chromatogram of Ocimum basilicum, L. distilled from plants treated by Fe + Zn + Mn (50 ppm) during the second cut in the second season 2015.

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Treatments	Control	Fe	Zn	Mn	Fe+Zn	Fe+Mn	Mn+Zn	Fe+Mn+Zn
Compound								
α–Thujene	-	-	-	-	-	-	-	0.06
α –Pinene	2.41	2.76	2.55	2.53	1.67	2.25	2.49	3.04
α –Phellandrene	-	-	-	0.77	-	-	-	-
Camphene	0.21	0.17	0.20	-	0.10	0.21	0.23	0.27
α –Myrcene	0.76	0.78	0.74	0.53	0.66	0.44	0.48	0.56
Sabinene	0.65	0.89	0.73		0.53	0.65	0.71	0.90
Camphor	1.33	0.95	0.88	0.74	1.37	0.91	1.16	1.73
<b>D-Limonene</b>	0.29	0.33	0.30	0.26	0.29	0.21	0.27	0.41
O-Cymene	-	-	-	-	0.06	-	-	0.07
1,8 Cineole	12.79	20.74	13.17	18.59	23.28	17.59	16.88	25.66
Trans Sabinene	0.31	0.39	0.29	0.43	-	0.35	0.30	0.88
Hydrate								
Propionate	0.31	0.37	0.32	-	-	-	-	-
L-Linalool	27.52	35.98	28.16	35.40	42.29	38.06	37.11	52.69
<b>Cis-Ocimene</b>	0.34	-	-	-	0.09	-	-	0.07
<b>Ç-Terpinene</b>	-	0.19	-	-	0.12	-	-	0.12
α -Terpineol	0.65	1.11	0.97	1.28	2.60	1.22	1.30	1.05
1-4-Terpineol	0.48	1.27	0.60	0.94	1.49	0.50	0.49	-
α -Terpinolene	-	-	6.90	-	0.12	-	-	0.15
Linalool Oxide	-	-	-	-	0.34	-	-	0.40
Estragole	9.94	6.07	12.33	7.74	10.44	15.00	4.22	3.21
<b>Bornyl Acetate</b>	3.69	3.68	5.02	4.44	3.43	5.56	4.57	3.48
2-Oxabicyclo	0.50	-	0.65	0.65	-	-	0.54	0.13
Caryophyllene Ovide	0.96	0.60	-	-	-	-	-	-
12.								
Cyclohexanediol	0.83	0.53	0.95	0.44	0.36	0.43	-	-
Cyclohexane	1.66	0.64	0.73	0.52	0.49	0.49	0.67	0.33
Trans-	1.20	-	2.27	0.61	0.49	0.67	0.60	0.28
Caryophyllene					0.07			0.07
α -Copaene	-	-	-	-	0.07	-	-	0.07
a -Bourdonene	- 1 <i>5</i> 2	0.15	0.18	-	0.09	-	-	0.10
numulene	1.55	1.06	1.91	1.22	0.45	1.02	1.22	0.25
Camphon	-	-	0.23 0.75	0.21	-	0.10	- 0.45	-
Bicycle	- 9.17	- 6.14	-	- 4.66	3.03	5.63	6.39	-

**Table (7).** The main components in essential oil of *Ocimum basilicum* plant<br/>by GC-Mass analysis during the second cut in the second season<br/>of 2015.

Table (7). Cont.								
D-Germacrene -	3.52	2.16	3.56	-	0.31	1.71	1.97	-
Trans-à-								2.25
Bergamotene	-	-	-	-	-	-	-	2.55
Trans-á-	0.74		0.57	0.28	0.10			0.07
Farnesene	0.74	-	0.57	0.58	0.10	-	-	0.07
Azulene	0.60	0.90	0.55	0.37	-	0.32	0.47	-
Cis-á-Farnesene	-	0.41	-	-	-	0.37	0.50	-
Cyclohexane	-	-	-	-	-	0.47	0.62	1.04
Naphthalene	0.64	0.15	0.23	2.93	-	0.14	0.19	-
α -Cadinol	11.01	5.45		10.23	-	5.46	9.97	-
(+) Spathulenol		0.15	0.19	0.26	-	0.16	0.25	-
Cubenol	1.34	0.74	9.16	1.25	-	0.71	1.20	-
1-Naphthalenol	-	-	-	1.10	-	-	-	-
Cyclohexane	-	-	-	-	-	0.60	-	-
Çmuurolene	4.59	-	3.82	-	0.76	-	3.04	0.31
ç-Cadinene	-	-	-	-	-	2.39	-	-
Caryophyllene				0.22			0.87	
Oxide	-	-	-	0.23	-	-	0.0/	-
Bicycle	-	-	-	-	0.10	-	0.43	-

Means having the same letter within the same column are not significantly different according to LSD for all-pairwise comparisons test at 5% level of probability.

#### 3. Mineral Content

#### 3.1. Macroelements percentage (N, P and K)

The results of table (8) indicate that there were differences in the percentage of N, P and K in the leaves of sweet basil plant, as a result of spraying with microelements. Moreover, results show that the highest N, P and K percentages per plant were observed in case of spraying Fe separately, comparing to Zn or Mn alone. Meantime, spraying plant with Zn + Fe + Mn together was the most effective treatment in increasing each of N, P and K percentages per plant and these increases were significant in both cuts and seasons.

The obtained results for the effect of Zn on the minerals percentages are in harmony with these mentioned by Heidarian et al. (2011) on *Glycine max* plant and Abd El-Aziz and Balbaa (2007) on *Salvia farinacea*. They concluded that application of Zn increased the N, P and K percentages in leaves. Similar results for the positive effect of Mn were obtained by Abd El-Aziz (2000) on basil plant and Eisa (2000) on fennel plantd. Who found that Mn application increased the N, P and K percentage in plant leaves.

The positive effect of Fe on the percentages of N, P and K are in harmony with those obtained by El-Mekawy et al. (2009) on *Achillea fragrantissima* and Nasiri et al. (2010) on *Matricaria chamomilla*. They

concluded that Fe application increased the N, P and K percentage in plant leaves.

**Table (8).** Effect of some microelements on nitrogen, phosphorus, potassiumpercentages in leaves of *Ocimumbasilicum* during two seasons of2014 and 2015.

Characters	Nitrogen		Phosphorus		Potassium	
	perce	ntage	perc	entage	percentage	
Treatments	$1^{st}$	$2^{nd}$	$1^{st}$	2 <sup>nd</sup>	$1^{st}$	$2^{\mathrm{nd}}$
	Season	Season	Season	Season	Season	Season
Control (distilled water )	1.117 G	1.230 G	1.233 G	1.400 G	0.124 E	0.136 E
Fe (50 ppm)	1.900 D	2.087 D	2.200 D	2.333 D	0.224 C	0.247 C
Mn (50 ppm)	1.300 F	1.420 F	1.467 F	1.566 F	0.138 E	0.150 E
Zn (50 ppm)	1.747 E	1.923 E	1.967 E	2.167 E	0.195 D	0.215 D
Fe+Mn (50 ppm)	2.250 BC	2.473 BC	2.533 B	2.767 BC	0.259 B	0.285 B
Fe+Zn (50 ppm)	2.337 B	2.570 B	2.633 B	2.867 B	0.269 B	0.296 B
Mn+Zn (50 ppm)	2.163 C	2.380 C	2.367 C	2.667 C	0.238 C	0.261 C
Fe+Mn+Zn (50 ppm)	2.460 A	2.707 A	2.833 A	3.033 A	0.290 A	0.318 A

Means having the same letter within the same column are not significantly different according to LSD for all-pairwise comparisons test at 5% level of probability.

The simulative effect of the mixture of Zn, Mn and Fe application on the mineral percentage (N, P and K) was also reported by Said-Al-Ahl and Mahmoud (2010) on *Ocimum basilicum* plant. They found that, spraying plants with Zn, Mn and Fe either alone or in mixture increased the contents of N, P and K percentage in plant tissue, and the best mixture was the application of the three microelements together to plants, followed by the coupling of Zn+Mn or Mn+ Fe. The stimulating effect of Zn, Mn and Fe may be due to their effect on enhancing the plant metabolism (Amberger, 1974 and Miller et al., 1995).

#### 3.2. Microelements concentration in leaves (Zn, Mn and Fe)

Data in table (9) presents that, the effect of spraying plants with Zn, Mn and Fe on the concentrations of these elements in the leaves of sweet basil plant. It is obvious that Zn, Mn and Fe sprayed each alone resulted in highly significant increase of Zn, Mn and Fe concentrations as compared with the control. This means that, Fe was the most effective element. Also, all microelements mixtures resulted in high significant increase of Zn, Mn and Fe concentrations compared with each element separately. It is also obvious that Zn+ Fe had produced significantly higher values of Zn, Mn and Fe than Zn + Mn or Mn + Fe. The mixture of the three elements had significantly increased Zn, Mn and Fe concentrations compared with any

single elements. The treatment of Zn + Mn+ Fe together gave the highest values followed by Zn+ Fe, Mn+ Fe and Zn+Mn.

Similar findings for the positive effect of zinc in Zn, Mn and Fe concentrations were stated by Grejtovsky et al. (2006) on *Matricaria chamomilla*., Abd El-Aziz and Balbaa (2007) on *Salviafa rinacea* and Akhtar et al. (2009) on *Mentha pipeta*. They found that, the concentrations of Zn, Mn and Fe were significantly increased as a result of Zn application.

**Table (9).** Effect of some microelements on Zn, Mn and Fe concentrations inleaves of Ocimum basilicum, during two seasons of 2014 and2015.

Characters	Characters Iron (p)		pm) Manganese (ppm)			(ppm)
	$1^{st}$	$2^{nd}$	$1^{st}$	$2^{nd}$	$1^{st}$	$2^{nd}$
Treatments	Season	Season	Season	Season	Season	Season
Control (distilled water)	188.61 H	207.47 H	47.68 H	52.44 H	37.51 H	41.26 H
Fe (50 ppm)	321.57 E	353.73 E	81.28 E	89.41 E	63.95 E	70.34 E
Mn (50 ppm)	226.33 G	248.96 G	57.21 G	62.93 G	45.01 G	49.51 G
Zn (50 ppm)	293.75 F	323.12 F	74.25 F	81.67 F	58.41 F	64.25 F
Fe+Mn (50 ppm)	371.01 C	408.11 C	93.78 C	103.16C	73.78 C	81.16 C
Fe+Zn (50 ppm)	388.49 B	427.34 B	98.20 B	108.00 B	77.26 B	84.98 B
Mn+Zn (50 ppm)	356.87 D	392.56 D	90.20 D	99.23 D	70.96 D	78.07 D
Fe+Mn+Zn (50 ppm)	408.84 A	449.72 A	103.34A	113.60 A	81.30 A	89.43 A

Means having the same letter within the same column are not significantly different according to LSD for all-pairwise comparisons test at 5% level of probability.

The obtained results for the effect of Mn on Zn, Mn and Fe concentrations are in accordance with those of Pande et al. (2007) on mint plant and Said-Al-Ahl and Omer (2009) on *Coriandrum sativum*. They stated that spraying plants with Mn at 50 ppm increased Zn, Mn and Fe concentrations in the leaves.

The presented data for the effect of iron agreed with those of Baloch et al. (2008) and Abd El-Wahab (2008), who reported that micronutrients such as Fe, Mn and Zn have important roles in plant growth and yield of aromatic and medicinal plants. The activator effect of the mixture of Fe, Mn and Zn application on the mineral concentrations (Fe, Mn and Zn) was also reported by Abd El-Aziz (2000) on basil plant and Eisa (2000) on fennel plant. They stated that spraying with Zn, Mn and Fe singly or in mixture at 50 ppm caused an increasing in these microelements in the plant tissues. These results may be due to the effect of these elements on the plant growth as well as the leaves yield per plant.

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# تأثير العناصر الصغرى على النمو والمحصول والتركيب الكيماوي لنبات الريحان

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أجريت هذه الدراسة في محطة بحوث القنطرة شرق التابعة لمركز بحوث الصحراء بمحافظة شمال سيناء - مصر خلال الموسمين المتتاليين ٢٠١٤ و ٢٠١٥، بهدف دراسة تأثير الرش بالعناصر الصغرى (الحديد، الزنك، المنجنيز، الحديد + الزنك، الحديد + المنجنيز، الزنك + المنجنيز، الحديد + الزنك + المنجنيز] بتركيز ٥٠ جزء في المليون لكل منهم على النمو والمحصول والمكونات الرئيسية لزيت نبات الريحان. وقد أدت المعاملة المركبة من كل من الحديد والمنجنيز والزنك بتركيز ٥٠ جزء في المليون لكل منهم إلي زيادة في النمو الخضري للنبات والمحتوى من الزيت وتركيز كل من النيتروجين، الفوسفور، البوتاسوم، الحديد، المنجنيز والزنك في أنسجة النبات. وقد أدت المعاملة أيضًا إلى زيادة في تركيز مركب اللينالول إلي ٢٠٦٥٪ و مركب ١٨ سينيول إلي ٢٢ ٢٦٪