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EFFECT OF NANO-MICROELEMENTS ON GROWTH, YIELD ANDESSENTIAL OIL PRODUCTION OF SWEET MARJORAM (*ORIGANUM MAJORANA*) PLANTS

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Abstract

A field experiment was carried out during the two successive seasons of 2017/2018 and 2018/2019, to evaluate the effect of nano micro-elements on improving the productivity of marjoram plant. The plants were monthly foliar sprayed with nano-Fe, nano-Mn and nano-Zn h at two levels 50 and 100 ppm. The data showed that plant height, number of branches number, fresh and dry weights of herb in second cut were higher than that of the first one. Treating plants with nano- Zn at 100 ppm and nano-Mn at 50 ppm produced the tallest plants. Nano-Zn was the most effective on increasing formation of branches fresh and dry weights of herb, in both cuts of both seasons. All treatments of nano micro-elements increased the content chl. a, b and carotene and total carbohydrates, compared with the control and the high level (100ppm) was more effective than the low one and nano-Zn, followed by nano Mn were the most effective. As regards the essential oil % and oil yield, nano -Zn at 100ppm was more effective than nano-Fe and nano -Mn. Nano-Fe (100ppm) and nano-Mn (100 ppm), significantly increased the biosynthesis of the essential oils, in the second cut of the second season, over control. The essential oil yield (ml) per plant was greatly affected by the application of nano-Fe, Mn and Zn, in both cuts of both seasons. The maximum oil yields (ml) were recorded with nano -Zn (100 and 50ppm), followed by nano-Mn (100ppm) and nano-Fe at 100ppm was more effective than at 50 ppm. The non-sprayed plants showed the lowest essential oil % and yield. A total of 30 compounds of marjoram essential oil were identified by GC-MS (oil profile), the main constituents were; sabinene, a-terpinene, y-terpinene, p-cymene, terpinene, linalool, cis-sabinene hydrate, linalyl acetate, terpinen-4-ol and y-terpineol. The most prominent component was 1-4-terpineol (27.71 to 37.54%), in the first cut and from 28.15% to 36.38%, in the second one, the maximum % was recorded with nano-Mn (100ppm) in the first cut and nano Fe (50 ppm) in the second cut. The trans-sabinene hydrate ranged from 5.41to10.80%, in the first cut and from 2.95 to 5.02 %, in the second one, the corresponding percentages of cis-sabinene hydrate were 13.02, 20.44, 12.22 and 14.27%, respectively. The treatment of nano- Mn exhibited the highest % of these components. The highest sabinene % was found in plants treated with nano Fe (50 ppm) in the first cut and nano -Zn in the second one. Nano-Mn (50 ppm) gave the highest P-cymene % in the first cut and nano-Zn (100ppm) in the second one.

Key word: Nano microelements growth, essential oil, marjoram.

Introduction

Sweet marjoram (*Origanum majorana* syn. *Marjorana hortensis*, family Lamiaceae) is one of the most important economic medicinal and aromatic plant, it grows well in Egypt. It is an important source of national income,beside its importance in pharmaceutical industries, it is grown almost all over the world (El-Asmawy *et al.*, 2007 and Verma (2010). Sweet marjoram is a hardy perennial herbaceous plant native to Mediterranean countries. he dried leaves are used commonly for flavoring many food products, it contains terpenoids, phenols and flavonoids as major constituents (Janicsak *et al.*, 1999) and others like steroids, fatty acids and vitamins (Baâtour *et al.*, 2011). It is used as a spice and condiment and possess high antioxidant activity (Triantaphyllou *et al.*, 2001; Juliani & Simon, 2002), as a spice in sausages, culinary dishes and commercial foods (Pizzas, sausages,..), in folk medicine and processed vegetables (Burdock, 1995 and Babili *et al.*, (2011), as stimulant, digestive, carminative, diuretic, antispasmodic, antioxidant and regulates menstruation (Yadava and Khare, 1995, Sivropoulou *et al.*, 1996) in the treatment of anxiety (Rogoz *et al.*, 2003). It is rich in essential oils up to 2% (Baratta *et al.*, 1998) and phenolic compounds (Ezzeddine *et al.*, 2001 and Busattaa, *et al.*, (2008). The oil can be beneficial in cases of nervous tension, respiratory congestion, painful muscles and joints, digestive problems, relaxation,relieving stress and supports release of mental and emotional tension. As a natural antioxidant and it is an aromatic culinary herb whose fresh or dry leaves can be used to flavor salads, meat and vegetable dishes, fresh herb has high levels of vitamin-A, C, beta-carotene, xanthins (powerful flavonoid antioxidants), that play a role in aging and various disease processes as anti-inflammatory and anti-bacterial properties. (Hossain *et al.,* 2008 and Radha and Padma 2011).

GLC -analysis of essential oil revealed that the main component was terpinen-4-ol up to 30.0% (Abbassy *et al.,* (2009). According to Soliman *et al.,* (2009), AbdEl –Wahab (2013) and AbdEl –Wahab (2017) the major compounds are terpinen-4-ol, γ-terpinene, trans-sabinene hydrate and linalool, trans-sabinene hydrate acetate and thujanol. On the other hand, Verma (2010) revealed that oil composition varied with respect to harvest cut height and the major constituents were sabinene-hydrate, terpinen-4-ol, sabinene and terpineol. In the last few years there has been a growing interest in the use of nano -fertilization for enhancing the productivity of medicinal and aromatic crops,due to the high efficiency and the homogenous distribution of the nutrients within the plants (Torabian *et al.,* 2017). The application of many micronutrients improves the plant's productivity (Hendawy and Khalid 2005) and can result in a quick positive response (Fernández *et al.,* 2013 and Sida-Arreola *et al.,* 2017), becoming an effective and better nutrient management, reducing the amount of fertilizers in plant nutrition (Solanki *et al.,* 2015 and Ghorbanpour *et al.,* 2017), it is an effective tool to achieve more production of crops (Jyothi and Hebsur, 2017). Nano-material is needed in minute quantity to enhance crop growth and yield significantly (quantity and quality) which prevent undesirable nutrient losses to soil(Ram, *et al.,* 2018). Iron is an element essential for plant growth and development, it involves in chlorophyll formation, an internal component of cytochrome oxidase and essential for the nitrate and sulfate reduction, nitrogen assimilation and energy generation (Honarjoo *et al.,* 2013). Zinc is an important micronutrient, it activates the functions of many enzymes,the synthesis of tryptophan and auxin, cell division and protein synthesis, it is necessary for maintaining the integrity of bio membranes (Marschner, 2012and Torabian *et al.,* 2016). Nano-Zn has favorable effects in creation of active Zn- phosphate inside the plant (Lv *et al.,* 2015). Iron and zinc have a pivotal role in different plant growth and developmental stages and RNA and DNA biosynthesis (Blasco *et al.,* 2015). Manganese (Mn) is an essential element for the biosynthesis of fatty acids, acyl lipids and proteins (Ness and Woolhouse, 1980), for photosynthesis and ATP synthesis (Ducic and Polle, 2005 and Millaleo *et al.,* 2010), for the biosynthesis aromatic amino acids and flavonoids (Lidon *et al.,* 2004), it involves in respiration, photosynthesis, synthesis of amino acids and activation of IAA (Burnell,1988 and Lidon *et al.,* 2004).

The stimulating effects of Nano- Fe, Zn and Mn as foliar spray on growth and yield of different medicinal and aromatic plants was reported by many investigators, in this regard, Nasiri, *et al.,* (2010) showed that essential oil percentage and yield of chamomile increased by foliar application of Fe and Zn.Elfeky, *et al.,* (2017) on *Ocimum basilicum* evaluated the effectiveness of foliar spray of nano-iron on growth and essential oil constituents, they found that it significantly enhanced total chlorophyll, total carbohydrate, essential oil %, Fe-content, plant height, No. of branches and leaves, fresh and dry weights. Moreover, Khater. (2015) on peppermint found that magnetite nanoparticles increased significantly plant growth. On *Zingiber officinale,* Siva and Benita (2016) stated that nano iron at 100ppm increased root and protein production. Torabian *et al.,* (2017) stated that foliar sprays of nano-Fe increased height and biological yield of sunflower plants. Askary *et al.,* (2017) reported that nano-Fe increased fresh and dry weights of Mentha piperita.On the other side, Weisany *et al.,* (2014) noted that foliar treatment with nano-Zn increased K content of soybean. Vafa, *et al.,* (2015) stated that treating savory (*Satureja hortensis*) with 200 ppm nano-Zn gave the tallest plants, heaviest fresh and dry weights, number of leaves, chlorophyll and essential oil contents. Sprays of nano- Zn (50-100 ppm) on *Spinacia oleracea* increased leaf area, chlorophyll and nitrogen contents (Kisan *et al.,* 2015). The stimulating effects of Nano-Zn on the growth parameters may be due to the high absorption efficiency and the high specific surface which increases IAA-levels within the plant tissues,stimulates new roots formation which improves the minerals absorption rate. Pessarakli (2016).On Holy basil, Moghimipour, *et al.,* (2017) found that foliar application of nano zinc chelate at 1-15 g/l significantly increased all measured growth traits and essential oil content and yield. Mohsenzadeh and Moosavian (2017) on *Rosmarinus officinalis* stated that nano-zinc oxide positively affected the enzymes activity and the content of chlorophyll. Rezaei-Chiyaneh *et al.,* (2018) treated *Nigella sativa* with foliar nano(iron, zinc and manganese, alone ortogether) at 2 g/l., they found significant effects on plant height, seed yield /plant and essential oil % and yield. *Abdel Wahab and Taha*. (2018) on *Eruca sativa* revealed that, nano Fe and Zn foliar treatments significantly increased fresh weight and photosynthetic rate and chemicals constituents (carbohydrate, flavonoids, crude protein, total fatty acids, IAA) and oil yield. Abdel wahab, *et al.*, (2017) on *Carum carvi* and Al-Juthery, *et al.*, (2019) on *Helianthus tuberosus* sprayed plants with nano micronutrients (Fe, Zn, Cu and Mn), they found superiority in the nutrient use efficiencies of the single spray treatments. Moreover, Hassanpouraghdam, *et al.*, (2019) on *Rosmarinus officinalis* reported that nano-Fe and Zn foliar application had a positive influence on the above-ground plant growth. Abdelkader, *et al.*, (2019) on *Foeniculum vulgare* stated that spraying plants with nano micronutrients (250, 500 and 1000 mg/l) improved vegetative growth (height and herb), yield and fruit yield/plant and 1000 mg/l had the greatest significant effect.

Materials And Methods

A field experiment was conducted at the Exp. Nursery of the Ornamental Hort. Depart. Fac. Agric., Cairo Univ., Egypt, during the seasons of 2017-2018 and 2018-2019 to study the response of growth, yield and essential oil productivity of *Origanummajorana* L. (Sweet majoram, syn. *Majorana hortensis* Moench.) to foliar individual applications of nano- Fe, -Zn and-Mn and assessing their effects on oil yield and composition.

Plant Materials and Procedure

Uniform marjoram seedlings of 10-12 cm length (from the Dept. of Medicinal and Aromatic Plants, Ministry of Agric., Egypt) were individually transplanted into the field (clay loam soil) at 50 cm between rows and 30 cm between plants. After a month,the plants were monthly foliar sprayed with nano-Fe, -Zn and- Mn (obtained from NRC, Dokky). The plants were sprayed with these nano-micronutrients at 50 or 100 ppm, besides the control plant. In both seasons, the plants were fertilized after each cut of the herb, with 200 kg as ammonium sulphate, 100 kg as calcium superphosphate and 50 kg as potassium sulphate per feddan. All agricultural practices of plants were done. The physical and chemical analyses of the soil were determined according to Jackson 1973. The physical composition were: 21.2% fine sand, course sand 8.30% silt, 34.5% clay and 1.88% organic matter. The chemical analysis were as follows: pH = 7.8; E.C (dS/m) = 1.5; and available N. 45ppm; available-P 29 ppm; K 1.5 meq/l.

Harvesting: The marjoram plants were harvested twice/year, *i.e.* in April and September, in both seasons. The plants were harvested by cutting the vegetative parts (herb) at about 10 cm above the soil surface leaving small branches with some leaves for regrowth.

Data recorded: For the two cuts of the both seasons, the following data were recorded at harvesting time: Plant height, No. of branches, fresh and dry weights of herb/ plant (g/plant).The herb was dried at 70°C until a constant weight was obtained and then the herb dry weight was recorded. Extraction and GLC analysis of essential oil: Oil percentage (based on air dried herb) was determined as indicated by Egyptian Pharmacopeia (1984) by distilling the herb for 2.5-3.0 hours and the oil yield/plant was calculated by multiplying oil% by dry weight of herb (ml/plant).GC-MS analysis: it was done for the two cuts of the second season only, it was carried out using gas chromatography-mass spectrometry instrument stands at the Depart. of Med.& Aromatic Plants, NRC, with the following specifications : Instrument : a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TG-5MS column (30m×0.25mm i.d., 0.25 µm film thickness), using helium as carrier gas at a flow rate of 1.0 ml/min and a split ratio of 1:10,using the following temperatures program: 60°C for 1 min; rising at 3.0°C/min to 240°C and held for 1 min. The injector and detector were held at 240°C. Diluted samples (1:10 hexane, v/v) of 0.2 µL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Most of the compounds were identified using the analytical method: mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library), according to Adams (2007).

C- Determination of chemical constituents:- Chlorophylls (a and b) and carotenoids contents (mg/g F.W) were estimated according to Saric *et al.*, (1967). The total carbohydrates content was determined in dried herb sample using the procedure of Herbert *et al.*, (1971).

Experimental design: The layout of the experiment was a complete randomized block design, with 3 replicates (each replicate contain 6 plants). So, this study contained 7 treatments (including the control).

Statistical analysis: Data of the vegetative growth, essential oil and chemical compositions were statistically analyzed. An analysis of variance (ANOVA) was carried out and the means of the recorded data were compared using L.S.D. test at the 5% level, as described by Steel and Torrie (1980).

Results and Discussion

Vegetative growth

Data in table 1 show the effect of nano–iron, nano-zinc and nano- manganese on plant height (cm), in the two cuts during the two seasons of 2017/18 and 2018/19. In the first season, all treatments of nano –particles significantly increased plant height over the control, in the two cuts. Nano-Zn at 100ppm gave the tallest plants (50.16 cm)in the first cut, followed by nano-Mn at 50 ppm (47.33cm). In the second cut, the tallest plants was recorded with nano-Zn (100 ppm) and nano-Mn(50 ppm). In the second season, all treatments of nano - microelements, significantly increased the plant height over the control in the first cut, whereas as in the second cut, all treatments (except the high level of nano-Fe and nano-Mn) significantly increased it, the tallest plants were obtained with nano-Zn at100 ppm, in the first cut and nano-Mn (50ppm) in the second one.

Concerning the effect on the number of branches per plant, the data revealed that, in both seasons, most of treatments significantly increased it, as compared the control. Spraying plants with nano-Zn gave the highest number of branches/plant. Regarding the response of fresh and dry weights of herb to foliar application of nano-Fe,-Zn and -Mn, during both seasons, it was found that nano -Zn (50 and 100 ppm) were the most effective in increasing the plant fresh weight in both cuts. The data show that the dry weight of herb followed a pattern of response similar to that of fresh weight table 2, moreover, the dry weight of herb was the heaviest in plants treated with nano-Zn. The non-sprayed plants showed lowest dry herb. The marked efficiency of the nano-Zn in increasing the dry herb is most probably due to enhancing some enzymes (as P-mobilizing) as mentioned by Raliya and Tarafdar (2013). Zinc is an important micronutrient which activates many enzymes and auxin, cell division and protein synthesis. (Marschner, 2012, Torabian *et al.*, 2016 and Vojodi Mehrabani *et al.*, 2017). Nano-Zn has favorable effects in creation of active Zn- phosphate inside the plant (Lv *et al.*, 2015). Manganese (Mn) is an essential element for the biosynthesis of fatty acids, acyl lipids and proteins (Ness and Woolhouse, 1980), for photosynthesis (Millaleo *et al.*, 2010) and involves in respiration, photosynthesis, synthesis of amino acids and activation of IAA (Lidon *et al.*, 2004). In addition, Misra

Table 1: Effect of nano-particles of iron, zinc and manganese on plant height and number of branches /plant, in the two cuts, during the two seasons of 2017/18 and 2018/19.

Nano Treatments	Plant height (cm)				No. of main branches/plant			
	First season, 2018/19		Second season, 2017/18		First season, 2018/19		Second season, 2017/18	
	First cut	Second cut	First cut	Second cut	First cut	Second cut	First cut	Second cut
Control	39.84	41.28	37.90	39.00	14.95	16.73	16.98	18.69
Nano-Fe 50 ppm	44.67	49.95	42.90	44.00	16.98	19.40	25.48	20.93
Nano-Fe 100ppm	42.67	48.33	44.50	41.67	18.49	21.66	25.20	19.40
Nano-Mn 50 ppm	47.33	51.03	45.09	47.33	16.07	24.39	24.26	24.16
Nano-Mn 100ppm	43.00	48.86	44.29	43.67	17.58	21.35	22.44	24.68
Nano-Zn 50 ppm	46.67	47.52	46.60	45.67	25.03	25.59	27.67	27.48
Nano-Zn 100ppm	50.16	52.82	48.90	48.33	26.67	27.94	29.78	28.51
L.S.D at 5%	4.87	4.63	5.35	5.80	2.80	3.59	3.18	4.26

Table 2: Effect of nano-particles of Fe,Zn and Mn on herb fresh and dry weights(g/plant)in the two cuts, during the two seasons of 2017/18 and 2018/19.

Nano Treatments	Fresh weight of herb g/plant				Dry weight of herb g/plant			
	First season,		Second season,		First season,		Second season,	
	First cut	Second cut	First cut	Second cut	First cut	Second cut	First cut	Second cut
Control	112.42	121.85	135.74	148.46	30.84	38.94	40.10	40.08
Nano-Fe 50 ppm	134.39	129.62	157.15	178.43	44.13	47.84	50.60	54.20
Nano-Fe 100ppm	147.74	135.70	168.20	182.99	47.20	48.68	52.08	56.16
Nano-Mn 50 ppm	149.63	142.54	157.37	186.52	49.37	50.01	47.35	53.73
Nano-Mn 100ppm	158.11	149.57	167.38	188.43	52.83	53.22	5211	54.20
Nano-Zn 50 ppm	168.01	175.68	185.14	190.97	61.30	64.27	68.31	70.32
Nano-Zn 100ppm	176.29	183.72	192.97	194.94	64.25	65.48	69.98	72.92
L.S.D at 5%	15.35	17.49	14.56	21.10	7.80	8.77	7.55	10.48

and Sharma (1991) on Japanese mint, reported that zinc application increased significantly the fresh and dry herb production. On geranium (*Pelargonium odoratissimum*), Lamia, *et al.*, (2017) reported that foliar spray of Zn affected significantly plant height, fresh and dry weights. HassanPouraghdam, *et al.*, (2019) on *Rosmarinus officinalis* reported that nano-Fe and Zn foliar application had a positive influence on the above-ground plant growth. Abdelkader, *et al.*, (2019) on *Foeniculum vulgare* stated that nano micronutrients improved vegetative growth (height and herb).

Pigment content: In most cases, the foliar application of nano micro-elements increased the content of chlorophyll a, b and carotenoids as compared with the control. The high level was more effective, in this respect,than the low one.

-Total carbohydrates: The foliar application of all nano micro-elements increased the accumulation of t total carbohydrates in the herb (in most cases), as compared with the control. Spraying plants with nano Zn followed by nano Mn enhanced the accumulation of total carbohydrates in the herb. In this regard, Misra, *et al.*, (2005) reported that zinc is involved in carbon assimilation, saccharides accumulation and chlorophyll biosynthesis. Abdel Wahab and Taha. (2018) reported that nano- Fe and -Zn foliar treatments significantly increased the photosynthetic rate and chemicals constituents (carbohydrate, flavonoids, crude protein, total fatty acids, IAA) and oil yield. The stimulating effects of Nano-Zn may be due to the high absorption efficiency of the element and the high specific surface which increases IAA-levels within the plant tissues,stimulates new roots formation which improves the minerals absorption rate (Pessarakli, 2016). Moreover, Abdel Wahab and Taha. (2018) on *Eruca sativa* reported that nano- Fe and -Zn foliar treatments significantly increased the photosynthetic rate and chemicals constituents (carbohydrate, flavonoids, crude protein, total fatty acids, IAA).

Essential oil % and yield

Data in table 4 show that the essential oil% was higher in the second cut (September) than in the first one (April), this can be attributed to the impact of temperature since the oil of the second cut was obtained from plants grew under high temperatures than those grown in the spring. Evans (1996) revealed that the number of oil glands in the leaves is not constants and increased with leaf expansion, so that the application of nutrient increased the amount of essential oil increased in plant. Nano-Zn (50 and 100 ppm) was the most effective in increasing the essential oil%, in both cuts in both seasons. Spraying sweet marjoram plant with 100 ppm nano-Zn was more effective than the low one giving 1.608, 1.716, 1.725 and

Table 3: Effect of nano-Fe, -Zn and- Mn on the contents of chlorophyll(a, b),carotenoids and total carbohydrates of marjoram leaves during 2017/18 and 2018/19.

Nano Treatments	Chlorophyll-a (mg/g FW)				Chlorophyll-b (mg/g FW)			
	First season, 2017/18		Second season, 2018/19		First season, 2017/18		Second season, 2018/19	
	First cut	Second cut	First cut	Second cut	First cut	Second cut	First cut	Second cut
Control	0.896	0.999	1.074	0.825	0.347	0.321	0.434	0.320
Nano-Fe 50 ppm	0.990	1.063	1.182	0.902	0.455	0.325	0.458	0.419
Nano-Fe 100ppm	1.004	1.177	1.135	0.987	0.497	0.431	0.453	0.420
Nano-Mn 50 ppm	1.015	1.086	1.061	0.990	0.452	0.432	0.515	0.520
Nano-Mn 100ppm	1.061	1.168	1.184	1.042	0.457	0.430	0.542	0.423
Nano-Zn 50 ppm	1.158	1.260	1.167	0.988	0.479	0.443	0.591	0.620
Nano-Zn 100ppm	1.222	1.247	1.188	1.060	0.549	0.480	0.613	0.620
L.S.D at 5%	0.134	0.137	0.125	0.117	0.055	0.043	0.075	0.080
Treatments	Carotene (mg/g FW)				Carbohydrates (% D.W)			
Control	0.473	0.453	0.523	0.522	18.28	20.63	19.63	19.87
Nano-Fe 50 ppm	0.545	0.624	0.512	0.533	19.39	23.78	22.59	20.83
Nano-Fe 100ppm	0.535	0.651	0.563	0.543	19.65	22.90	21.77	21.84
Nano-Mn 50 ppm	0.470	0.630	0.44	0.570	20.31	19.13	17.22	22.86
Nano-Mn 100ppm	0.480	0.677	0.492	0.552	21.34	25.80	21.92	24.72
Nano-Zn 50 ppm	0.536	0.617	0.545	0.580	23.90	24.30	23.95	23.58
Nano-Zn 100ppm	0.535	0.690	0.515	0.542	25.49	27.41	23.95	24.87
L.S.D at 5%	0.052	0.046	0.055	0.068	3.23	4.00	3.75	3.54

Table 4: Effect of nanoFe,-Zn zinc and-Mn on essential oil % and oil yield /plant of marjoram herb, in the two cuts, during the two seasons of 2017/18 and 2018/19.

Nano Treatments	Oil % D.W				ml/plant			
	First season, 2017/18		Second season, 2018/19		First season, 2017/18		Second season, 2018/19	
	First cut	Second cut	First cut	Second cut	First cut	Second cut	First cut	Second cut
Control	1.196	1.394	1.302	1.375	0.369	0.543	0.522	0.551
Nano-Fe 50 ppm	1.40	1.586	1.575	1.607	0.618	0.759	0.797	0.871
Nano-Fe 100ppm	1.470	1.674	1.598	1.683	0.694	0.815	0.832	0.945
Nano-Mn 50 ppm	1.325	1.455	1.362	1.628	0.654	0.728	0.645	0.875
Nano-Mn 100ppm	1.352	1.612	1.514	1.763	0.714	0.858	0.789	0.956
Nano-Zn 50 ppm	1.549	1.710	1.690	1.676	1.179	1.154	1.099	0.950
Nano-Zn 100ppm	1.608	1.716	1.725	1.770	1.291	1.207	1.122	1.034
L.S.D at 5%	0.230	0.256	0.253	0.244	0.188	0.175	0.160	0.169

1.770 %, in both cuts in both seasons, respectively. The non-sprayed plants showed the lowest percentages of essential oil in dried herb, in both seasons. In this regard, Misra, *et al.,* (2005) reported that essential oil production of plants is strongly influenced by several factors, as macro and micronutrients,which are very important in producing high quality of essential oil and yield, it is involved in carbon assimilation and finally carbon utilization in volatile oil biosynthesis. Moghimipour, *et al.,* (2017) studied the effect of foliar application of nano zinc (0, 0.5, 1 and 1.5 g/l) on essential oil content of Holy basil, they reported that the highest value of essential oil content was obtained in plants treated with nano zinc chelate. On the other side, there were marked increases in the essential oil percentages in response to the application of nano-Fe at 100 ppm and nano-Mn at 100 ppm, in the second cut of the second season. These treatments increased significantly the biosynthesis of the essential oils, as compared with the control. Fe act either as metal components of various enzymes or as functional, structural, or regulatory cofactors, thus, it is associated with saccharide metabolism, photosynthesis and protein synthesis (Marschner, 2012). Evans (1996) revealed that the number of oil glands in the leaves is not constants and increased with leaf expansion, so that the application of nutrient increased the amount of essential oil increased in plant. Misra *et al.,* (2006) reported that the essential oil biosynthesis of basil plants was strongly influenced by iron application. Zehtabe-Salmasi *et al.,* (2008) on mint reported that essential oil yield was increased by the application of iron., but, on *Origanum vulgar*e plants, Yeritsyan and Economakis (2002) reported that essential oil content was reduced by the application iron. Abd El-Wahab (2008) reported iron, have important roles the yield of M. pipperita. Nasiri *et al.,* (2009) reported that essential oil percentage and yield of *M. chamomilla* increased by iron application. Akhtar, *et al.,* (2009) indicated that the essential oil biosynthesis of peppermint was strongly affected by the application of Fe and Zn. Misra *et al.,* (2005) reported that essential oil biosynthesis of geranium was strongly influenced by zinc deficiency.

The essential oil yield of Sweet marjoram (ml plant^{-1}) was affected by the foliar application of nano-Fe,-Mn and -Zn, in both seasons table 4. The maximum oil yield per plant was recorded with nano-Zn (100 ppm), in both seasons, followed by nano-Zn at 50ppm and nano-Mn (100ppm). Spraying sweet majoram plant with the high level of nano-F (100ppm) was more effective than the low one, in both seasons. The non-sprayed plants showed the lowest yield of essential oil/plant. In this respect, Amuamuha *et al.,* (2012) on *Calendula officinalis*, found that nano-iron significantly increased the essential oil yield the second harvest to the highest level. Iron is required in the biosynthetic pathway and the application of micronutrients is very important in the production of high yield of oil with high quality products (Sawan *et al.,* 2001).

Chemical composition of essential oil

GC-MS analysis was done to evaluate the effect of each nano-element on the main constituents of the essential oil of marjoram table 5. The identified main constituents were: sabinene, a-terpinene, y-terpinene, p-cymene, terpinolene, linalool, cis-sabinene hydrate, linalyl acetate, terpinen-4-ol and y-terpineol. The component of 1-4-terpineol was found to be the first major compound (27.71-37.54%) in the first cut and it ranged from 28.15% to36.38%, in the second one. These findings are in agreement with those of Omer (1998) on Origanum syriacum and Omer *et al.,* (1994) on marjoram. The maximum content of this compound was recorded in the essential oil of plants sprayed with nano-Mn (100ppm) in

Table 5: Effect of nano-iron, zinc and manganese on the percentages of main componentsof essential oil of marjoram herb , in the two cuts, during the second season 2018/19.

Main components of essen-tial oil	Treatments of nano-iron, nano-zinc and nano-manganese						
	Con-trol	Fe 50 ppm	Fe 100 ppm	Mn 50 ppm	Mn 100 ppm	Zn 50 ppm	Zn 1000 ppm
	First cut						
Sabinene	2.64	7.06	4.55	5.55	2.66	5.93	5.01
a-terpinene	6.08	1.40	3.39	1.13	3.76	2.50	2.81
y-terpinene	2.50	1.53	1.39	0.76	1.89	0.96	0.99
P-cymene	3.05	8.93	9.61	12.15	3.46	11.94	10.80
Cis sabinene hydrate	16.19	13.02	15.06	17.28	20.44	14.05	13.77
Trans sabinene hydrate	8.89	5.41	10.56	10.80	10.75	8.87	10.07
Linalool	2.84	4.72	3.24	2.53	2.69	2.77	3.34
1-4-terpineol	36.36	30.03	28.60	30.63	37.54	30.57	27.71
A-terpineol	5.58	5.70	6.25	5.01	5.04	5.68	5.76
Linalyl acetate	2.29	1.42	2.56	1.82	1.33	2.49	2.77
	Second cut						
Sabinene	4.75	6.13	8.14	7.27	6.42	7.03	8.39
a-terpinene	4.26	3.77	3.65	3.59	3.77	3.46	3.36
y-terpinene	1.85	1.66	1.90	1.87	2.06	2.18	2.61
P-cymene	6.96	6.54	8.77	8.39	7.95	8.73	10.60
Cis sabinene hydrate	14.18	12.22	14.04	12.64	14.27	12.80	12.48
Trans sabinene hydrate	3.82	2.95	3.19	4.41	5.02	4.73	4.13
Linalool	6.31	5.85	6.10	8.20	8.02	7.52	3.19
1-4-terpineol	32.40	36.38	32.34	30.50	28.57	28.15	28.65
A-terpineol	6.27	7.49	5.36	5.67	5.72	5.95	5.55
Linalyl acetate	1.37	0.80	0.48	0.57	1.30	0.57	0.62

the first cut and nano-Fe (50 ppm) in the second one and the minimum content was recorded with plants that received nano- Zn at high level and Zn at the low level, in the first and second cuts, respectively. The percentages of trans- sabinene hydrate and cis-sabinene hydrate were the highest in plants treated with nano-Mn, in both cuts and the minimum content in plants treated with nano- Fe (50 ppm).Sabinene% increased to the highest% in plants treated with nano Fe at 50 ppm and nano-Zn. P-cymene% was the highest by treatment of 50 ppm nano-Mn in the first cut, whereas 100 ppm nano-Zn was the most effective in the second cut. All nano-treatments decreased the percentages of a-terpinene and linalyl acetate, in both cuts as compared with the control. Picacaglia and Marotti (1993) stated that the differences in relative amounts of some components as γ–terpinene and p-cymene, in essential oils of Satureja montana could be attributed to the effects of environmental conditions. El-Ghandour *et al.*, (2009) stated that the essential oil of marjoram plants contained the following eleven main compounds namely trans-sabinene hydrate, cis-sabinene hydrate, linalyl acetate, eugenol, linalool, a-terpineol, terpinene-4-ol and thymol. The main components were terpinene 4-ol, a-terpineol, cis -sabinene hydrate, trans-sabinene linalyl acetate. It was reported that Zn treatment caused a decrease in oxygenated mono-terrenes and hydrogenated mono-terpenes of essential oil of Mentha spicata (Dehabadi, *et al.*, 2010) Ghannadnia (2014) 0n Cuminum cyminum revealed that Mn treatments (up to 160ppm) increased the monoterpene concentrations. Yadegari (2016) on lemon balm (*Melissa officinalis* L.) sateted that the most of essential oils components were affected by Mn treatment. Mn was more effective at 150 ppm and Pirzad andVaièiulytë *et al.,* (2017) on Thymus pulegioides reported that the presence of higher amounts of manganese in soil stimulated the biosynthesis of main compounds in essential oils (geraniol). Barin (2018) on Pimpinella anisum reported that zinc influenced the primary metabolic pathways which lead to the biosynthesis of active components of essential oil.

Conclusions

From our results, it can be recommended the foliar application of nano micro-elements (Fe, Zn and Mn) for improving the productivity of marjoram plant (herb and essential oil%, yield and components) The high level of these elements 100ppm was the most effective for increasing oil percentage and oil yield/plant. The application of nano-Zn and Mn, gave the best result in case of herb fresh yield. The essential oil yield and composition responded well to nano-Zn and nano Mn. GC-MS analyses of essential oil identified thirty components in our study and the most component of oil was terpinen-4-ol, followed by cis-and trans-sabinene hydrate, sabinene and p-cymene.

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