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Effect of foliar spraying with some Micro-elements on the chemical composition and essential oil of Hyssop (Hyssopus officinalis) plant

Abd El-Ghafour El-Sayed[1]; Ahmed El-Liethy[2]; Saber Hendawy[3] Rehab Farag[4]

[1] Professor of Medicinal and Aromatic Plants, Faculty of Agriculture, Cairo University. Mail: abdelghafour_awad@yahoo.com
[2] Professor of Medicinal and Aromatic Plants, Faculty of Agriculture, Cairo University. E-mail: el-leithyahmed@yahoo.com
[3] Professor of Medicinal and Aromatic Plants, National Research Centre. E-mail: hendawysaber@yahoo.com
[4] Master student, Heliopolis university. E-mail: rehab.ibrahim@sekem.org
corresponding author:-eng Rehab Farag,Master student,Heliopolis university for sustaina Development ble. E-mail: rehab.ibrahim@sekem.org

Abstract

Background: *Hyssopus officinalis L*. belongs to Lamiaceae. The herb has a long history of medicinal uses whereas essential oil is widely being used in cosmetics, food ,and pharmaceutical industries worldwide, as well as fresh herb is usually used in cooking. This work was conducted to investigate the effect of Micro-elements on the growth and essential oil of Hyssop *(Hyssopus officinalis)*, cultivated under Egyptian condition. This two-year randomized complete block experiments were performed foliar spraying with Zinc (Zn), Magnesium (Mg), Manganese (Mn), and Iron (Fe) on *Hyssopus officinalis* L. **Results:** The study revealed that Zinc and Magnesium had pronounced effects, enhancing plant height, aerial parts, whole plant dry weight, and branching. Moreover, these micro-elements positively impacted flowering and various chemical constituents, including chlorophyll, carotenes, potassium, protein, phosphorus, nitrogen, and total carbohydrates. Foliar application of Zn, Mn, and Fe significantly increased essential oil percentage, resulting in a notable 35% boost compared to the control. The dominant compound in the essential oil composition was trans-3-pinanone (63.37%). **Conclusion:** Micro-nutrients specially Zinc and Magnesium emerged as key influencers on growth characteristics and positively affected hyssop's chemical composition; chlorophyll A & B, carotenoids, and total carbohydrates with improving essential oil yield and its components that emphasizies the potential of micro-nutrients applications to optimize the cultivation of this valuable herb.
Keywords: *Medicinal plant, Lamiaceae family, active ingradiant, GC MS analysis*

1. Background

Herb hyssop (*Hyssopus officinalis L.*, family Lamiaceae) is a brightly colored herbaceous plant of the genus *Hyssopus* native to Southern Europe, the Middle East, and the region surrounding the Caspian Sea **(Busari, 2006)**. The Hyssopus genus consists of herbs or subshrubs that are primarily cultivated but can also be found in the wild. The flower cluster is 20 to 25 cm long resembling thorns and comprises 4 to 10 groups of flowers, at the end. The *H. Officinalis* root has a taproot with multiple heads. The stems are between 0.5 and 0.7 m either upright or leaning and divided into woody branches. Leaves are arranged opposite each dark green with smooth edges, elongated or oblong shaped slightly pointed at the tip measuring 2 to 4 cm in length and 0.5 to 1 cm in width. The Hyssopus L. Genus includes around 10 to 12 species primarily found in the Mediterranean

*Corresponding author e-mail: *rehab.ibrahim@sekem.org*
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region extending towards Central Asia **(Fathiazad et al., 2011)**. *H. Officinalis L*., also known as hyssop has long been valued for its benefits. It is used as a carminative, tonic, antiseptic, expectorant and cough suppressant. Despite its taste *H. Officinalis* is commonly used in the food industry to provide a refreshing minty flavor and enhance dishes as a condiment **(Fathiazad et al., 2011)**. In addition, *H. Officinalis* has been used for centuries in medicine **(Fathiazad et al., 2011)**. Is highly valued in the cosmetic, food and pharmaceutical industries worldwide. In medicine it is believed that *H. Officinalis* possesses properties that can soothe and expectorate as well as suppress coughs and stimulate the gastrointestinal system. It is commonly utilized in sauce recipes. Serves as a flavoring ingredient

Kazazi et al., (2007)., in the food industry. Moreover fresh *H. Officinalis* herb is frequently added to preparations **(Fernández-López et al., 2003)..**

For example, Utilized extensively, The main component of H. officinalis is its dried leaves. Za'atar; a well-known herbal mix of the Middle East. Additionally, The herb known as *H. officinalis* serves the purpose of adding flavor to liqueur and is a key component in the sanctioned recipe of Chartreuse **(Kazazi et al., 2007)**. Beekeepers commonly utilize this herb to generate honey that possesses a delightful aroma and a luscious taste.Also, *H. officinalis* is widely used to add flavor to liqueurs and is an important ingredient in the official recipe of Chartreuse **(Kazazi et al., 2007)**. Beekeepers often use it to produce honey with a unique and pleasant aroma. Moreover, its leaves can be used as a fragrant seasoning, providing a slightly bitter taste due to its tannins, along with a strong minty scent **(Paun et al., 2014)**.

Micro-elements play an important role in the growth and development of all crop plants. In the case of medicinal plants that synthesize essential oils,and nutrients can effectively increase oil yield and quality **(Zheljazkov *et al*. 2010, 2011)**. Micro-nutrients are elements that are essential for plant growth but are required in quite smaller amounts than those of the primary nutrients, nitrogen, phosphorus, and potassium. They play an indispensable role in cell division and development of meristematic tissues, stimulate photosynthesis, respiration, energy, and nucleotide transfer reactions and fasten the plant maturity **(Marschner, 1998).**

Furthermore, the growth parameters (plant height, bulb diameter, number of leaves per plant, fresh bulb weight, total fresh and dry weight per plant) were positively affected by the application of micro-nutrients, although micro-nutrients are needed in relatively very small quantities for adequate plant growth and production, their deficiencies induce a great disturbance in the different physiological and metabolic processes inside the plant **(Khalil *et al*., 2008)**. Micro-nutrients such as zinc, magnesium, manganese, and iron have important roles in plant growth and yield of aromatic and medicinal plants **(El-Wahab, A., and Mohamad, A. 2008)**. Moreover, In addition, it led to an increase in shoot and root dry weight **(Younis *et al*. 2013; Yarnia *et al*. 2012)** of *Ocimum basilicum* L according to research conducted by Said-Al Ahl in 2010, the addition of micro-elements had a significant impact when compared to the control group. This impact was observed in terms of increased plant height, number of branches, and fresh weight. It is worth noting that the highest plant height and the average number of branches were observed in all cuttings during both seasons when the on soil iron and salinity spray mixture was used.Results showed that simultaneous foliar application of Fe, Zn, and Mn had positive effects on the quantitative and qualitative traits of Hyssop. Therefore, micro-nutrients could be applied as a suitable strategy to reduce chemical inputs toward sustainable agriculture and to achieve optimum biological yield in hyssop **(Amini, 2019)**.

This study, conducted over two years from 2018 to 2020, focused on the effects of essential microelements, including Zn, Mg, Mn, and Fe, on various agronomic and chemical characteristics of Hyssopus officinalis L. The research aimed to understand how these elements could enhance the plant's growth and oil production. The study took place in an organic farm located in Sharkia, Egypt, with assessments conducted in Egypt. Two successive seasons (2018/2019 and 2019/2020) were selected for the experiments to study the effect of microelements and their interaction on vegetative growth, oil production, and some chemical components of Hyssopus officinali L plants.

METHODS

This study was conducted during two successive seasons (2018/ 2019 and 2019/ 2020) in organic farm located in, Sharkia governorate, Egypt to study the effect of Micro-elements and their interaction on vegetative growth, oil production, and some chemical components of *Hyssopus officinali* L plants.

1. Layout of the first experiment

A randomized complete block design with three replicates was applied. The block contains six lines. Three concentrations (50, 100, and 150 ppm) of micro-elements (Zinc, Manganese, Magnesium, and iron) were randomly distributed.

The experiment was conducted on a total area of $120m^2$ plots, each containing a line of 20m long and 60cm wide, and on which replicates had an area of 3 meters. Each line was distanced from one another by 1m while the distance between plants (Spacing between holes) was 0.30m to be finalized with 20 cultivated plants in each replicate. Under these specifications, a feddan would contain 24000 plants.

2. Plant material and experimental procedures

Seeds of *Hyssopus officinalis* were obtained from the German-based company “Pharmasaat " PHARMASAAT Arznei- und Gewürzpflanzen Saatzucht GmbH Theseeds were sown in the nursery on September 15th and remained for 40 days until the seedlings were fit for transplanting in the permanent land. The soil was previously prepared by adding 10 tons/feddan of compost. The seedlings were transferred on October 26th to the permanent land which was appropriately prepared for cultivation. Plants reached 15cm in height at the beginning of January and most of them got to 25cm at the start of April.

The source of microelements is Future Chemicals Company, Egypt headquartered, in the Fourth Industrial Zone.

The micro-elements were purchased from Future Chemicals Company, the Fourth Industrial Egypt.

The first spraying of treatments took place on the 5th of January in when plants were two months after the transplantation whereas the second spray was applied on the 10th of April, before the flowering stage.

Preparation of spraying solutions

The concentrations of 50, 100, and 150 ppm of micronutrients were prepared by mixing 20L of tap water with 1, 2, and 3g of each micronutrient, respectively.

The soil analysis was carried out at the soil lab research, National Research Centre, Egypt, and the following data were obtained.

Table (1) Main characteristics of soil

Specification	Value
1-**Mechanical analysis**	
Sand %	81.8
Silt %	16.79
Clay%	1.23
Texture	Sand
2-**Chemical analysis**	
pH 1:2.5 Ext.	7.56
Ec 1:2.5dSm^{-1}	2.22
Soluble cations meq/ L	
Na^+	5.5
Ca^{++}	4.55
Mg^{++}	1.65
Soluble anions meq/ L	
$CO3^{-1}$	0.00
HCO^{-3}	3.49
Cl^{-1}	6.54
SO^{-4}	3.2
Available mg/Kg	
P	8.6
K	176
Total N	**96**

Table (2) Main characteristics of compost

pH (1:10)	8.33
EC (1:10)	2.79 dSm^{-1}
Ammonium Nitrogen	77.3 ppm
Nitrate Nitrogen	20.2ppm
Total Nitrogen%	0.69%
Organic Matter%	20.30%
Organic Carbon%	11.80%
Ash percentage%	79.70%
Organic Carbon: Nitrogen Ratio	17:01
Total phosphor%	0.16%
Total Potassium%	1.64%

Compost analysis was done in the soil analysis laboratory at Heliopolis University

The following growth parameters were recorded during both seasons:

1. During the growing seasons, the main plant stem's height was measured twice (90 and 120 days) from the date of sowing.
2. At two-time points, specifically 90 and 120 days after the date of sowing, the number of main lateral branches per plant were counted.
3 Measurement in grams per plant was done for the fresh and dry weight of the herb.
4. The flower's fresh and dry weights were measured in grams per (g/plant)
5. Aerial parts fresh and dry weight (g/plant).

Samples of herb, flowers, and aerial parts of The hyssop plant (*Hyssopus officinalis*) of each treatment were air-dried and then oven-dried at 40˚C to a constant weight. Actually the herb was dried, ground into powder and kept in a desiccator for further chemical analysis. The dried herb was then powdered and put in a canister ready to be used for chemical analysis (Ahmed (2018).

The following chemical components were determined after each season:

1. Chlorophyll A, B, and carotenoids (%):

Chlorophyll a, b, and total carotenoid contents were determined according to **the method of Moran and Porath (1980).**

2. Total carbohydrates (%):

The total carbohydrate percentage of each treatment was determined according to **Dubois *et al*. (1956).**

3. The percentage of volatile oil (%).

The hydrodistillation method using a Clevenger-type apparatus was used for the extraction of the air-dried plant materials for 3 hours. The obtained essential oil was kept in a firmly sealed vial at 4°C until they were analyzed **(Singh *et al* 2008).**

4. GC-MS analysis

The herb essential of promising treatments that produced the greatest vegetative growth production were analyzed using GC/Ms.

The GC-MS analysis of *H. officinalis* essential oil was carried out at Zn, Mg, Mn and Fe at 150 ppm treatments In the Department of Medicinal and Aromatic Plants Research at the National Research Center, we use a gas chromatography mass spectrometry instrument. It's a TRACE GC ULTRA GAS Chromatograph by THERMO Scientific Corp., USA, and it works with an ISQ Single Quadrupole Mass Spectrometer. This GC MS machine includes a TG 5MS column, with dimensions like 30 m in length, 0.25 mm internal diameter, and a film thickness of 0.25 µm. When we use it, we pick Helium as the carrier gas and keep a flow rate of 1.0 ml per minute. The split ratio marked is 1:10. The temperature settings variety is 80˚C for one minute, rises 4.0˚C per minute to 300 ˚C, and maintains for five minutes. The injector and detector were held at 240 ˚C. Diluted samples (1:10 hexane, v/v) of 0.3 µL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70ev, using a spectral range of m/z 35-500. Most of the compounds were identified using the analytical method: mass spectra (authentic chemicals, Wiley spectral library collection, and NSIT library) and confirmed with published data **(Adams, 2001).**

5. Statistical analysis

We used a process called "randomized complete blocks design." After that, we checked the data. We referenced Duncan's multiple range test (DMR) and the Costat program (version 6.4; CoHort Company, Birmingham, UK, 1998 – 2008). Cardinali and Nason explained these in 2013.

RESULTS

1. Growth characteristics

1.1 **Plant height (cm)**

Generally, in the first season, spraying of all micro-elements; Zn, Mg, Mn, and Fe, at all concentrations induced plant height especially the greatest concentration (150ppm) as the plant height reached 59.15, 64.34, 49.07, and 58.14cm, respectively, as compared to (38.76cm). The increment in plant height for all elements was concentration-dependent. It is observed that plant elongation recorded the greatest values with Mg at all concentrations.

Data illustrated in **Table (3.4)** show that all treatments produced significant increments in plant height compared to the control plants in both seasons. It was observed that plant height increment was concentration dependent as increasing the applied concentration of Mg from 50ppm to 150ppm significantly increased the height from 42.09 to 64.34cm in the first season and from 47.02cm to 69.92cm in the second season compared to control plants (38.76 and 43.79cm for the first and second seasons, respectively). Zn application induced plant height more than Mg as it reached 56.45 and 72.58 at the same two concentrations, respectively. Although Mn application enhanced plant height, the recorded increments showed lesser values (47.02 and 58.31cm for applications of 50 and 150ppm, respectively) as compared to Mg and Zn at the same concentrations. On the other hand, an significant difference was recorded between plant heights with application of Fe at 50 ppm (45.97cm) and control (43.79cm).

1.2 Number of branches/plants

The effect of foliar spraying with micro-elements on branches number of Hyssop plants is presented in a **Table (3).** In general, several branches were significantly induced and the effect was increased by increasing the applied concentration to reach the maximum number at the greatest concentration (150 ppm). At 50ppm and 100ppm of applied microelement produced nearly the same effect (28, 32, 30, and 34 for Zn, Mg, Mn, and Fe at 50ppm as well as 38.5, 38.5, 32.34 and 38.5 at 100ppm, respectively). On the other hand, the greatest efficacy was observed at 150 ppm (56.98, 48.89, 40.81, and 40.81 for Zn, Mg, Mn, and Fe, respectively). It is worth mentioning that the obtained results of the second season came at the same trend as the first season.

1.3 Herb fresh weight (g/ plant)

All applied treatments produced an increase in the herb's fresh weight compared to the control plants, **Table (3.4).** It is clear that zinc application at 150 ppm induced fresh weight in both seasons, as the value reached 160.77 and 170.93g/ plant In the initial and subsequent seasons, correspondingly.In contrast, iron application recorded the least mean herb fresh weight increments in the two seasons (134 and 130.03g/ plant at 150 ppm, respectively). Meanwhile, Mg at the same concentration produced the greatest fresh weight in the first season (160.77g) and the third level of herb weight induction in the second season (144.16g) as compared to the other treatments. It was observed that plants sprayed with 150 ppm manganese presented a significant increase in the fresh weight (156.53g) and came after zinc (170.93g).

1.4 Herb dry weight (g/ plant)

Application of treatments produced an increase in Hyssop dry weight compared to the control plants. According to **Table (3)** In the first season, treatments with zinc and magnesium at 150 ppm resulted in the highest dry weight of the hyssop plant, with both treatments showing the same value of 61.83g in the second season. These results indicate that the application of 150 ppm produced the highest dry weight. The dry weight trend were observed in the initial season was similar to that seen in the subsequent season.

1.5 Flower fresh weight (g/ plant)

Data presented in Table (3) showed the effect of different micro-elements treatments on the fresh weight of the *Hyssopus officinalis* flower. Application of Zn and Mg produced the same level of efficacy on flower fresh weight as they showed close results in the first season. Application of Mg at a concentration of 150 ppm produced the maximum fresh weight of the flower in the first season (43.98g/ plant). In agreement, Mg at the concentrations 50, 100 and150 ppm produced the highest fresh weight (63.60, 62.55, and 64.28), respectively in the second season with insignificant differences between Mg concentrations results **(Table,4)** compared with the control (28.18, 42.13) for Earlier seasons used shorter sentences with more variation compared to later ones. However, spraying of iron showed promising results in increasing the flower's fresh weight as its results came at the second level after magnesium (51.18, 53.88, and 58.20g/ plant for the three concentrations, respectively) in the second season.

1.6 Flower dry weight (g/ plant)

Data presented in **Table (3)** for the first season clearly indicated that all sprayed elements improved flower dry weight per plant concentration independently. Additionally, the application of Zn at all concentrations produced less value as compared to Mg, Mn, and Fe. It presented 12.27 g flower dry weight per plant at 150ppm whereas Mn was more effective than Zn in enhancing flower dry weight (13.27g/ plant) followed by iron which represented 14.03 g flower dry weight/ plant. It is worth mentioning that Mg was the most promising element for improving flower dry weight as it reached 15.32g at 150ppm in the first season.

In the second season, flower dry weight was generally improved as the control plants reached 11.39g/ plant as compared to the first season (9.82g/ plant). Furthermore, the application of all elements enhanced flower dry weight concentration independently. Magnesium application played as flower dry weight enhancer as it recorded 17.19, 16.90, and 17.37g /plant for the three applied concentrations, respectively, followed by an iron application (13.83, 14.56, and 15.73, respectively) then zinc application (12.85, 14.06 and 15.73g/ plant, respectively) and manganese (11.53, 12.66 and 13.7g/ plant, respectively).

1.7 Aerial parts fresh and dry weight (g)

The fresh weight of the aerial parts of the plant, which includes the herb and flower, exhibited a similar trend to the individual weights of the herb and flower. Overall, the fresh weight significantly increased with the concentration of all applied elements. The highest weight was recorded at 150 ppm for all elements, with the following effectiveness order: Mg > Zn > Mn > Fe. Specifically, zinc was the most effective inducer for fresh weight (229g/plant at 150 ppm), followed by Mg and Mn (208g/plant and 207g/plant at 150 ppm, respectively), and finally Fe (188g/plant at 150 ppm). Similarly, the dry weight of the aerial parts was significantly influenced by foliar application of Zn, Mg, Mn, and Fe in a concentration-dependent manner. The trend of dry weight mirrored that of fresh weight, with the element inducing the highest fresh weight also causing the greatest dry weight. The effectiveness order of elements on dry weight was consistent with that of fresh weight: Mg > Zn > Mn > Fe. Thus, the trend of results for aerial parts' dry weight paralleled that of fresh weight, with the effectiveness of elements arranged as follows: Zn > Mg and Mn > Fe

Table (3) Effect of Micro-nutrients on the growth and yield parameters of Hyssopus *officinalis L.* (the First season,2018/2019)

Treatments		Plant height (cm)	Number of branches	Flower fresh weight(g)	Flower heads Dry wt.(g)	Total plant fresh weight (g) shoot fresh weight + flower fresh weight	Total plant dry weight (g) (shoot dry w + flower dry)
Control	**0 ppm**	38.76h	28.875f	28.18g	9.82d	132.76i	50.04g
	50 ppm	52.95d	28.875f	31.80f	11.08d	153.71g	57.97f
Zn	**100 ppm**	55.58c	38.5c	34.38e	11.98bcd	176.22df	66.53d
	150 ppm	59.15b	56.98a	35.20de	12.27bcd	195.97b	74.10b
	50 ppm	42.09g	32.34e	37.99c	13.24abcd	161.92f	60.90e
Mg	**100 ppm**	58.14b	38.5c	39.95b	13.92abc	176.16d	66.31d
	150 ppm	64.34a	48.895b	43.98a	15.32a	204.75a	77.16a
	50 ppm	40.08h	30.415ef	32.00f	11.15cd	151.60h	57.15f
Mn	**100 ppm**	44.73f	32.34e	36.44cd	12.70abcd	176.84d	66.70d
	150 ppm	49.07e	40.81c	38.09c	13.27abcd	188.03c	70.94c
	50 ppm	40.08h	34.65d	36.75cd	12.81abcd	160.69f	60.47e
Fe	**100 ppm**	42.64g	38.5c	36.34cd	12.66abcd	165.33e	62.27e
	150 ppm	58.14b	40.81c	40.26b	14.03ab	175.17d	65.92d
L.S.D.(5%)		*1.31*	*1.3*	*1.5*	*1.69*	*1.66*	*1.66*

Table (4) Effect of Micro-nutrients on the growth and yield parameters of *Hyssopus officinalis L.* (2nd Season)

Treatments		Plant height (cm)	Number of branches	Flower fresh weight(g)	Flower heads Dry wt.(g)	Total plant fresh weight (g) t	Total plant dry weight (g))
Control	**0 ppm**	43.79j	26.18i	42.13f	11.39e	147.22h	45.96h
	50 ppm	56.45g	33fg	47.53e	12.85de	170.77f	53.52fg
Zn	**100 ppm**	65.89c	52.8b	54.01c	14.60bcd	195.73c	61.37c
	150 ppm	72.58a	73.92a	58.20b	15.73abc	229.13a	72.14a
	50 ppm	47.02hi	31.02gh	63.60a	17.19a	176.79e	54.54ef
Mg	**100 ppm**	60.48e	34.54f	62.55a	16.90ab	188.75d	58.56d
	150 ppm	69.92b	52.8b	64.28a	17.37a	208.44b	64.95b
	50 ppm	47.02hi	29.7h	42.67f	11.53e	164.96g	51.89g
Mn	**100 ppm**	49.19h	36.08ef	46.86e	12.66de	189.53d	59.75d
	150 ppm	58.31f	44.44d	50.91d	13.76cde	207.44b	65.42b
	50 ppm	45.97i	34.54f	51.18d	13.83cde	166.26g	51.81g
Fe	**100 ppm**	49.19h	38.06e	53.88c	14.56bcd	177.93e	55.50e
	150 ppm	63.15d	47.74c	58.20b	15.73abc	188.23d	58.64d
L.S.D. (5%) for microelement		*1.66*	*1.4*	*1.43*	*1.69*	*1.81*	*1.60*

Chemical analysis

2.1 Chlorophyll

2.1.1 Chlorophyll A (mg/g)

Efficacy of micro-elements at different concentrations (50, 100, and 150ppm) on the chlorophyll production in *Hyssopus officinalis* plants was investigated and obtained results were tabulated (Table,5) for the two cultivated seasons. Data in **Table (5)** show that treatment with Mg at a concentration of 150 ppm produced a significant increment in chlorophyll A content in both seasons (0.858 and 0.891mg/ g, respectively). In agreement, zinc treatment produced significant gradual increments in the production of chlorophyll A (0.48, 0.65, and 0.72mg/ g for the three concentrations, respectively) and came at the second efficacy level after Mg, the same trend was true for the second season. On the other hand, the application of 150ppm of manganese presented 0.62 mg/ g whereas the middle-applied concentration (100ppm) of Fe was more promising (0.65 mg/ g) than an application of 150ppm (0.63mg/ g).

2.1.2 Chlorophyll B (mg/g)

Data in **Table (5)** showed that application of Zn and Mg at concentrations of 150 ppm (0.67 and 0.70mg/ g, respectively) also Fe at 100 ppm (0.63mg/ g) led to a noteworthy significant increment in chlorophyll B compared to the other treatments and the control (0.23mg/ g) in the 1st season. It could be concluded that all treatments produced significant increments in chlorophyll B content above the control.

2.1.3 Carotenoids (mg/g)

Table (5) shows the results obtained for the response of micro-nutrient foliar application on carotenoid production in the *Hyssopus officinalis*. It is evident from the results that the trend of data was as follows; zinc was the promising treatment for the production of carotenoids (0.366 mg/ g with an application of 150 ppm) followed by the application of Mg (0.337mg/ g at 150ppm) as compared to the control or all other treatments in the first and second seasons whereas iron application which showed its best results with application of 100ppm (0.32mg/ g) and finally manganese application at the third level of effect in enhancing carotenoid production with application of 100ppm (0.298mg/ g). This trend was true for the second season also **(Table 5).**

Table (5) Effect of Micro-nutrients on (chlorophyll) and carotenoids percentage of *Hyssopus officinalis L*

Treatments		First season 2018/2019			Second season,2019/2020		
		Chlorophyll mg/ g f.w		Carotenoids mg/g f.w	Chlorophyll mg/ g f.w		Carotenoids mg/g f.w
		A	B		A	B	
Control	**0 ppm**	0.36e	0.23e	0.09k	0.30f	0.21j	0.11g
	50 ppm	0.478d	0.35d	0.1759h	0.3922e	0.312g	0.1151g
Zn	**100 ppm**	0.652c	0.3324d	0.1942g	0.6301c	0.374e	0.1674f
	150 ppm	0.7182b	0.6741a	0.3656a	0.6857b	0.549b	0.3121a
	50 ppm	0.483d	0.323d	0.1183j	0.358f	0.3561f	0.099h
Mg	**100 ppm**	0.7523b	0.5117b	0.2015f	0.6421c	0.301h	0.178e
	150 ppm	0.8586a	0.7026a	0.3377b	0.8911a	0.5361c	0.2541b
	50 ppm	0.477d	0.197f	0.131i	0.3661ef	0.1804k	0.1821e
Mn	**100 ppm**	0.6058c	0.4211c	0.2989d	0.6112cd	0.3712e	0.2321c
	150 ppm	0.6208c	0.326d	0.2635e	0.5845d	0.372e	0.1992d
	50 ppm	0.233f	0.3064d	0.0925k	0.3402f	0.2554i	0.1102g
Fe	**100 ppm**	0.653c	0.6397a	0.3177c	0.6042cd	0.5643a	0.3121a
	150 ppm	0.634c	0.5064b	0.0944k	0.6223c	0.4734d	0.2811c
L.S.D.(5%) for microelements		*0.0473*	*0.0548*	*0.0049*	*0.0267*	*0.0066*	*0.0811i*

2.2 Total Carbohydrates (%)

The presented data in **Table (6)** show that a concentration-dependent effect of micro-nutrients on total carbohydrate content was observed with application of micro-nutrients and the results trend was Mg > Zn > Fe > Mn at the first season whereas results showed Mg > Fe > Zn > Mn trend for the second season. It is evident from the results that the application of Mg at 150 ppm produced the greatest total carbohydrate percentage in the first and second seasons (9 and 8.22% at 150 ppm).

Table (6) Effect of Micro-nutrients on carbohydrate percentage of *Hyssopus officinalis* L. at harvest time for the two seasons.

Treatments		First season 2018/2019	Second season 2019/2020
		Total Carbohydrates (%)	Total Carbohydrates (%)
Control	0 ppm	21.80g	21.80m
	50 ppm	23.90e	23.22i
Zn	100 ppm	25.90d	25.71f
	150 ppm	27.50bc	26.99d
	50 ppm	23.80e	23.52i
Mg	100 ppm	28.10b	27.97b
	150 ppm	29.00a	28.22a
	50 ppm	22.50f	22.13 l
Mn	100 ppm	25.30d	24.88h
	150 ppm	26.90c	26.54e
	50 ppm	22.90f	22.99k
Fe	100 ppm	25.50d	25.01g
	150 ppm	27.50bc	27.11c
L.S.D. (5%) for microelements		0.50	0.09

2.3 Essential oil (%)

As the Hyssopus plant is considered a source of essential oil, the role of micro-nutrients in the production and composition of essential oil of herbs and flowers were investigated and the results of essential oil % are shown in Table (7). In the first season, zinc showed promising herb oil production at 150 ppm (0.51%) followed by iron (0.48% for herb) magnesium (0.34% for herb), and then manganese (0.32% for herb). In the second season, Oil production from the *Hyssopus officinalis* increased compared to the first season for all plants, including both the control and the treatment groups, and this trend remained consistent.Regarding the compositions of extracted essential oil, the essential of promising treatments that produced the greatest vegetative growth production were analyzed using GC/Ms and the results were tabulated in **Table (8).** It is clear from the obtained results that monoterpene hydrocarbons are the major compounds of herb essential oil of control plants and constituted 64.18% including five compounds, the major compound is trans-3- pinanone (49.21%), whereas oxygenated monoterpene constituted 27.63% with five compounds, the major oxygenated monoterpene is 2(10) -Pinene, (1S,5S) -(-)- (14.16%) followed by α-phellandrene (9.17%) it has occurred from results of essential oil that application of micro-nutrients exhibited a different effect on the composition of essential oil. For example, magnesium application induced sesquiterpene biosynthesis to reach 27.74% as compared to control (5.83%). Hydrocarbons sesquiterpene comprise 13.33% and the major sesquiterpene hydrocarbon is Elixene (5.17%) whereas the oxygenated sesquiterpenes comprise (14.41%) and the major oxygenated sesquiterpene is Hedycaryol (5.79%).

On the contrary, iron application at 100 ppm induced the production of monoterpenes (92.29%) and especially the oxygenated monoterpenes (72.39) with major compound cis-3-pinanone (35.61%) more than hydrocarbons (20.23%) with major compound 2(10) -Pinene, (1S,5S) -(-)- (9.9%) as compared to control plants, moreover, it showed less induction for sesquiterpenes (7.71%) with major hydrocarbon β-Bourbonene (1.33%). In accordance, application of iron at 150 ppm showed the same trend of results of 100 ppm as it induced biosynthesis of monoterpenes (94.53%) especially oxygenated compounds (58.25%, the major compound is trans-3-pinanone; 48.34%) than hydrocarbons (36.28%, the major compound is 2(10) -Pinene, (1S,5S) -(-)- (21.09%).

On the other hand, zinc application at 150 ppm induced the production of sesquiterpene (10.20%) with major compound Germacrene (6.56%) whereas the mono terpene was insignificantly decreased to reach 89.79% as compared to control. Six oxygenated monoterpenes were detected and the major compound is trans-3-pinanone (49.21%) whereas five monoterpene hydrocarbons were detected and the major compound is 2(10) -Pinene, (1S,5S) -(-)-(17.77%).

Furthermore, the application of manganese at 150 ppm keeps the biosynthesis rate of monoterpene (91.97) close to control (91.81%) with major compounds trans-3-pinanone (41.66%) as oxygenated nature and 2(10) -Pinene, (1S,5S) -(-)- (22.98%) as hydrocarbon. Additionally, the production of sesquiterpene was induced to reach 8.03%, the major compound is β-Eudesmol (0.95).

In conclusion, the application of magnesium to hyssop plants promoted the biosynthesis of sesquiterpenes (27.74%) at the expense of monoterpenes (72.30%) whereas, application of iron at 100 ppm encouraged the biosynthesis of monoterpenes (92.29%) more than sesquiterpenes (7.71%) which was more promoted with increasing the applied concentration to 150 ppm to reach 94.53% for monoterpenes and 5.47% for sesquiterpenes. On the other hand, zinc application enhanced the biosynthesis of sesquiterpenes (10.20%) at the expense of monoterpene (89.79%). Moreover, slight induction of sesquiterpenes (8.08%) was recorded with the application of manganese at 150 ppm and monoterpenes remained at the same concentration with the same major compounds of control.

The obtained results showed that application of Zn, Mg, Fe and Mn significantly improved the agronomic characters of *Hysoppus officinalis*, e.g. plant height and weight, flowering, branching and . The results of our experiment showed similar effects of Zn, Mg, Mn, and Fe on vegetative characteristics such as, when the plant height was measured, we discovered that the zinc treatments at 50 ppm and 150 ppm concentrations were the best results when compared to the control and the other treatments. For the number of branches,our results recorded the highest measurements when all elements were treated (Zn, Mg, Mn and Fe) at a concentration of 150 ppm.The Our results also showed that the zinc and magnesium treatments at 150 ppm produced the greatest dry weight of the hyssop plant and these results are in line with previous experiments. Our results also showed that zinc at a concentration of 150 ppm gave the highest plant fresh weight, and this is in line with previous experiments. The positive effects of these elements on the plant height and weight of several plants were proved by many investigators **Yadegari 2017** on lemon balm plants.

DISCUSSION

Table (7) Effect of Micro-nutrients on essential oil percentage of *Hyssopus officinalis* L. at harvest time for the two seasons.

		First season 2018/2019	second season 2019/2020
Treatments		**Herb oil %**	**Herb oil %**
Control	**0 ppm**	0.251i	0.275k
Zn	**50 ppm**	0.265h	0.299i
	100 ppm	0.359e	0.372e
	150 ppm	0.505a	0.605a
Mg	**50 ppm**	0.294g	0.341g
	100 ppm	0.325f	0.392d
	150 ppm	0.339e	0.448c
Mn	**50 ppm**	0.264h	0.280jk
	100 ppm	0.263h	0.285jk
	150 ppm	0.320f	0.318h
Fe	**50 ppm**	0.295g	0.293ij
	100 ppm	0.385c	0.351f
	150 ppm	0.488b	0.554b
L.S.D.(5%) for micro- elements		*0.008*	*0.009*

Other results emphasized the promotive effect of these elements on rhizome essential oil of Japanese mint (Misra and Sharma, 1991). Generally, foliar fertilizer as Fe, Cu, Mn and Zn at 400 ppm resulted in highest improvement of growth character, yield and chemical constituents. On coriander, it was recorded that foliar application of Fe produced higher essential oil content followed by Zn (Yadegari et al 2015). Mazaheri et al. (2013) stated that spraying of Zn+Mn caused a significant increase in the essential oil yield of cumin in contrast to the control, A similar effect of micronutrient supply on this parameter was also reported on M. chamomilla (Nasiri et al. 2010), Coriandrum sativum (Said-Al Ahl and Omer, 2009), Mentha sp (Rawia et al. 2010) . It was reported by Askary et al. (2017) that Chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, and protein contents increased significantly under Fe2O3 nanoparticles treatment.

Table (8): The main essential oil constituents of *Hyssopus officinalis* L. as affected by micro-nutrients application

Rt (min.)	common name	Control	Mg at 150ppm	Fe at 150 ppm	Zn at 150 ppm	Mn 150 ppm	Chemical formula
3.87	α-Thujene	--	--	0.4	--	0.23	C10H16
4.04	α-Pinene	0.72	--	1.22	0.66	1.06	C10H16
4.98	Sabinene	2.44	0.82	3.74	2.29	0.27	C10H16
5.14	2(10) -Pinene, (1S,5S) -(-)-	14.16	5.39	21.09	17.77	22.98	C10H16
5.39	β-Pinene	1.14	0.89	1.53	1.23	1.68	C10H16
6.61	D-Limonene	--	--	--	--	--	C10H16
6.7	α-Phellandrene	9.17	13.13	8.3	7.22	16.53	C10H16
7.62	ϒ-Terpinene	--	--	--	--	0.24	C10H16
9.18	Linalool	--	0.57	0.28	0.45	--	C10H18O
10.78	2(10)-Pinen-3-ol, (1S,3R,5S)-(-)-	0.23	--	--	--	--	C10H16O
11.24	6-Isopropenyl-3-methoxymetoxy-3-methyl-cyclohexene	6.49	5.69	8.21	6.98	5.2	C12H20O2
11.66	3-Pinanone, cis	7.48	1.19	1.26	1.46	2.04	C10H16O
11.75	Pinocarvone	--	--	--	8.16	--	C10H14O
12.46	trans-3-Pinanone	49.21	44.62	48.34	42.50	41.66	C10H16O
13.2	Myrtenal	0.77	--	0.16	1.07	--	C10H14O
20.8	(-)-β-Bourbonene	0.33	0.99	1.0	0.83	2.44	C15H24
21.68	Isocaryophillene	--	--	--	--	--	C15H24
22.35	Caryophyllene	--	2.04	0.55	1.1	0.81	C15H24
23.93	Humulene	--	1.31	0.24	0.6	0.39	C15H24
24.1	Alloaromadendrene	0.36	0.72	--	--	0.39	C15H24
25.06	Germacrene D	1.1	3.13	0.77	1.97	1.6	C15H24
25.42	(+)-Ledene	--	--	--	--	0.24	C15H24O
25.66	Elixene	1.81	5.14	0.74	2.06	0.36	C15H24
28.06	Hedycaryol	2.45	5.79	1.16	1.67	0.44	C15H26O
29.17	(-)-Spathulenol	0.68	0.46	0.18	--	--	C15H24O
31.41	γ-eudesmol	--	--	--	0.58	0.46	C15H26O
31.86	tau.-Cadinol	--	0.58	--	--	--	C15H26O
32.36	β-Eudesmol	0.51	1.15	0.27	0.82	0.95	C15H26O
33.06	Elemol	0.48	3.12	0.56	0.57	--	C15H26O
35.95	Bisabolol oxide A	--	3.28	--	--	--	C15H26O2
Total of Monoterpens		91.81	72.3	94.53	88.72	91.89	
Oxygenated monoterpens		64.18	52.07	58.25	59.55	48.9	
Non oxygenated monoterpens		26.63	19.23	36.28	29.17	42.99	
Total of Sesquiterpens		7.72	27.71	5.47	10.2	8.08	
Oxygenated Sesquiterpens		4.12	14.38	2.17	3.64	2.09	
Non oxygenated Sesquiterpens		3.6	13.33	3.3	6.56	5.99	
Totalof identified compounds		99.53	100.01	100	98.92	99.97	

Conclusion

In conclusion, the two-year study investigating the impact of micro-elements (Zn, Mg, Mn, and Fe) on foliar spraying the agronomic and chemical characteristics of *Hyssopus officinalis* L. demonstrated that foliar application of these micro-elements plays pivotal role in enhancing various growth parameters; plant height, branch, as well as fresh and dry weights of herb and flowers, the effect was concentration dependent. In addition, magnesium showed notable increases in plant height, branching, and herbs fresh weight whereas. Zinc application enhanced flower fresh weight and stimulating the accumulation of essential oil. On the other hand, Iron exhibited concentration-dependent effect on monoterpenes and sesquiterpenes biosynthesis. Furthermore, micro-nutrients induced accumulation of chlorophyll, carotenoid and total carbohydrate.

List of Abbreviations

Key	Description
Zn	Zinc
Mg	magnesium
Mn	Manganese
Fe	Iron
ppm	Parts per million
mg	one thousandth (1/1,000) gram
PH	potential hydrogen
EC	electrical conductivity
ml	milliliter
µm	micrometer

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