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Role of some Micronutrients in Alleviating Water Stress and Enhancing Productivity of Marjoram Plants Grown in Sandy Soil

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ABSTRACT

This study examines the relationship between foliar application of two micronutrients, i.e., Fe or Mn (at rates 0, 100, 200, and 300 ppm) and different water stress levels (100% as control, 80 and 60% of ETo) during two seasons of 2022 and 2023. Water stress had a negative effect on growth characteristics and yield composition. Foliar application of Fe and Mn showed a positive effect on plant productivity as well as alleviating water stress. Spraying Fe or Mn (300 ppm for each) at 80% ETo led to increased plant height by 49.80 and 52.54%, number of branches by 52.08 and 65.43%, herb yield 25.40 and 29.38%% and oil yield 36.08 and 30.58%, respectively, compared with non-treated plants. Water stress and micronutrients have a positive effect on the antioxidant activity (DPPH%) of the essential oil. GC-MS analysis of essential oil showed that the monoterpenoid composition of marjoram was affected by water stress level, but the effects may have been indirect via an alteration in development rate. Increasing water stress initiated a reduction of monoterpene or sesquiterpene hydrocarbons and accelerated the transformation of hydrocarbons into oxygenated components.It could be concluded that foliar application of marjoram plants with Fe or Mn at 300 ppm obtained the optimal characteristics of marjoram plants under experimental conditions. On the other hand, to obtain high oil content with high antioxidant activity of essential oil it could be recommended to irrigate marjoram plants with 80% ETo and 300 ppm of Mn as foliar application.

Keywords: marjoram, water stress, micronutrients, GC–MS, DPPH %, essential oil.

INTRODUCTION

Sweet marjoram (*Majorana hortensis*), is an annual herb that is indigenous to tropical and warm temperate regions. It can also be found growing wild in Mediterranean countries. It is grown all over the world in a range of ecological conditions and is a member of the Lamiaceae family (Guenther, 1972). It has been used for centuries as an aromatic and medicinal plant. Marjoram herb is known to possess stimulant, antibacterial, antiviral, anti-spasmodic, anti-diabetic, anti-mutagenic and antioxidant properties (Marrelli *et al*., 2018). Marjoram's secondary metabolites, such as its herbal extract and essential oil, are primarily responsible for its medicinal value (Robya *et al*., 2013). Marjoram cultivation has rapidly increased in recent years because of its high standard of quality and conservation of natural resources. The production of phytopharmaceuticals in medicinal and aromatic herbs is strongly influenced by ecological and hereditary factors, as well as their interactions (Mohasseli and Sadeghi, 2019; Farouk and Omar, 2020).

Water stress is becoming the main ecological factor that is negatively affecting the sustainability of medicinal plant production worldwide (Farouk and Omar, 2020; Shehzad *et al*., 2020). Prolonged water stress can change a number of physio-biochemical and physicochemical processes, as well as the allocation patterns of photosynthetic materials and molecular responses (Farooq *et al*., 2020; Rady *et al*., 2020). Furthermore, water stress leads to the excess production of reactive oxygen species, which damages plant cells frequently and affects a number of molecular and biochemical functions (Farooq *et al*., 2020; Farouk and Omar, 2020). Under oxidative stress caused by water stress, plants have a defense strategy to eliminate reactive oxygen species through induction of enzymatic and non-enzymatic antioxidant defense enzymes, which protect the membranes and other vital substances (Farouk and Omar, 2020; Shehzad *et al*., 2020). These endogenous anti-drought compounds are insufficient to allow stressed crops to withstand prolonged drought; therefore, the effective induction of drought tolerance requires the external application of substances that induce stress tolerance (Farouk and Al-Huqail 2020).

Plants need small amounts of nutrients named micronutrients. These trace elements are necessary for plants and a deficiency in them decreases crop productivity. Micronutrients can stimulate specific physiological, biochemical, and metabolic processes under drought stress. Plant susceptibility to stress factors can be influenced by micronutrients either directly or indirectly through altering enzyme activity, modifying signal transduction pathways and/or generating specific metabolites (Hajiboland, 2012). Among the significant trace elements is iron and manganese.

Iron (Fe) is one of the important trace elements. It plays an important role in plant metabolic processes such as photosynthesis, respiration, ROS scavenging, osmoregulation and nitrogen assimilation. It plays an essential and significant role in many different enzymes; in particular, enzymes involved or help in process of respiration, which includes peroxidase, catalase and cytochrome oxidase. Fe is directly involved in the oxidation and reduction processes as well. Additionally, it is very critical in the chlorophyll synthesis and involved in the production of chlorophyll pigment molecules

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(Nikolic and Kastori. 2000). Fe nutrition plays a critical role in protecting plants from drought-induced oxidative stress (Abadia *et al.*, 1999).

Manganese (Mn) a significant trace element, works as an activator for various enzymes involved in a range of biological processes, including oxidation, reduction, decarboxylation and hydrolytic reactions in plants (Taiz *et al.*, 2015). Manganese plays a role in photosynthetic enzymes and respiration (Schmidt and Husted, 2019) and prohibits nitrate accumulation in plant tissues (Zarabimafi and Pour, 2014). Manganese application improving salt-stress tolerance of plants by enhancing their antioxidant defense, ion homeostasis, and glyoxalase systems (Rahman *et al.*, 2016). In a number of crops, Mn helps to improve their ability to tolerate salt stress (Ye *et al.*, 2020).

There is a scarcity on information on how micronutrients alleviate the effect of water stress levels. The objective of this research was to evaluate the role of Fe and Mn foliar application to maximize yield and productivity of *Majorana hortensis* plants grown in sandy soil.

MATERIALS AND METHODS

The site and climatic conditions

The current experiment was performed at Aly Mobarak Experimental Farm, Horticulture Research Station, El-Bustan area, El-Behiera Governorate, Egypt, during 2022 and 2023. The meteorological data of experimental site is presented in table (1). Some physical and chemical properties data of the experimental soil is presented in table (2).

Table 1. Meteorological data of experimental site during the two seasons.

Month		Season 2022								
		Feb.	**Mar.**	**Apr.**	**May**	**June**	**July**	**Aug.**	**Sep.**	**Oct.**
Temperature	Max	19.33	23.76	25.35	32.64	33.85	34.98	35.73	30.44	26.12
	Min	10.56	14.09	11.08	15.62	19.79	21.02	21.30	21.11	14.19
RH-AVG%		57.87	54.25	47.58	37.2	47.84	47.82	51.88	53.76	56.88
Wind speed (m/sec)		2.560	2.98	3.180	1.590	1.570	1.690	1.550	1.820	2.370
Radiation ($MJ/m^{2)}$)		12.63	17.34	22.24	25.41	26.58	27.49	26.53	20.37	17.36
Et_0 mm day^{-1}		3.220	4.150	4.340	6.100	6.320	6.440	6.510	5.250	4.090
		Season 2023								
Temperature	Max	21.46	26.37	28.14	36.23	37.57	38.83	39.66	33.79	28.99
	Min	11.40	15.22	11.97	16.87	21.37	22.70	23.00	22.80	15.33
RH-AVG%		58.50	55.59	49.39	39.18	48.67	49.65	53.03	53.06	54.43
Wind speed (m/sec)		2.760	3.220	3.270	1.720	1.700	1.830	1.670	1.970	2.560
Radiation ($MJ/m^{2)}$)		13.64	18.73	24.02	27.44	28.71	29.69	28.65	22.00	18.75
Et_0 mm day^{-1}		3.480	4.480	4.690	6.590	6.830	6.960	7.03	6.670	4.420

Table 2. Some physical and chemical properties of the experimental soil

Physical properties			
Soil layer depth (cm)	00-20	20-40	40-60
Texture	Sandy	Sandy	Sandy
Course sand (%)	48.66	55.71	37.76
Fine sand (%)	48.83	40.58	58.43
Silt+ clay (%)	2.51	3.71	3.81
Field Capacity, (%)	13.0	13.3	10.1
Wilting Point, (%)	4.6	4.5	4.4
Available water, (%)	8.3	8.1	8.0
Bulk density (t m^{-3})	1.69	1.68	1.67
Chemical properties			
$EC_{1:5}$ (dS m^{-1})	0.45	0.53	1.00
pH (1:2.5)	8.60	8.70	9.32
Total $CaCO_3$ (%)	7.00	2.34	4.66

Plant materials and experimental design

The seeds of marjoram were obtained from the Medicinal and Aromatic Plants Research Farm, El-Kanater el-khairia, El-Kalubia Governorate, Egypt. The seeds were sown in the nursery bed on February for both seasons. After 45 days, uniform seedlings of 10-15 cm in an average height with 3 pairs of leaves were transplanted to the soil prepared by adding manure at rate (20 m^3) and calcium super phosphate (300 kg/fed.), while ammonium sulphate (300 kg/fed.) and potassium sulphate (100 kg/fed.) were added during the season. All agricultural practices were applied according the Ministry of Agriculture.

Experiment treatments

Water treatments

Three irrigation treatments were calculated as a percentage of irrigation requirements as the following:

- 100% of ETo (2000 and 2400 m^3/fed. in the first and second seasons respectively) as control,
- 80% of ETo (1600 and 1920 m3/fed. in the first and second seasons respectively) and
- 60% of ETo (1200 and 1440 m3/fed. as in the first and second seasons respectively).

Evapotranspiration (ETo) mm/day was calculated by Penman Monteith equation (Allen *et al.*, 1998) using the climatologically data of El-Bostan area. Drip irrigation system was used with a flow rate of 4 liters/h. Once the plants were established, irrigation treatments were initiated at 2-day intervals.

Micronutrient application

Fe and Mn (EDTA-Chelated form, 8.5 and 14%, respectively) as foliar application at rates 100, 200, and 300 ppm were conducted three times during both seasons. The first dose was sprayed after four weeks from planting. The second was after three weeks from the first one. The third spray was implemented after four weeks from the first cut. On the other hand, the untreated plants which sprayed with water were used as control. The experiment was designed in a split plot arrangement with three replicates, the irrigation levels were located as main plots and micronutrient rates were located as sub- plots.

Harvesting

The two cuts of marjoram herbs for each treatment were obtained on June and September by cutting the aerial parts at 10 cm above the soil surface during every season of the two experimental seasons.

Recorded data

Vegetative growth Characteristics

At each cut during the two seasons, plant height (cm), number of branches per plant, the weight of fresh herbs of plant was recorded and the yield of herb fresh weight per fed. was calculated. The herbs were washed and air dried, so, it was dried in the oven at 70 °C until constant weight was obtained. Then, the dry weight of each herb was recorded and the yield of herb dry weight per fed. was calculated.

Determination of essential oil and its components

Samples of fresh herb was accurately weighed and subjected to hydrodistillation with sterile water for 3 h using a Clevenger-type apparatus as reported by Erich (1982). The percentage of oils calculated and then the oil content per plant and yield of volatile oil per fed. were calculated. Furthermore, the volatile oil major compounds (in second cut of second season) were determined by GC-MS analysis, according to the program described by Chamorro *et al*., (2008).

Determination of Antioxidant activity of essential oil

Free radical scavenging activity of Marjoram oil was spotted using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as a source of free radicals was carried out as described previously by Burits and Bucar (2000).

Statistical analysis

The data recorded on the tested characteristics were subjected to an analysis of variance (ANOVA) as a factorial experiment in split plot design, and Least Significant Difference (L.S.D) test at the 0.05 level was used to compare the differences between means of the treatments according to Steel *et al*., (1997).

RESULTS AND DISCUSSION

Vegetative growth characteristics

The effects of irrigation levels and the rates of Fe or Mn as foliar spraying as well as their interactions on some vegetative growth parameters such as plant height and branches number of marjoram per plant for both cuts during two seasons were shown in tables 3 and 4. The results clearly showed that, decreasing irrigation levels resulted in a significant decrease in the investigated traits. The maximum reduction was obtained from plants irrigated with 60% followed by 80% as compared with well-watered plants in both cuts during the two seasons. Foliar application of Fe or Mn at the various rates of 100, 200, and 300 ppm caused a significant increase in the plant height and branches number as compared with the untreated plants. The highest foliar application rate of Fe and also Mn (300 ppm) led to increase in the plant height, from average of 13.33 to 68.35 % (for the first and second cuts respectively) in the plant irrigated with 80% ETo and about 28.94 (for the 1st cut) to 55.35% (for the 2nd cut) in the plant irrigated with 60% of ETo. In terms of number of branches increased by average of 28.22 (for the first cut) to 60.14 % for the second cut in plant irrigated with 80% ETo and 59.31 to 85.62% for the first and second cuts respectively in the plant irrigated with 60% of ETo compared with untreated plant at the same level of irrigation. These results may be attributed to application of Fe or Mn which increased plant height, number of branches and other morphological traits. This is due to Fe and Mn plays a crucial role in regulating several metabolic activities, Said-Al Ahl and Mahmoud (2010) mention that on basil plant. Saini and Singh (2017) found that foliar spray of Fe was promote plant height, branch per plant on *Vigna radiata* plant. While Emam (2020) found that Mn gave more growth parameter on *Linum usitatissimum* plant.

Table 3. Effect of different irrigation levels and micro elements Fe or Mn rates on plant height of sweet marjoram.

Micro element rates (M) (ppm)	First season (2022)							
	First cut				Second cut			
	Irrigation levels (I)							
	100%	80%	60%	Mean	100%	80%	60%	Mean
Control	34.66	30.00	25.33	30.00	22.42	15.80	14.37	17.53
Fe (100)	36.33	31.00	30.66	32.66	26.80	20.41	16.99	21.40
Fe (200)	40.00	33.50	31.33	34.94	28.53	22.85	19.52	23.63
Fe (300)	42.33	34.00	32.66	36.33	31.43	25.38	21.96	26.26
Mn (100)	36.33	32.66	27.00	32.00	25.61	23.54	18.35	22.50
Mn (200)	38.72	34.00	31.00	34.57	30.11	25.72	20.83	25.55
Mn (300)	40.53	35.66	33.50	36.56	31.49	26.60	22.13	26.74
Mean	38.41	32.97	30.21		28.06	22.90	19.16	
LSD (0.05)								
I		0.96				0.89		
M		1.35				1.28		
I X M		2.34				2.29		
	Second season (2023)							
Control	35.49	30.75	26.02	30.75	24.08	21.52	16.55	20.72
Fe (100)	37.75	32.22	30.09	33.35	25.61	23.54	20.33	23.16
Fe (200)	39.95	34.42	31.11	35.16	27.84	25.72	24.11	25.89
Fe (300)	44.00	36.00	34.25	38.08	31.49	27.93	25.09	28.17
Mn (100)	34.5	32.13	30.59	32.41	29.30	24.81	21.21	25.11
Mn (200)	38.33	35.05	34.45	35.94	30.55	27.90	24.33	27.59
Mn (300)	46.56	37.15	35.41	39.71	32.01	28.11	25.71	28.61
Mean	39.51	33.96	31.70		28.70	25.65	22.48	
LSD (0.05)								
I		1.43				1.25		
M		2.19				1.92		
I X M		3.79				3.27		

Table 4. Effect of different irrigation levels and micro elements, Fe and Mn rates on number of branches of sweet marjoram.

Micro element rates (M) (ppm)	First season (2022)							
	First cut				Second cut			
	Irrigation levels (I)							
	100%	80%	60%	Mean	100%	80%	60%	Mean
Control	22.25	15.83	13.06	17.05	28.11	24.55	18.44	23.70
Fe (100)	23.51	20.23	17.35	20.36	31.00	27.33	20.33	26.22
Fe (200)	25.27	24.30	20.41	23.33	34.66	30.66	24.33	29.88
Fe (300)	30.78	24.44	24.25	26.49	38.00	33.50	27.00	32.83
Mn (100)	28.11	20.78	25.34	24.74	34.00	29.60	22.50	28.70
Mn (200)	27.50	23.55	21.74	24.26	37.00	32.66	26.50	32.05
Mn (300)	31.30	25.35	23.49	26.71	41.50	35.00	29.00	35.17
Mean	26.96	22.07	20.81		34.90	30.47	24.01	
LSD (0.05)								
I		1.36				2.10		
M		2.07				3.20		
I X M		3.59				5.55		
	Second season (2023)							
Control	23.05	18.88	14.18	18.70	30.05	24.88	19.18	24.70
Fe (100)	25.57	23.25	18.34	22.39	32.57	27.25	21.34	27.05
Fe (200)	27.70	25.95	20.20	24.62	36.70	31.66	24.20	30.85
Fe (300)	30.62	27.13	22.59	26.78	39.44	31.90	29.59	33.64
Mn (100)	26.95	23.11	19.11	23.06	33.17	28.33	22.66	28.05
Mn (200)	28.03	25.40	21.41	24.95	38.45	31.40	25.33	31.73
Mn (300)	32.11	29.41	24.15	28.56	41.50	32.33	28.90	34.24
Mean	27.72	24.73	20.00		35.98	29.68	24.46	
LSD (0.05)								
I		1.42				1.89		
M		2.16				2.89		
I X M		3.74				5.01		

Yield parameters

Fresh herb yield (Herb yield)

Biomass accumulation exhibited to different water stress levels and micronutrients rates were recorded and presented in tables 5 and 6. The obtained results showed that, increasing water stress caused a significant decrease in fresh herb yield, the maximum reduction was obtained by 60% ETo. Foliar application of microelements Fe and Mn have a significant influence on marjoram biomass accumulation was observed in first and second cut at both seasons. Fe and also Mn at 300 ppm rate showed the highest effect on fresh herb yield. The maximum results obtained in the fresh herb yield on the plants irrigated by 80% ETo and spraying with Mn at 300 ppm rate by more than 99.11% as compared with the untreated plants in second cut at second season. The improvement in growth parameters and herb yield by micronutrients application might exist as a result of enhanced photosynthetic and other metabolic activities which cause an increase in different plant metabolites responsible for cell division and elongation (Hatwar *et al.*, 2003).

Table 5. Effect of different irrigation levels and micro elements, Fe and Mn rates on fresh herb yield/ plant (g) on sweet marjoram.

Micro element rates (M) (ppm)	First season (2022)							
	First cut				Second cut			
	Irrigation levels (I)							
	100%	80%	60%	Mean	100%	80%	60%	Mean
Control	61.70	50.69	42.55	51.65	95.40	76.39	47.07	72.95
Fe (100)	63.60	58.76	46.71	56.36	110.74	90.04	58.25	86.34
Fe (200)	68.73	60.33	53.97	61.01	124.59	108.22	73.53	102.11
Fe (300)	70.11	62.93	55.47	62.84	145.44	110.01	87.53	114.33
Mn (100)	65.35	55.11	50.55	57.00	110.63	95.11	60.87	88.87
Mn (200)	72.44	62.15	54.44	63.01	131.55	111.24	76.29	106.36
Mn (300)	75.35	63.55	54.41	64.44	155.04	114.59	90.15	119.93
Mean	68.18	59.07	51.16		124.77	100.80	70.53	
LSD (0.05)								
I		2.23				5.22		
M		3.4				7.97		
I X M		5.90				13.81		
	Second season (2023)							
Control	62.95	52.23	45.86	53.68	99.41	60.55	57.71	72.56
Fe (100)	66.68	54.66	47.76	56.37	115.49	75.43	70.43	87.12
Fe (200)	70.53	57.00	50.93	59.49	130.90	91.39	85.91	102.73
Fe (300)	80.85	60.73	56.20	65.93	150.55	110.56	90.41	117.17
Mn (100)	67.41	53.11	48.46	56.33	125.16	88.52	80.22	97.97
Mn (200)	73.43	55.51	52.34	60.43	138.59	95.55	85.35	106.50
Mn (300)	84.55	63.11	60.41	69.36	159.17	120.55	95.41	125.04
Mean	72.34	56.62	51.71		131.32	91.79	80.78	
LSD (0.05)								
I		2.24				5.62		
M		3.49				8.59		
I X M		5.92				14.88		

Table 6. Effect of different irrigation levels and micro elements, Fe and Mn rates on herb fresh yield/fed. (t.) on sweet marjoram.

Micro element rates (M) (ppm)	First season (2022)							
	First cut				Second cut			
	Irrigation levels (I)							
	100%	80%	60%	Mean	100%	80%	60%	Mean
Control	1.17	0.96	0.81	0.98	1.81	1.45	0.89	1.39
Fe (100)	1.21	1.12	0.89	1.07	2.10	1.71	1.11	1.64
Fe (200)	1.31	1.15	1.03	1.16	2.37	2.06	1.40	1.94
Fe (300)	1.33	1.20	1.05	1.19	2.76	2.09	1.66	2.17
Mn (100)	1.24	1.05	0.96	1.08	2.10	1.81	1.16	1.69
Mn (200)	1.38	1.18	1.03	1.20	2.50	2.11	1.45	2.02
Mn (300)	1.43	1.21	1.03	1.22	2.95	2.18	1.71	2.28
Mean	1.30	1.12	0.97		2.37	1.92	1.34	
LSD (0.05)								
I		0.04				0.10		
M		0.06				0.15		
I X M		0.11				0.26		
	Second season (2023)							
Control	1.20	0.99	0.87	1.02	1.89	1.15	1.10	1.38
Fe (100)	1.27	1.04	0.91	1.07	2.19	1.43	1.34	1.66
Fe (200)	1.34	1.08	0.97	1.13	2.49	1.74	1.63	1.95
Fe (300)	1.54	1.15	1.07	1.25	2.86	2.10	1.72	2.23
Mn (100)	1.28	1.01	0.92	1.07	2.38	1.68	1.52	1.86
Mn (200)	1.40	1.05	0.99	1.15	2.63	1.82	1.62	2.02
Mn (300)	1.61	1.20	1.15	1.32	3.02	2.29	1.81	2.37
Mean	1.37	1.08	0.98		2.50	1.74	1.53	
LSD (0.05)								
I		0.04				0.11		
M		0.08				0.16		
I X M		0.11				0.28		

Dry herb yield

Data regarding dry herb yield (g) per plant and yield of dry herb (ton) per fed. under irrigation levels, microelements as foliar spraying and their interaction are illustrated in Tables 7 and 8. Data showed that, the dry matter yield was exhibited similar trends to fresh herb yield. Increasing deficit irrigation treatments decreased dry matter yield. The greatest reduction was obtained from plants irrigated by 60% ETo registered 46.77% deficiency on marjoram dry yield /fed. followed by 80% ETo registered 25.81% deficiency on marjoram dry yield /fed. as compared with well-watered plants, in first cuts during second seasons. However, microelements treatments led to enhancing dry matter yield.

Increasing of Fe and Mn concentrations from 100 to 300 ppm was capable of enhancing marjoram yield /fed. Results revealed that Mn (300ppm) was the most beneficial treatment on dry matter yield (the increase was move closer 96.55% as compared with the control in second cut at second season) followed by Fe at 300ppm rate gave increased by 82.76% as compared with untreated plant in second cut at second season.

Table 7. Effect of different irrigation levels and micro elements, Fe and Mn rates on dry herb yield/plant (g) on sweet marjoram.

Micro element rates (M) (ppm)	First season (2022)							
	First cut				Second cut			
	Irrigation levels (I)							
	100%	80%	60%	Mean	100%	80%	60%	Mean
Control	16.04	12.17	10.21	12.81	24.80	18.33	11.77	18.30
Fe (100)	16.54	14.10	11.21	13.95	28.79	21.61	14.56	21.65
Fe (200)	17.87	14.48	12.95	15.10	32.39	25.97	18.38	25.58
Fe (300)	18.23	15.10	13.31	15.55	37.81	26.40	21.88	28.70
Mn (100)	16.99	12.95	12.13	14.02	28.76	22.35	14.61	21.91
Mn (200)	18.83	14.92	13.07	15.61	34.20	26.70	18.31	26.40
Mn (300)	19.59	15.25	13.06	15.97	40.31	27.50	21.64	29.82
Mean	17.73	14.14	12.28		32.44	24.12	17.31	
LSD (0.05)								
I		0.56				1.28		
M		0.86				1.96		
I X M		1.48				3.39		
	Second season (2023)							
Control	15.11	13.06	11.92	13.36	23.86	15.14	15.00	18.00
Fe (100)	16.00	13.67	12.42	14.03	27.72	18.86	18.31	21.63
Fe (200)	16.93	14.25	13.24	14.81	31.42	22.85	22.34	25.53
Fe (300)	19.40	15.18	14.61	16.40	36.13	27.64	23.51	29.09
Mn (100)	16.18	13.28	12.60	14.02	30.04	22.13	20.86	24.34
Mn (200)	17.62	13.88	13.61	15.04	33.26	23.89	22.19	26.45
Mn (300)	20.29	15.78	15.71	17.26	38.20	30.14	24.81	31.05
Mean	17.36	14.16	13.44		31.52	22.95	21.00	
LSD (0.05)								
I		0.56				1.40		
M		0.86				2.14		
I X M		1.49				3.70		

Generally, it could be concluded that water stress had a negative effect on plant growth parameters and herb yield of marjoram compared to well-watered control plants. Increased water stress resulted in a decrease in plant height, branches number and yield of fresh and dry herb. This reduction is frequently attributed to drought-induced disorders in all morphological and biochemical characteristics, such as prevention of water and photo-assimilates translocation (Blum, 2017); hindered photosynthetic ability and ion uptake, as well as an excessive generation of reactive oxygen species

(Shehzad *et al*., 2020; Siddiqui *et al*., 2020). Moreover, this decline may be explained by a decrease in the growth of leaf cells or even by a lower rate of cell division in the plant (Sayyari and Ghanbari, 2012 and Tadesse, 1997).

Table 8. Effect of different irrigation levels and micro elements, Fe and Mn rates on dry herb yield/fed. (t.) on sweet marjoram.

Micro element rates (M) (ppm)	First season (2022)							
	First cut				Second cut			
	Irrigation levels (I)							
	100%	80%	60%	Mean	100%	80%	60%	Mean
Control	0.30	0.23	0.19	0.24	0.47	0.35	0.22	0.35
Fe (100)	0.31	0.27	0.21	0.27	0.55	0.41	0.28	0.41
Fe (200)	0.34	0.28	0.25	0.29	0.62	0.49	0.35	0.49
Fe (300)	0.35	0.29	0.25	0.30	0.72	0.50	0.42	0.55
Mn (100)	0.32	0.25	0.23	0.27	0.55	0.42	0.28	0.42
Mn (200)	0.36	0.28	0.25	0.30	0.65	0.51	0.35	0.50
Mn (300)	0.37	0.29	0.25	0.30	0.77	0.52	0.41	0.57
Mean	0.34	0.27	0.23		0.62	0.46	0.33	
LSD (0.05)								
I		0.01				0.02		
M		0.02				0.04		
I X M		0.03				0.06		
	Second season (2023)							
Control	0.29	0.25	0.23	0.25	0.45	0.29	0.29	0.34
Fe (100)	0.30	0.26	0.24	0.27	0.53	0.36	0.35	0.41
Fe (200)	0.32	0.27	0.25	0.28	0.60	0.43	0.42	0.49
Fe (300)	0.37	0.29	0.28	0.31	0.69	0.53	0.45	0.56
Mn (100)	0.31	0.25	0.24	0.27	0.57	0.42	0.40	0.46
Mn (200)	0.33	0.26	0.26	0.29	0.63	0.45	0.42	0.50
Mn (300)	0.39	0.30	0.30	0.33	0.73	0.57	0.47	0.59
Mean	0.33	0.27	0.26		0.60	0.44	0.40	
LSD (0.05)								
I		0.01				0.03		
M		0.03				0.05		
I X M		0.30				0.07		

Furthermore, a reduction in water causes a plant's absorption of sunlight and photosynthesis to decrease due to a reduction in leaf area, which in turn causes a reduction in dry matter and the production of plant yield (Shao *et al*., 2008). These results are in agreement with those reported by Awad Alla *et al*., (2022) on basil, Farouk *et al*., (2020) on marjoram and Ahmed *et al*., (2014) on pepper.

On the contrary, treating marjoram plants with Fe or Mn as foliar application had a positive effect on vegetative growth parameters, herb yield, oil yield per plant and per feddan relative to untreated plants. The improvement in the growth parameters and the herb yield by the micronutrients application might exist as a result of enhanced photosynthetic and other metabolic activity which caused an increase in different plant metabolites responsible for cell division and elongation (Hatwar *et al*., 2003).

Essential oil yield and productivity

Essential oil content

Data in Table 9 revealed that water stress significantly increased essential oil % compared with well-watered control plants in both cuts during experimental seasons. The highest percentage of essential oil was obtained from plants which irrigated by 60% ETo followed by 80% ETo. However, no significant differences were observed between the two highest water stress treatments during the two seasons. The increases in the percentage of essential oil under water deficit may be attributed to a higher density of oil glands, primarily due to the decrease in the quantity and area of leaves per plant under deficit (Zali *et al*., 2018).

As spraying micro elements concentrations increased marjoram oil content at all water stress levels, except Mn at 300 ppm gave significantly augment oil percentage (0.61%) followed by Fe at same rate (0.59%) compered untreated plant. There were non-significant variations found between these concentrations in the two cuts during both experimental seasons.

Emam (2020) found that spraying Mn on *Linum usitatissimum* led to more growth and high oil content. Where Mn is acts as an activator and cofactor of about 30 enzymes such as Mn SOD, oxalate oxidase (OxOx), catalase, malic enzyme and phosphoenol, playing a vital role in oxido-reduction, decarboxylation and hydrolytic reactions. Four atoms of Mn are required in OEC located at PSII. Thus, this makes Mn an important metal which is required for oxygen evolution (DalCorso *et al.* 2014; Schmidt and Husted 2019).

Table 9. Effect of different irrigation levels and micro elements, Fe and Mn rates on essential oil content (%) in fresh herb of sweet marjoram

Micro element rates (ppm)	First season (2022)							
	First cut				Second cut			
	Irrigation levels (I)							
	100%	80%	60%	Mean	100%	80%	60%	Mean
Control	0.38	0.41	0.43	0.41	0.40	0.45	0.50	0.45
Fe (100)	0.43	0.43	0.44	0.43	0.45	0.49	0.52	0.49
Fe (200)	0.46	0.52	0.53	0.50	0.48	0.51	0.53	0.51
Fe (300)	0.47	0.53	0.55	0.52	0.51	0.53	0.55	0.53
Mn (100)	0.45	0.48	0.48	0.47	0.48	0.50	0.55	0.51
Mn (200)	0.47	0.52	0.54	0.51	0.50	0.52	0.54	0.52
Mn (300)	0.48	0.54	0.56	0.53	0.52	0.56	0.57	0.55
Mean	0.44	0.49	0.51		0.47	0.51	0.54	
LSD (0.05)								
I		0.04				0.03		
T		0.06				0.06		
I X T		0.10				0.10		
	Second season (2023)							
Control	0.40	0.43	0.44	0.43	0.47	0.50	0.51	0.49
Fe (100)	0.45	0.46	0.46	0.46	0.49	0.52	0.55	0.52
Fe (200)	0.48	0.48	0.49	0.48	0.54	0.56	0.56	0.55
Fe (300)	0.51	0.53	0.53	0.52	0.55	0.60	0.61	0.59
Mn (100)	0.49	0.50	0.51	0.50	0.52	0.53	0.55	0.53
Mn (200)	0.50	0.52	0.53	0.52	0.54	0.56	0.58	0.56
Mn (300)	0.51	0.55	0.56	0.54	0.60	0.60	0.63	0.61
Mean	0.47	0.50	0.50		0.52	0.55	0.57	
LSD (0.05)								
I		0.02				0.02		
M		0.05				0.03		
I X M		0.09				0.06		

Oil yield

Data presented in Tables 10 and 11 showed that water stress significantly reduced yield of essential oil per plant and per fed. compared with well-watered control plants in both cuts during experimental seasons. The greatest essential oil yield obtained from plants which irrigated with well-water, which increased by more than 57% compared with higher deficiency water in soil (60% of ETo). The reduction in yield of essential oil under water deficit probably due to the decline in productivity of biomass (Mohasseli and Sadeghi, 2019; Farouk and Omar, 2020). Similar results were obtained by AwadAlla *et al*., (2022) and Farouk *et al*., (2020).

However, our data noticed that a significant impact of micro elements treatments on yield of essential oil. The data revealed that foliar application of Fe at 300ppm was able to enhance the oil yield as compared with the control in plant exposed to the same degree of stress. Which increased by average of 43.33 and 119.13 % (for the 1st and 2nd cuts respectively) in plant irrigated with 80% ETo and 47.78 and 104.70% in plant irrigated with 60% ETo. While the plant sprayed with Mn at 300ppm increased the yield of essential oil by average of 54.33 and 138.96 % (for the 1st and 2nd cuts respectively) in plant irrigated with 80% ETo and 56.06 and 118.34% in plant irrigated with 60% ETo.

Table 10. Effect of different irrigation levels and micro elements, Fe and Mn rates on the essential oil yield/plant (ml) in fresh herb of sweet marjoram.

Micro element rates (M) (ppm)	First season (2022)							
	First cut				Second cut			
	Irrigation levels (I)							
	100%	80%	60%	Mean	100%	80%	60%	Mean
Control	0.23	0.21	0.18	0.21	0.38	0.34	0.24	0.32
Fe (100)	0.27	0.25	0.21	0.24	0.50	0.44	0.30	0.41
Fe (200)	0.32	0.31	0.29	0.31	0.60	0.55	0.39	0.51
Fe (300)	0.33	0.33	0.31	0.32	0.74	0.58	0.48	0.60
Mn (100)	0.29	0.26	0.24	0.27	0.53	0.48	0.33	0.45
Mn (200)	0.34	0.32	0.29	0.32	0.66	0.58	0.41	0.55
Mn (300)	0.36	0.34	0.30	0.34	0.81	0.64	0.51	0.65
Mean	0.31	0.29	0.26		0.60	0.52	0.38	
LSD (0.05)								
I	0.03				0.05			
M	0.04				0.07			
I X T	0.07				0.12			
	Second season (2023)							
Control	0.25	0.22	0.20	0.23	0.47	0.30	0.29	0.35
Fe (100)	0.30	0.25	0.22	0.26	0.57	0.39	0.39	0.45
Fe (200)	0.34	0.27	0.25	0.29	0.71	0.51	0.48	0.57
Fe (300)	0.41	0.32	0.30	0.34	0.83	0.66	0.55	0.68
Mn (100)	0.33	0.27	0.25	0.28	0.65	0.47	0.44	0.52
Mn (200)	0.37	0.29	0.28	0.31	0.75	0.54	0.50	0.59
Mn (300)	0.43	0.35	0.34	0.37	0.96	0.72	0.60	0.76
Mean	0.35	0.28	0.26		0.70	0.51	0.46	
LSD (0.05)								
I	0.02				0.04			
M	0.04				0.05			
I X M	0.06				0.09			

The findings also demonstrated that water deficit caused a significant increase in essential oil percentage related with a decrease in essential oil yield compared with well-watered plants. The increases in the percentage of essential oil under water deficit may be attributed to a higher density of oil glands, primarily due to the decrease in the quantity and area of leaves per plant under deficit (Zali *et al*., 2018). During water stress in sunflower, Mn application induced the highest positive effect on yield parameters and enhanced the proline and carbohydrate content (Babaeian *et al.* 2011**)**.

The increment in the oil percentage and the oil yield due to application of Fe or Mn can be explained by the increases in the plant growth occurred by these micronutrients. This result is consistent with the findings of AwadAlla *et al*., (2022) and Said-Al-Ahl and Mahmoud (2010).

Generally, results demonstrated that for all treatments, the values of the vegetative growth, herb yield parameters, the oil percentage, the oil yield per plant and the oil yield per fed. that recorded with Mn were greater than that of Fe in almost cases. However, there were non-significant found between these two micronutrients.

Table 11. Effect of different irrigation levels and micro elements, Fe and Mn rates on the essential oil yield/fed. (L.) in fresh herb of sweet marjoram.

Micro element rates (M) (ppm)	First season (2022)							
	First cut				Second cut			
	Irrigation levels (I)							
	100%	80%	60%	Mean	100%	80%	60%	Mean
Control	4.45	3.95	3.48	3.96	7.25	6.53	4.47	6.08
Fe (100)	5.20	4.80	3.90	4.63	9.47	8.38	5.76	7.87
Fe (200)	6.01	5.96	5.43	5.80	11.36	10.49	7.40	9.75
Fe (300)	6.26	6.34	5.80	6.13	14.09	11.08	9.15	11.44
Mn (100)	5.59	5.03	4.61	5.07	10.09	9.04	6.36	8.50
Mn (200)	6.47	6.14	5.59	6.06	12.50	10.99	7.83	10.44
Mn (300)	6.87	6.52	5.79	6.39	15.32	12.19	9.76	12.42
Mean	6.12	5.68	4.87		12.19	10.00	7.62	
LSD (0.05)								
I	0.48				0.89			
M	0.73				1.37			
I X M	1.26				2.37			
	Second season (2023)							
Control	4.78	4.27	3.83	4.30	8.88	5.75	5.59	6.74
Fe (100)	5.70	4.78	4.17	4.88	10.75	7.45	7.36	8.52
Fe (200)	6.43	5.20	4.74	5.46	13.43	9.72	9.14	10.77
Fe (300)	7.83	6.12	5.66	6.54	15.73	12.60	10.48	12.94
Mn (100)	6.28	5.05	4.70	5.34	12.37	8.91	8.38	9.89
Mn (200)	6.98	5.48	5.27	5.91	14.22	10.17	9.41	11.26
Mn (300)	8.19	6.59	6.43	7.07	18.15	13.74	11.42	14.44
Mean	6.60	5.35	4.97		13.36	9.77	8.83	
LSD (0.05)								
I	0.47				0.67			
M	0.71				1.03			
I X M	1.24				1.78			

Essential oil constituents

Table 12 showed the major constituents and their relative percentages of marjoram essential oils as affected by irrigation levels and microelements. GC–MS analysis cleared that Terpinen-4-ol, Sabinene, Terpinolene, α- Terpinene and α- Pinene as the main constituents followed by α- Thujene, Bornyl acetate, Linalool, Caryophyllene and Cineole. The major compounds were increased by increasing water stress. The maximum percentage of these compounds was obtained from plants which irrigated with 60% followed by 80% and 100%, respectively. Our data also observed that, the highest percentages of major compounds were obtained from plants which sprayed with Mn followed by Fe at the same rate of concentration (300 ppm).

GC–MS analysis of essential oil showed that the monoterpenoid composition of marjoram was affected by water stress level, but the effects may have been indirect via an alteration in development rate. Increasing water stress initiated a reduction of monoterpene or sesquiterpene hydrocarbons and accelerated the transformation of hydrocarbons into oxygenated components. Results indicated that, there were variations in the main compounds % of marjoram essential oil resulted from different irrigation levels and spraying with micro elements. The differences in the composition of marjoram essential oil are mostly related to the growing conditions (Alavi *et al*., 2013). The effects of water stress are not limited to plant growth and essential oil yield but also extend to oil quality, and changes in EO yield and composition due to water stress may be due to effects on enzyme activity (Hendawy and Khalid, 2005).

Table 12. The effects of water stress and different micronutrients (at 300ppm) on the main chemical components (%) of marjoram essential oil by GC-MS

Main compounds	Water stress based on ETo								
	100%			80%			60%		
	control	Fe	Mn	control	Fe	Mn	control	Fe	Mn
α-Phellandrene	1.40	1.86	1.95	0.69	0.84	0.93	0.49	0.97	1.12
α-Thujene	2.67	3.55	3.42	2.98	4.99	4.45	0.03	0.63	1.08
α-Pinene	1.25	1.77	1.61	1.10	1.35	1.28	0.90	1.04	1.01
β-Pinene	2.2	2.75	2.98	1.74	1.92	1.81	1.32	1.5	1.88
Sabinene	9.24	9.05	10.28	3.29	4.8	4.11	2.25	2.81	3.96
β-Myrcene	1.25	3.46	2.78	1.07	1.19	1.43	0.96	1.26	1.46
γ-Terpinene	2.88	3.72	3.34	1.84	2.5	2.39	1.34	1.63	1.98
Terpinolene	5.6	6.01	7.96	5.46	8.4	9.37	4.84	5.33	6.36
α-Terpinene	3.77	1.14	2.03	4.56	4.67	4.46	2.10	2.35	3.91
cis-Sabinene hydrate	3.35	4.4	4.02	3.24	4.81	3.37	5.29	5.86	6.65
Terpinen-4-ol	18.25	19.86	20.19	24.37	25.43	26.42	28.45	31.35	29.85
Linalool	2.55	2.08	2.33	2.91	3.37	4.66	3.06	5.44	6.72
Cineole	1.77	2.28	2.04	2.16	3.77	3.02	3.10	4.73	3.94
β-Terpineol	5.83	6.45	6.94	7.96	8.55	9.09	8.99	10.02	9.53
Carvone	1.89	1.21	1.99	2.96	2.87	2.96	3.57	3.73	3.25
Thymol	0.70	0.80	0.90	1.00	1.05	1.09	1.12	1.14	1.17
cis-p-Menth-2-en-1-ol	1.09	1.64	1.91	2.11	2.33	2.45	2.19	2.53	2.44
trans-Sabinene hydrate	1.35	2.97	2.54	1.87	2.00	2.45	2.32	2.98	2.59
4-Terlx-nyl acetate	1.25	1.58	1.87	1.34	1.69	1.99	1.56	2.05	2.19
4-Terlx-nyl acetate	0.25	0.73	0.97	0.58	0.99	1.02	1.15	1.57	1.69
Linalyl acetate	1.67	1.89	1.81	1.07	1.23	1.84	2.06	2.84	2.50
β-Caryophyllene	2.93	2.78	2.53	1.04	1.57	1.40	0.48	0.78	0.93
Bornyl acetate	0.15	0.26	0.23	0.2	0.28	0.25	0.25	0.31	0.34
Other compounds	16.51	10.70	11.18	14.46	3.40	4.52	11.18	3.15	1.45
Hydrocarbons components	33.19	36.09	38.88	23.77	32.23	31.63	14.71	18.3	23.69
Alcohols and ether	34.19	39.68	39.97	44.62	50.26	51.46	53.4	62.91	61.72
Aldehydes-Ketones	1.89	1.21	1.99	2.96	2.87	2.96	3.57	3.73	3.25
Phenols	0.70	0.80	0.90	1.00	1.05	1.09	1.12	1.14	1.17
Acids and Esters	3.32	4.46	4.88	3.19	4.19	5.10	5.02	6.77	6.72
Oxygenated components	40.1	46.15	47.74	51.77	58.37	60.61	63.11	74.55	72.86
Total	89.80	92.94	97.80	90.00	94.00	96.76	89.00	96.00	98.00

Fe. = chelated EDTA-Fe (300 ppm), and Mn. = chelated EDTA-Mn (300 ppm)

Antioxidant activity of essential oil

Data illustrated in Table 13 showed the effects of irrigation rats, spraying with micro elements as well as their interactions on the antioxidant activity of the essential oils which determined by the scavenging activity of the stable free radical DPPH. The results indicated that under all treatments, the essential oil of marjoram showed a high radical scavenging activity (ranged from 74.17 to 81.72%). Water stress had a significant effect on the antioxidant activity. Increasing deficit irrigation resulted in an increase in antioxidant activity of essential oil comparing to well-watered plant, water stress prompts oxidative stress at the cellular and intercellular levels, since secondary metabolites possess strong antioxidative properties, it may be related to a counteract mechanism to the deleterious effects of reactive oxygen species (Farooqi *et al*.,2005). On the other hand, spraying plants with Fe or Mn caused a significant increase in antioxidant activity of essential oil. Increasing of Fe and Mn rates from 100 to 300 ppm resulted in an increase in antioxidant activity of essential oil as compared with the untreated plant. The maximum antioxidant activity was obtained from plants which treated with Mn followed by Fe at rate 300 ppm. The interaction between the irrigation treatments and Mn or Fe spraying had a significant impact on antioxidant activity of essential oil. The greatest antioxidant activity was obtained when the plants irrigated with 60 % and spraying with 300 ppm of Mn. These results are in accordance with Khoshru *et al.*, (2023) mention that, Mn is involved in the synthesis and activation of antioxidants, such as ascorbate peroxidase and catalase, which help plants combat oxidative stress. These enzymes scavenge harmful reactive oxygen species generated during various metabolic processes.

Table 13. The effect of irrigation levels and micronutrients (at different concentrations) as well as their interactions on Antioxidant activity of essential oils

Micro element rates (ppm)	DPPH inhibition%			
	Irrigation levels			
	100%	80%	60%	Mean
Control	74.17	75.78	78.88	76.27
Fe 100	74.86	76.32	79.09	76.75
Fe 200	75.14	77.83	79.55	77.50
Fe 300	76.86	78.13	79.98	78.32
Mn 100	75.06	77.22	79.45	77.24
Mn 200	76.95	78.89	80.54	78.79
Mn 300	77.37	80.04	81.72	79.71
Mean	75.77	77.74	79.89	
LSD (0.05)				
I		0.97		
M		0.78		
I X M		1.02		

Findings also showed that, for all treatments the essential oil has antioxidant activity which could be explained by the similar chemical composition of volatile oils with varied relative especially the major compounds (Elansary, 2015). Moreover, the antioxidant activity of essential oil of marjoram can be attributed to the considerable content of major constituents including terpinen-4-ol, α-terpinol, γ-terpinene, spathulenol, linalyl acetate, sabinene hydrate, piperitol, β-caryophyllene and α-pinene (Gharib *et al.,* 2013). In our study the antioxidant activity of essential oil increased with increasing water stress. This increment is due to the rise in the contents of Terpinen-4-ol, Sabinene, Terpinolene, α- Terpinene and α- Pinene under water stress. Likewise, increasing dose of Mn followed by Fe as foliar application increased the antioxidant activity in essential oil. The rise in contents of these major constituents is responsible for the increase in antioxidant activity in essential oil. Similar results were obtained by Elansary, (2015); Gharib *et al*., (2013) and Wei and Shibamoto (2007).

CONCLUSION

The current study revealed that water stress significantly reduces the growth characters as well as the herb yield of marjoram. Also foliar application of Mn or Fe at 300 ppm at its mitigate the harmful effects of stress. On the other side, to obtain a high essential oil percentage and high antioxidant activity of essential oil by irrigated water at 60 and 80% of ETo combined with spraying Mn at 300 ppm. It could be recommended that irrigate from at moderate water stress and foliar application of Fe and Mn at 300 ppm rate gave maximize yield and quality of marjoram grown in sandy soil.

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دور بعض العناصرالغذائية الصغرى فى تخفيف الاجهاد المائى وتعزيز انتاجية نباتات البردقوش المزروعة فى التربة الرملية

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الملخص

أجري هذه البحث بهدف دراسة العلاقة بين الرش الورقي بعنصري الحديد أو المنجنيز (بمعدلات 0 و100 و200 و300 جزء في المليون) ومستويات مختلفة من الإجهاد المائي (60، 80 و100% على أساس البخرنتح المرجعي) خلال موسمين متتاليين 2022 و2023م، على المحصول وإنتاج الزيت وتركيبة الزيت لنباتات البردقوش. كان للإجهاد المائي تأثير سلبي على خصائص النمو وكمية المحصول. أظهر الرش الورقي للحديد والمنجنيز تأثيرًا إيجابيًا على إنتاجية النبات بالإضافة إلى تخفيف الإجهاد المائي. وأدى رش بـ الحديد أو المنجنيز بمعدل 300 جزء في المليون لكل منهما على حدى عند مستوى ري (80٪ على أساس البخرنتح المرجعي) إلى زيادة ارتفاع النبات بنسبة 49.80 و 52.54٪ وعدد الفروع بنسبة 52.08 و 65.43٪ ومحصول العشب بنسبة 25.40 و 29.38٪ ومحصول الزيت بنسبة 36.08 و 30.58٪ على التوالي مقارنة بالنبات غير المعالج في نفس مستوى الإجهاد المائي. الإجهاد المائي وعنصري الحديد والمنجنيز لهما تأثير إيجابي في نشاط مضادات الأكسدة للزيت العطري. وبالمثل، كان لرش بعنصري الحديد والمنجنيز تأثير إيجابي على جميع الصفات محل الدراسة. أظهر تحليل GC-MS للزيت العطري أن الإجهاد المائي أثر على المركبات أحادي التربينويد في زيت البردقوش ، ولكن ربما كانت هذه التأثيرات غير مباشرة خلال مراحل النمو. وأدت زيادة فتره الإجهاد المائي إلى بدء تقليل الهيدروكربونات أحادية التربين أو السيسكويتربين وتسريع تحويل مركبات الهيدروكربونات إلى مركبات أكسدة. يمكن الاستنتاج أن الرش الورقي لنباتات المردقوش بالحديد أو المنغنيز بتركيز 300 جزء في المليون أدى إلى الحصول على زياده كمية وجوده المحصول لنباتات البردقوش في ظل الظروف التجربة أو الظروف المماثلة لها. من ناحية أخرى، الحصول على أعلى محتوى زيتي مع أعلى نشاط مضاد للأكسدة للزيت العطري، يمكن التوصية بري نباتات البردقوش بـ (80٪ على أساس البخرنتح المرجعي) والرش الورقي بعنصر المنجنيز بتركيز 300 جزء في المليون.

الكليمات الدالة: البردقوش، الإجهاد المائي، العناصر الصغرى، جي سي-MS، DPPH %، الزيت العطري