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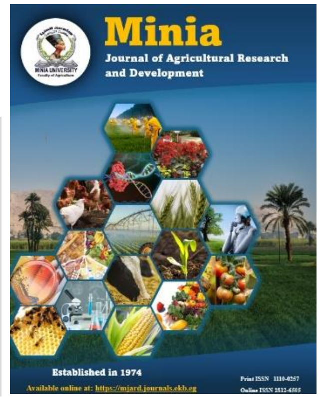

Effect of Type and Method of Application of Humic Substances on *Melissa Officinalis* Plants

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ABSTRACT

Melissa officinalis (lemon balm) is an important aromatic plant due to its essential oil (EO) content. *Melissa officinalis* plants were treated with two types of humic substances (HS): humic acid (HA) or fulvic acid (FA) at 0, 250, 500 or 750 ppm. The application was carried out three times before the 1st cut and repeated after the 2nd and 3rd ones as foliar spray (FS), soil addition (SA) or (FS+SA). Generally, plants fresh and dry weights, EO yield were significantly varied among the method of application and the applied concentration. Both of method of application and the concentration had a remarkable effect on plant dry weight and EO production. Moreover, results showed a significant interaction among the investigated factors for most assessed traits. The lowest dry herb yield (397 – 415g/plant) being for the control plants however, the highest yields (479 and 467 g/plant) were assessed for plants treated with (750 ppm of HA or FA) as a FS respectively without significant difference between them. The response of EO accumulation to the HS concentration was more pronounced as the control plants had 0.403 ml/plant which increased gradually and significantly by increasing HS concentration to achieve 0.561 ml/plant with the highest. Therefore, it is recommended to treat lemon balm plants with FA at 750 ppm as SA to improve EO production. Moreover, higher concentrations of HS should be tested for improving plant growth and EO production.

Keywords: Lemon balm, humic acid, fulvic acid, and method of application

INTRODUCTION

Melissa officinalis L. which known as lemon balm or sweet balm and belongs to the Lamiaceae family is an annual herb native to the Eastern Mediterranean and Western Asia however, it is grown all over the world **(Meftahizade et al., 2010).** *Melissa officinalis* is a source of light-yellow color essential oils (EO) that almost accumulate at 0.02-0.30%, in all over-

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ground parts **(Moradkhani et al., 2010)**. Lemon balm EO consisting of monoterpenes and sesquiterpenes moreover, the plant contains phenolic acids, flavonoids, and tannins **(Carvalho et al., 2011)**. Due to its diverse biological activities lemon balm plant is used as a flavor and in many medicines manufacture, perfumes, and cosmetics products **(Abdellatif et al., 2021)**

Some new natural elicitors can be applied to minimize artificial chemical application **(Canellas et al., 2015).** Humic substances (HS) are neutrally complex organic molecules present everywhere in the environment **(Vista, 2015)**. They can be divided according to their molecular weights and degree of solubility into two groups: a) fulvic acids (FA) which has a lower MW is rich in phenolic, carboxylic acid, and ketone groups, that makes them dissolve in all pH levels and b) humic acid (HA) is only soluble at alkaline pH as it has more aromatic alkyl units **(De Moura et al., 2023** and **Livens, 1991)**.

Studies evaluating the effect of HS on many medicinal plants demonstrate that they can significantly contribute to the accumulation of many active of bioactive substances e.g. EOs (**Pereira et al., 2024**). This study compared various ways of applying HS included soil addition (SA), fertigation or foliar spray (FS). However, the application technique did not have significant effects on the biosynthesis of secondary metabolites **Drosos et al.** (**2017)** thought that the soil application could act as soil conditioners which might have an indirect effect on plant growth and productivity**.** Higher content and concentration EOs of many aromatic plants such as *Mentha* spp, *Anethum graveolens* showed a positive correlation between the dry or fresh biomass improvement and EO content following HS application (**Hendawy et al., 2015** and **El-Gohary et al., 2014).**

Therefore, the aim of this study was investigating the role of two different types of HS; HA and FA as well as method of application which were FS and/or SA on growth and EOs production of lemon balm plants.

MATERIAL AND METHODS

Experimental design

A randomized complete block design field experiment in split-split arrangement **(Gomez** and **Gomez, 1984)** with 3 replicates was carried out during 2021/2022 and 2022/2023 seasons at Hort. Dept., Fac. of Agric., Minia Univ. The physical and chemical analysis of the experimental soil following **Jones (2018)** is presented in Table 1. Seeds were kindly provided by Dept. of Med. and Arom. Plant Res., Hort. Res. Inst., Agric. Res. Centre, Cairo, Egypt. Seedlings (45-days old) were transplanted on the commencement of Dec. in plots (2.5m length X 2m width) included 24 rows with 60 cm distance between the rows and 30 cm between the hills within the row. The main plot included 2 types of HS; HA and FA. The sub-plot included three methods of application FS, SA, and FS+SA. Whereas the sub-sub plot included the concentrations of the HS (control, 250, 500 and 750 ppm). Hence, the experiment included 24 treatments. Plants which treated with FS+SA received the same concentration of HS as a FS and SA at the same time. The control plants treated with the tap water. There was one row for each HA concentration included 6 plants.

Table 1: Physical and chemical analysis of the experimental soil (average of both seasons).

Soil characters	**Value**	**Soil characters**		**Value**
Soil type	Clayey loam	Avail. P (%)		15.40
Sand (%)	28.59	Exch. K (mg/100g)		2.45
Silt (%)	30.29	Exch. Ca (mg/100g)		31.43
Clay	41.12	Exch. Na (mg/100g)		2.46
Organic Matter (%)	1.65	DTPA Ext.(ppm)	Fe	8.39
$CaCO_3$ (%)	2.10		Cu	2.04
pH (1:2.5)	7.79		Zn	2.81
EC (mmhos/cm)	1.06		Mn	8.19
Total N (%)	0.08			

Treatments

The commercial products Umia 80 Saigner (70% HA acid, 10% K_2O) and Be-gold contain (70% of FA and 10% K_2O_2) (Poligono industrial Llanos de la Estación, Tomas Edison, Zaragoza – Spain) were used as a source of HA and FA, respectively. Foliar spray treatment was done using a hand sprayer till run off three times. For SA a 50 ml of HS containing the proper concentration of HA or FA was added around the base of the plant and mixed with soil. Plants were treated with HS three times with three weeks intervals, commenced on 19th Feb. The application was repeated three times with two weeks intervale after the 2nd and 3rd cut commenced on 14th Jun and on 20th Aug respectively.

Harvesting and estimation of growth attributes

Lemone balm plants were cut at about 7 cm above the soil surface three times during each season at 1st week of April, 1st week August, and 2nd week of October for both seasons. Just after each cut the fresh weights were assessed before air draying for several days to estimate the dry weights.

Essential oils estimation

A sample of 20 g of the dry herb was used to determine the EOs% using hydro distillation in a Clevenger apparatus for 3 h **(British Pharmacopoeia, 1963).** The yield of EOs for each cut was estimated then the yield of the three cuts were calculated.

Photosynthetic pigments:

The three photosynthetic pigments chlorophyll a and b, as well as the carotenoids contents were determined in fresh leaves of plants as described by **Ritchie (2008).** Fresh leaves collected from the middle part of the branches, three weeks after the last treatments A photosynthetic pigment which extracted from leaf sample which weighted of 0.5 g using, 10 ml of 99.5 % Methanol alcohol. The extraction was measured using a spectrophotometer at wavelengths of 666, 653 and 470 nm. Then concentrations of chlorophyll a and b and carotenoids were calculated.

Statistical analysis

Data were subject to the analysis of variance (ANOVA) then LSD 5 % between the means according to **Mead et al. (1993)** were calculated using **MSTAT program** (version 4.0) edited in 1986 by the MSTAT development team, Michigan University and Agricultural University of Norway **MSTAT-C (1986)**.

RESULTS AND DISCUSSION

Plant fresh weight

Results presented in Table 2 shows the significant responses of the total herb fresh weight (three cuts) of lemon balm plants to the three investigated factors in both seasons. Moreover, there were significant interactions between and among these factors. Nevertheless, very slight variation

(1.31 and 1.40 kg/plant) was noticed between the method of application however, more remarkable variation (1.18 and 1.45 kg/plant) was found between the concentrations in the 1st season with a similar trend in the 2nd season.

Overall, the control plants had the lowest weights (average of 1.18 kg/plant) but the plants treated with 750 ppm of FA as a SA had the highest weight 1.56 kg/plant followed by 1.54 kg/plant for the treatment 750 ppm of HA as a SA (Table 2). Similar observations were found in the 2nd season. Nevertheless, regardless of the type of HS as well as the method of application increasing the concentration from 500 to 750 ppm did not significantly increase the total herb fresh weights.

Table (2): Effect of method of application and concentrations of humic substances; humic acids (HA) and fulvic acids (FA) on the total fresh weights yield (kg/plant) of three cuts of *Melissa officinalis* during two seasons.

Humic substances (A)	methods of application* (B)	Humic substance concentrations (ppm) (C)				Mean (B)	Mean (A)
		Control	**250**	**500**	**750**		
First season (2021/2022)							
Humic acid	**FS**	1.19	1.30	1.44	1.46	**FS**	**1.37**
	SA	1.18	1.43	1.43	1.54	**1.31**	
	FS+SA	1.19	1.40	1.39	1.48	**SA**	
Fulvic acid	**FS**	1.17	1.33	1.28	1.31	**1.40**	**1.33**
	SA	1.20	1.42	1.46	1.56	**FS+SA**	
	FS+SA	1.18	1.42	1.38	1.32	**1.34**	
Mean (C)		**1.18**	**1.38**	**1.39**	**1.45**		
LSD 0.05	**A: s, B: 0.02, C: 0.03, AB: 0.08, AC: 0.06, BC: 0.09, ABC: 0.18**						
Second season (2022/2023)							
Humic acid	**FS**	1.19	1.30	1.39	1.43	**FS**	**1.36**
	SA	1.20	1.41	1.39	1.50	**1.32**	
	FS+SA	1.21	1.41	1.38	1.50	**SA**	
Fulvic acid	**FS**	1.20	1.39	1.30	1.35	**1.38**	**1.35**
	SA	1.20	1.40	1.43	1.53	**FS+SA**	
	FS+SA	1.20	1.44	1.38	1.34	**1.36**	
Mean (C)		**1.20**	**1.39**	**1.38**	**1.44**		
LSD 0.05	**A: s, B: 0.02, C: 0.03, AB: 0.08, AC: 0.06, BC: 0.09, ABC: 0.18**						

*** FS: foliar spray and SA: soil addition**

Plant dry weight

In both seasons plant dry weights did not significantly affect with the type of HS however, both of method of applications and the concentrations had significant effects of that trait (Table 3). Similar to the fresh weights the variation among methods of application was slight (441–445 g/plant) however, wide variation (407-462 g/plant) was estimated due to HS concentrations. The ANOVA showed a significant interaction among the three factors in the 1st season but there was significant interaction only between the type of HS and the method of application in the 2nd one (Table 3). In the 1st one, the lowest herb yield being for the control plants (397- 415 g/plant) however, the highest yields (479 and 467 g/plant) were assessed for plants treated with (750 ppm of HA as FS) and (750 ppm of FA as FS) respectively without significant difference between them. Under the same type of HS and method of application only increasing HA from 500 to 750 ppm as a combined treatment had a significant increase in plant dry weights.

Table (3): Effect of method of application and concentrations of humic substances; humic acids (HA) and fulvic acids (FA) on the total dry weights yield (g/plant) of three cuts of *Melissa officinalis* during two seasons.

Humic substances (A)	methods of application* (B)	Humic substance concentrations (ppm) (C)				Mean (B)	Mean (A)
		Control	250	500	750		
First season (2021/2022)							
Humic acid	**FS**	408	448	454	479	**FS**	**438**
	SA	415	457	442	459	**441**	
	FS+SA	405	425	418	450	**SA**	
Fulvic acid	**FS**	402	441	432	467	**445**	**441**
	SA	412	461	455	461	**FS+SA**	
	FS+SA	397	456	446	457	**432**	
Mean (C)		**407**	**448**	**441**	**462**		
LSD 0.05	**A: ns, B: 4, C: 5, AB: 8, AC: 10, BC: 15, ABC: 30**						
Second season (2022/2023)							
Humic acid	**FS**	401	447	454	479	**FS**	**439**
	SA	414	455	444	459	**439**	
	FS+SA	404	429	435	452	**SA**	
Fulvic acid	**FS**	409	439	428	456	**445**	**438**
	SA	409	456	449	471	**FS+SA**	
	FS+SA	399	452	444	446	**433**	
Mean (C)		**406**	**446**	**442**	**461**		
LSD 0.05	**A: ns, B: 5, C: 6, AB: 10, AC: ns, BC: ns, ABC: 36**						

*** FS: foliar spray and SA: soil addition**

Similar results regarding the effect of HS on plant fresh and dry weight have been obtained by **Nourdoz et al. (2021)** on *M. officinalis* plants which treated with FA at as a FS. On other medicinal and aromatic plants **Abdel-Rahman et al., 2024, Samy et al., 2024**, **Alhoushi**, **2024, Mirzapour et al., 2022,** and **Bahadour et al., 2024**) concluded that HS especially HA and FA significantly improved vegetative growth traits due to their capability to enhance root system development which consequently improv access soil of nutrient and water uptake **(Nardi et al., 2002).** Although many researchers showed differences between HA and FA based on molecular weight and degree of solubility **(Schnitzer** and **Schulten, 1998).** But both of HA and FA are believed to improve plant growth by enhancing nutrient availability, improving soil structure, and stimulating microbial activity (**Canellas** and **Olivares, 2014** and **Fitzpatrick** and **Goh, 1997)**. In some cases, the growth-promoting effects of both substances may be similar **(Chen** and **Aviad, 1990)**. This could explain why no significant difference in growth was observed between the two substances based on plant dry weights. The other reason for similar response to HA and FA could be due to plant itself as some species can respond differently to HA and FA while others may exhibit similar responses to both substances. It is possible that lemon balm did not exhibit a clear significant growth enhancement from either treatment **(Nardi et al., 2002** and **Basu** and **Kumar 2013**).

The significant effect of different method of application on plant response to HS has been observed by **Chen** and **Aviad (1990), Ahmad et al. (2022)** on fenugreek, **Abdellatif et al. (2017)** on tomato. The significant differences among the method of application based on growth attributes of lemon balm plants can indeed be expected and widely explained by **Canellas** and **Olivares (2014) and Nardi et al. (2002).** For example, FS allows for direct absorption through the leaf surface, providing quicker results and influencing photosynthesis and nutrient uptake. However, SA targets the root system, affecting nutrient availability, soil aggregation, and microbial interactions over a longer time. but the uptake might be slower compared to foliar application which finally can influence growth parameters like root development and biomass production **(Zandonadi et al., 2013).**

Essential oil accumulation

In both seasons the ANOVA showed that all investigated factors had a significant effect of total EO yield (Table 4) with similar trend on both of them. Plants treated with FA had higher EO yield (0.520 ml/plant) than HA-treated ones (0.485 ml/plant). Moreover, plants treated with HS as SA or FS+SA had the same EO yield (0.507 ml/plant) whereas FS treatment was significantly the lowest (0.494 ml/plant). The response of EO accumulation to the concentration of HS was more pronounced as the control plants had 0.403 ml/plant which increased gradually and significantly by increasing HS concentration increased to achieve 0.561 ml/plant with the highest concentration of HS.

Results highlighted a significant interaction among the investigated factors in both seasons. However, unlike the 1st season increasing the concentration from 500 to 750 ppm under the same type and method of application significantly increased the EO yield. In that season the lowest EO yield (0.398-0.409 ml/plant) was assessed for control plants however the highest yield (0.603 to 0.622 ml/plant) being for plants treated with FA at 750 ppm regardless the method of application (Table 4).

Abd El-Monem and **El-Khamis (2017)** found that HA and FA significantly improved vegetative growth, and subsequently higher EO yields of thymus. Also, the studies of **Kaur** and **Arora (2017), Pahlevani** and **Khalvandi (2016), Sharma** and **Sharma (2014),** and **Zandonadi et al. (2013)** demonstrated how HA application can enhance plant growth and, consequently, increase EO of different aromatic plants. Certainly, the production of EO is influenced by the overall vigor growth following HS application. Generally, improving vegetative growth can positively affect the plant's ability to synthesize and produce EO **(Bishnoi** and **Rathi, 2012)**.

That simply because plant biomass is crucial for the plant's energy production, which, in turn, supports its biosynthesis of various metabolites, including EO. **Dawood et al. (2019) Bayat et al. (2021)** and **Zhang** and **Lin (2015)** suggested that the increment in EO production following HS application could be due to the hormonal actions in photosynthesis, antioxidant activity, plant cell membranes permeability and transport nutrients to places of metabolic requirement.

Table (4): Effect of method of application and concentrations of humic substances; humic acids (HA) and fulvic acids (FA) on the total essential oils yield (ml/ plant) of three cuts of *Melissa officinalis* during two seasons

Humic substances (A)	Methods of application* (B)	Humic substance concentrations (ppm) (C)				Mean (B)	Mean (A)
		Control	250	500	750		
First season (2021/2022)							
Humic acid	**FS**	0.400	0.452	0.502	0.561	**FS**	**0.485**
	SA	0.410	0.504	0.510	0.526	**0.494**	
	FS+SA	0.404	0.497	0.504	0.546	**SA**	
Fulvic acid	**FS**	0.396	0.530	0.528	0.579	**0.507**	**0.520**
	SA	0.406	0.559	0.562	0.576	**FS+SA**	
	FS+SA	0.399	0.568	0.560	0.579	**0.507**	
Mean (C)		**0.403**	**0.518**	**0.528**	**0.561**		
LSD 0.05	**A: s, B: 0.004, C: 0.007, AB: 0.008, AC: 0.014, BC: 0.021, ABC: 0.042**						
Second season (2022/2023)							
Humic acid	**FS**	0.398	0.467	0.473	0.533	**FS**	**0.476**
	SA	0.409	0.482	0.488	0.534	**0.495**	
	FS+SA	0.402	0.470	0.485	0.566	**SA**	
Fulvic acid	**FS**	0.406	0.538	0.542	0.602	**0.512**	**0.536**
	SA	0.399	0.577	0.586	0.622	**FS+SA**	
	FS+SA	0.401	0.574	0.583	0.603	**0.510**	
Mean (C)		**0.403**	**0.518**	**0.526**	**0.577**		
LSD 0.05	**A: s, B: 0.006, C: 0.008, AB: ns, AC: 0.016, BC: 0.024, ABC: 0.048**						

*** FS: foliar spray and SA: soil addition**

Photosynthetic pigments

Chlorophyll a was significantly increased following FA application in both seasons compared with HA. Plants which treated with HS as a SA had the lowest chlorophyll a content in the 1st season, but in the 2nd season this treatment had significantly the highest chlorophyll a content (2.833 mg/g fresh weights). Significant decreases on chlorophyll a content were estimated by increasing the applied concentration of HS over than 250 ppm. However, plants treated with 250 ppm of HS had significantly higher chlorophyll a content (3.096 mg/g fresh weight) than the control treatment (2.553 mg/g fresh weight) (Table 5). There were significant interactions among the three investigated factors and the minimum chlorophyll a content was estimated for the control plants (2.471-2.649 mg/g fresh weight) however, the highest value (3.385 mg/g) was for plants treated with 250 ppm of FA as integrated treatment in the 1st season but in the 2nd, one plants treated with 250 ppm of FA as SA had the highest content (3.495 mg/g fresh weight). In both seasons almost for most cases results showed that no significant difference was observed by increasing HS concentration under the same method of application which means no need to increase the concentration over than 250 ppm.

Table (5): Effect of methods of application and concentrations of humic substance; humic and fulvic acids on the chlorophyll a (mg/g FW) of *Melissa officinalis* during two seasons.

Humic substances (A)	Methods of application * (B)	Humic substance concentrations (ppm) (C)				Mean (B)	Mean (A)
		Control	250	500	750		
First season (2021/2022)							
Humic acid	**FS**	2.471	3.247	2.938	2.554	**FS**	**2.735**
	SA	2.537	3.384	2.710	2.250	**2.768**	
	FS+SA	2.649	3.318	2.634	2.127	**SA**	
Fulvic acid	**FS**	2.521	2.630	3.295	2.491	**2.681**	**2.745**
	SA	2.577	2.610	2.828	2.551	**FS+SA**	
	FS+SA	2.561	3.385	3.087	2.399	**2.770**	
Mean (C)		**2.553**	**3.096**	**2.915**	**2.396**		
LSD 0.05	**A: s, B: 0.041, C: 0.062, AB: 0.082, AC: 0.124, BC: 0.186, ABC: 0.372**						
Second season (2022/2023)							
Humic acid	**FS**	2.101	2.145	2.547	2.663	**FS**	**2.491**
	SA	2.022	2.799	3.32	2.273	**2.526**	
	FS+SA	2.045	2.451	2.437	3.093	**SA**	
Fulvic acid	**FS**	2.225	2.941	2.749	2.843	**2.833**	**2.820**
	SA	2.146	3.495	3.341	3.267	**FS+SA**	
	FS+SA	1.989	3.074	2.805	2.97	**2.608**	
Mean (C)		**2.088**	**2.818**	**2.866**	**2.852**		
LSD 0.05	**A: s, B: 0.041, C: 0.062, AB: 0.082, AC: 0.124, BC: 0.186, ABC: 0.372**						

*** FS: foliar spray and SA: soil addition**

Chlorophyll b content of lemon balm leaves was significantly affected with all applied factors. But unlike chlorophyll a more chlorophyll b content was assessed on plants treated with HA than FA in both seasons. FS-treated plants were the best treatment in the 1st season (0.954 mg/g fresh weight) but SA application significantly augmented chlorophyll b content (0.938 mg/g fresh weight) than the other two methods (Table 6). There were significant interactions between and among these factors. Overall, in the 1st season control plants had the lowest content (0.807-0.893 mg/g fresh weight) which increased to 1.275 and 1.153 mg/g without significant differences between them for plants treated with (HA at 250 ppm as FS) and (FA at 250 ppm as integrated treatment. Similar observations were estimated in the 2nd season (Table 6).

Table (6): Effect of methods of application and concentrations of humic substances; humic and fulvic acids on the chlorophyll b (mg/g FW) of *Melissa officinalis* during two seasons.

Humic substances (A)	Methods of application* (B)	Humic substance concentrations (ppm) (C)				Mean (B)	Mean (A)
		Control	250	500	750		
First season (2021/2022)							
Humic acid	**FS**	0.878	1.275	0.978	0.829	**FS**	**0.929**
	SA	0.827	1.140	0.974	0.729	**0.954**	
	FS+SA	0.807	1.121	0.859	0.729	**SA**	
Fulvic acid	**FS**	0.893	1.089	0.874	0.817	**0.901**	**0.920**
	SA	0.878	0.854	0.914	0.888	**FS+SA**	
	FS+SA	0.832	1.153	1.021	0.827	**0.918**	
Mean (C)		**0.853**	**1.060**	**0.973**	**0.813**		
LSD 0.05	**A: s, B: 0.018, C: 0.024, AB: 0.036, AC: 0.048, BC: 0.072, ABC: 0.142**						
Second season (2022/2023)							
Humic acid	**FS**	0.794	0.977	0.995	0.981	**FS**	**0.965**
	SA	0.707	1.137	1.108	1.108	**0.871**	
	FS+SA	0.847	1.012	0.952	0.959	**SA**	
Fulvic acid	**FS**	0.720	0.878	0.856	0.765	**0.938**	**0.834**
	SA	0.654	0.933	1.104	0.754	**FS+SA**	
	FS+SA	0.682	0.808	0.835	1.021	**0.890**	
Mean (C)			**2.088**	**2.818**	**2.866**	**2.852**	
LSD 0.05	**A: s, B: 0.018, C: 0.024, AB: 0.036, AC: 0.048, BC: 0.072, ABC: 0.142**						

The carotenoids content was significantly higher for plants treated with HA only in the 2nd season. In that season unlike the 1st one SA application significantly forced the plants to have more carotenoids that the combined treatment However, in both seasons FS-treated plants had the lowest carotenoids content 1.289 and 1.265 mg/g fresh weight, respectively. In both seasons there were significant difference on carotenoid contents between plants treated with 500 or 750 ppm of HS however, both of them had significantly higher contents than the control or 250 ppm-treated plants (table 7). Overall, in the 1st season untreated plants had the lowest content (1.018 -1.092 mg/g fresh weight) while the highest value for HA application (1.708 mg/g fresh weight was for plants treated with 750 ppm as a FS. Similar trends were estimated in the 2nd season.

Table (7): Effect of methods of application and concentrations of humic substances; humic and fulvic acids on the carotenoids (mg/g FW) of *Melissa officinalis* during two seasons

Humic substances (A)	Methods of application* (B)	Humic substance concentrations (ppm) (C)				Mean (B)	Mean (A)
		Control	**250**	**500**	**750**		
First season (2021/2022)							
Humic acid	**FS**	1.054	1.217	1.125	1.708	**FS**	
	SA	1.018	1.252	1.437	1.566	**1.289**	**1.302**
	FS+SA	1.092	1.232	1.315	1.602	**SA**	
Fulvic acid	**FS**	1.055	1.221	1.549	1.382	**1.311**	
	SA	1.073	1.377	1.467	1.304	**FS+SA**	**1.305**
	FS+SA	1.058	1.295	1.512	1.364	**1.308**	
Mean (C)		**1.058**	**1.266**	**1.401**	**1.488**		
LSD 0.05	**A: s, B: 0.021, C: 0.016, AB: 0.042, AC: 0.032, BC: 0.048, ABC: 0.096**						
Second season (2022/2023)							
Humic acid	**FS**	1.231	1.261	1.403	1.461	**FS**	
	SA	1.225	1.647	1.678	1.589	**1.265**	**1.414**
	FS+SA	1.147	1.467	1.510	1.344	**SA**	
Fulvic acid	**FS**	1.184	1.182	1.154	1.243	**1.466**	
	SA	1.151	1.615	1.422	1.404	**FS+SA**	**1.298**
	FS+SA	1.187	1.214	1.260	1.556	**1.335**	
Mean (C)		**1.188**	**1.398**	**1.405**	**1.433**		
LSD 0.05	**A: s, B: 0.021, C: 0.016, AB: 0.042, AC: 0.032, BC: 0.048, ABC: 0.096**						

*** FS: foliar spray and SA: soil addition**

Rahgoshahi et al. (2023), **Al-Ghezi** and **Obaid (2021)** and **Ekin** and **Yildirim (2016)** on different aromatic plants highlighted the role of HS on photosynthetic pigments biosynthesis by improving the uptake of many essential nutrients which are critical for its formation. Moreover, HA may also influence the production of growth hormones such as auxins, which could enhance chlorophyll biosynthesis (**da Silva et al., 2023** and **Tahir** and **Shah, 2019).** When photosynthetic pigments are optimized photosynthesis often have vegetative growth larger leaf areas and better-developed root systems, which improve their ability to capture light and nutrients. This increased photosynthetic capacity can provide the energy needed for the synthesis of EO **(Shah** and **Ali, 2018).**

So that the study suggested that treating lemon balm plants with HA or FA at 750 ppm as SA could significantly improve its biomass and EO production. The application should be done three times before the 1st cut and repeated after each one.

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الملخص العربى

تأثير نوع وطريقة استخدام المواد الدبالية على نباتات الميليسا

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المليسا نبات عطري مهم بسبب محتواه من الزيوت العطرية. تم معاملة النبات بنوعين من المواد الدبالية وهما حمض الهيوميك وحمض الفولفيك بتركيزات ٠ أو ٢٥٠ أو ٥٠٠ أو ٧٥٠ جزء في المليون. تم إجراء التطبيق ثلاث مرات قبل الحش الأول، وتكرر ثلاث مرات بعد الحش الثاني والثالث. وكانت المعاملات في صورة على رش ورقي أو إضافة ارضية أو في صورة معاملة مزدوجة (رشا مع المعاملة الأرضية). بشكل عام، تباينت الأوزان الطازجة والجافة للنباتات خلال موسم النمو وإنتاج الزيوت العطرية بشكل معنوى بين طريقة التطبيق والتركيز المستعمل. وكان لكل من طريقة التطبيق والتركيز تأثير ملحوظ على الوزن الجاف للنبات وإنتاج الزيوت العطرية. علاوة على ذلك، أظهرت النتائج تفاعلًا كبيرًا بين العوامل المدروسة لمعظم الصفات المقيمة. كان أقل إنتاج للأعشاب الجافة (٣٩٧-٤١٥ جم/نبات) في نباتات المجموعة المقارنة، بينما تم تقييم أعلى إنتاج (٤٧٩ و٤٦٧ جم/نبات) للنباتات المعاملة بـ ٧٥٠ جزء في المليون من حمض الهيوميك أو حمض الفولفيك كمحلول رش على التوالي دون فرق معنوى بينهما. كانت استجابة تراكم الزيوت العطرية لتركيز حمض الفولفيك أكثر وضوحًا، حيث احتوت نباتات المجموعة الضابطة على ٠.٤٠٣ مل/نبات، والتي زادت تدريجيًا وبشكل ملحوظ بزيادة تركيز حمض الفولفيك لتصل إلى ٠.٥٦١ مل/نبات، وهو أعلى تركيز. لذلك، يُنصح بمعالجة نباتات المليسا بحمض الفوليك بتركيز ٧٥٠ جزء في المليون كإضافة ارضية لتحسين إنتاج الزيوت العطرية. علاوة على ذلك، يجب اختبار تركيزات أعلى من حمض الفولفيك لتحسين نمو النبات وإنتاج الزيوت العطرية.