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THE EFFECT OF SUBSTRATE, ORGANIC MATTER, AND SALINITY ON THE CONTENTS OF ESSENTIAL OIL AND ACTIVE INGREDIENTS OF LEMON BALM (*Melissa officinalis* L.)

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ABSTRACT

To investigate the effect of substrate, organic matter, and salinity on the amount of essential oil and active ingredients of lemon balm (*Melissa officinalis* L.) a factorial experiment was conducted in a completely randomized design. Based on the results, the highest percentage of essential oil was observed in the combined substrate of arable soil + compost + vermicompost. The highest percentages of compounds including Trans-carveol, Isoborneol, and Carvacrol acetate were observed in the salinity of 80 mM in the combined substrate of arable soil + compost + vermicompost. The highest percentage of compounds including citronellol and γ-Terpinene were recorded under compost substrate. In the control and with a combined substrate of arable soil + compost, the highest percentage of compounds, including 1,3,8,-P-menthatriene was observed. Application of HA could increase the main constituents, including Trans-carveol, γ-Terpinene, Isoborneol, Citronellol, and Carvacrol acetate in lemon balm.

Key words: compost, vermicompost, sodium chloride, essential oil compounds

INTRODUCTION

Medicinal plants are economically important plants that are used raw or processed in traditional and modern industrial medicine. These plants are sources of active ingredients and essential oils that can be converted into a variety of medicines, some of which are life-saving [Banerjee and Roychoudhury 2018].

Lemon balm belongs to the family Lamiaceae and is a valuable medicinal plant that is native to southern Europe and western Asia. Most of its medicinal properties have been attributed to its essential oils. The essential oil of this plant is widely used in pharmaceutical, food, and cosmetic industries [Menezes et al. 2015].

In medicinal and aromatic plants, the production of secondary metabolites, including essential oils and their constituents, is controlled by genetic factors, but environmental factors, especially stressful conditions, play a major role in the quantity and quality of these substances. There is ample evidence of multiplication of secondary metabolites under environmental stress, but some research has shown that this effect is not permanent, and even decreases in secondary metabolites

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under environmental stress were observed [Akula and Ravishankar 2011].

Salinity stress creates major constraints on growth, development, productivity, and product quality in many parts of the world by disrupting the physiological function of plants. In this context, stressed plants may be considered as potential sources of secondary metabolites for economic use [Valifard et al. 2018]; but this depends on the sensitivity of plants to salinity.

In previous reports, the essential oil and its main constituents in lemon balm (*Melissa officinalis* L.) [Khalid and Cai 2011, Ahmed et al. 2017], basil (*Ocimum basilicum* L.) [Bahcesular et al. 2020], parsley (*Petroselinum crispum* Mill.) [Varlvaro et al. 2016], European marjoram (*Origanum majorana* L.) [Baâtour et al. 2011], and pennyroyal (*Mentha pulegium* L.) were enhanced under the severity of salinity [Aziz et al. 2008].

The arable land properties are another factor affecting the production and quality of secondary metabolites in the cultivation of medicinal plants, so the use of organic fertilizers by improving the physical and chemical properties of arable land increases the yield of medicinal plants. Vermicompost is a bio-organic fertilizer containing a highly biologically active mixture of bacteria, egg capsules of earthworm, enzymes, plant debris, and animal manure [Lakhdar et al. 2009]. Vermicompost contains nutrients needed for plant growth in the absorbable forms such as nitrate, magnesium, phosphate, exchangeable calcium, soluble potassium, and other substances compared to other organic fertilizers. On the other hand, vermicompost is a suitable solution to combat salinity due to having a suitable and wide contact surface for microbial activities and thus preparation of various nutrients during the plant growth period [Hosseinzadeh et al. 2016]. The results of researchers' studies on the medicinal plant of lemon beebrush, showed that the use of appropriate amounts of vermicompost (10%) improved the growth and the level of phenolic compounds in plants under salinity stress [Mohsenzadeh and Zamanpour Shahmansouri 2019]. They also reported that vermicompost is a better fertilizer than compost. Gohari et al. [2019] also reported the use of vermicompost as a suitable method to reduce the negative effects of toxicity of sodium chloride on growth, and the quantitative and qualitative traits of the Moldavian dragonhead plant under irrigation conditions with salt water, and for sustainable agricultural development.

Modern agriculture is emerging as a science with strong potential for redesigning more sustainable crop systems and increasing the role of microbiological factors in arable land. Among the microorganisms that can help increase agricultural production in terms of efficiency, environmental compatibility and cost-effectiveness are effective microorganisms. These effective microorganisms are inoculants composed of fungi and bacteria isolated from arable soil that can coexist in the fermented liquid medium [Bonfim et al. 2011].

Iran is one of the best regions in the world in terms of climate and geographical location for the cultivation of medicinal plants, but a large part of the country is under salinity stress. It is necessary to use appropriate strategies to reduce the negative effects of salinity and cultivate plants that tolerate salinity. The production of vermicompost is increasing rapidly. Due to the favorable effect of this material on characteristics of arable land, growth, and development of crops and also considering the importance of plant cultivation, this study has tried to investigate the effects of compost and vermicompost fertilizers on the essential oil components of lemon balm under salinity stress.

MATERIALS AND METHODS

To investigate the effect of substrate, salinity, and organic matter on the amount of essential oil and active ingredients of the lemon balm plant, a factorial experiment was conducted in a completely randomized design with 3 replications. Factors of this experiment included: substrates, organic matter and salinity. Three types of substrates, including the composition of arable soil + compost (SC), arable soil + vermicompost (SV), and arable soil + compost + vermicompost (SCV) all in equal proportions (Tab. 1). Organic matter consisted of – zero, HA, and EM (both in a proportion of 5 per thousand). EM is a combination of 120 species of aerobic and anaerobic microorganisms that are composed of 3 main groups of yeasts, photosynthetic bacteria and lactic acid bacteria, which are often used in the production of food products and are very useful for health. Salinity in 3 levels, including (control treatment, 40 and 80 mM).

Table 1. Results of physicochemical analysis of soil, compost, and vermicompost

Sample	pH	EC (ds/m)	Organic matter (%)	N (%)	P (ppm)	K (ppm)	Fe (ppm)	Zn (ppm)	Mn (ppm)	Cu (ppm)	B (ppm)
Soil	7.95	1.53	0.9	0.9	6.2	1.37	3.2	0.29	1.4	0.14	0.12
Compost	7.42	4.06	17.34	1.73	0.87	0.15	517	68.5	185	8.5	39.5
Vermicompost	7	13.02	34.68	3.46	1.23	1.5	0.69	207.5	395	40	68.5

To produce seedlings, first, the seeds were soaked in water for 48 hours, and then the seeds were planted in seedling trays containing cocopeat. After growth, the prepared seedlings were transplanted (3) in 5-liter pots containing the desired substrates and after the establishment of plants (3 plants per pot), salinity treatment was applied as irrigation water. After applying salinity, every 10 days, organic matter at a concentration of 5 per thousand was given to the plants with irrigation water with suitable electrical conductivity. Plants were allowed to grow (outdoor with shading, with a temperature 26–34°C and relative humidity 65–75%) until the first flower appeared. The plants were harvested and then the samples (aerial parts of the plant) were immediately transferred to a shady and dry place with a temperature of 25 to 26°C with ventilation and then dried using an oven at a temperature of 40°C for 48 hours. After drying, the samples were collected in separate bags and ground into small pieces by a mill, and finally transferred to the laboratory for extraction and analysis. After that, the amount and composition of essential oils were measured.

Essential oil measurement. 25 g of dry matter was weighed from each replication per treatment. It was mixed with 300 ml of water and then the essential oil of the plant was extracted by water distillation using a Clevenger apparatus for 3 hours. The obtained essential oil was dehydrated using sodium sulfate and the percentage of essential oil was calculated.

Isolation and identification of the contents of essential oil compounds. For this purpose, Davies's method, 1990 using gas chromatography (Hewlett-Packard-5890) with an ion trap system was used.

Assay of active ingredients in essential oils. For this purpose, the method of Young-Cheol et al. [2005] was used to determine the concentrations using a gas chromatograph apparatus (Hewlett-Packard 6890) based on the retention time of the output peaks compared to standard samples.

Statistical analysis. This study was performed as a factorial experiment in a completely randomized design with 3 replications. The first factor included the substrates at three levels, the second factor included organic matter at two levels, and the third factor included salinity at 3 levels. To assess the normality of the obtained data, Kolmogorov-Smirnov and Shapiro-Wilk tests were used using SPSS 20.0 software and after making sure the data were normal, data were analyzed using SAS 9.1 statistical software. The means were compared based on the protected LSD test (PLSD) at $p < 0.05$. Data were analyzed using SAS 9.1 software. The charts were drawn using Excel 2007.

RESULTS

Percentage of essential oils. Comparison of the individual effect of substrate showed that the amount of essential oil in the combined substrate of SCV (4.47%) was significantly highest and in arable soil + compost (SC) was (3.53%) the lowest (Fig. 1). With increasing salinity, the percentage of essential oil decreased so that its level in the control was 4.2%, and at salinity levels of 40 mM and 80 mM were 3.99 and 3.79%, respectively (Fig. 2).

Essential oil compounds. Based on the results of Table 2 in this study, 34 compounds were identified in the essential oil of lemon balm. The major constituents of lemon balm were Trans-carveol, Isoborneol, Carvacrol acetate, Citronellol, 1,3,8-P-menthatriene, γ-Terpinene, Isopulegol, and γ-3-Carene. The effects

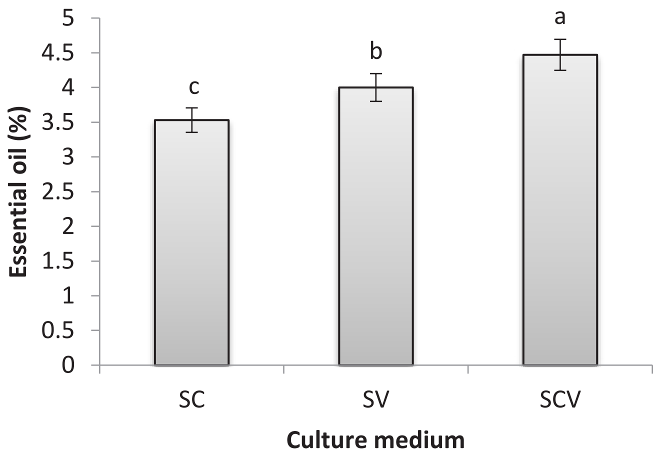

Fig. 1. Individual effect of substrate on essential oil of lemon balm. SC = soil + compost (1 : 1), SV = soil + vermicompost (1 : 1), SCV = soil + compost + vermicompost (1 : 1 : 1)

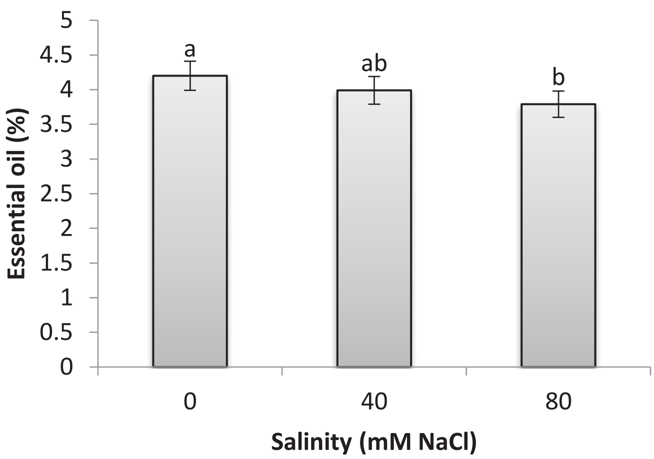

Fig. 2. Effect of salinity on essential oil amount of lemon balm

of substrate, salinity, and organic matter on these compounds are briefly discussed below.

Trans-carveol. The amount of Trans-carveol in the combined substrate of arable soil + compost + vermicompost (SCV) was (19.95%) significantly highest and in the substrate of SC (13.97%) was the lowest (Fig. 3a). With increasing salinity, the amount of trans-carveol increased significantly, so that the control was 15.41% and salinity levels of 40 and 80 mM were 16.80 and 18.95%, respectively (Fig. 4a). The use of HA resulted in a significant increase in Trans-carveol (17.99%) compared to the control treatment (Fig. 5a). In the substrate of arable soil + vermicompost (SV) as well as SCV with increasing salinity, the amount of Trans-carveol increased significantly. The highest amount of Trans-carveol (21.8%) was observed in the substrate containing SCV at the highest salinity level, and the lowest (12.56%) in the substrate containing arable soil + compost for the control (Tab. 2).

Isoborneol. Based on the results, the substrate had a significant effect on the amount of Isoborneol, so the amount of the compound in the combined substrate of arable SCV (11.46%) was the highest (Fig. 3b). With increasing salinity, the amount of Isoborneol increased

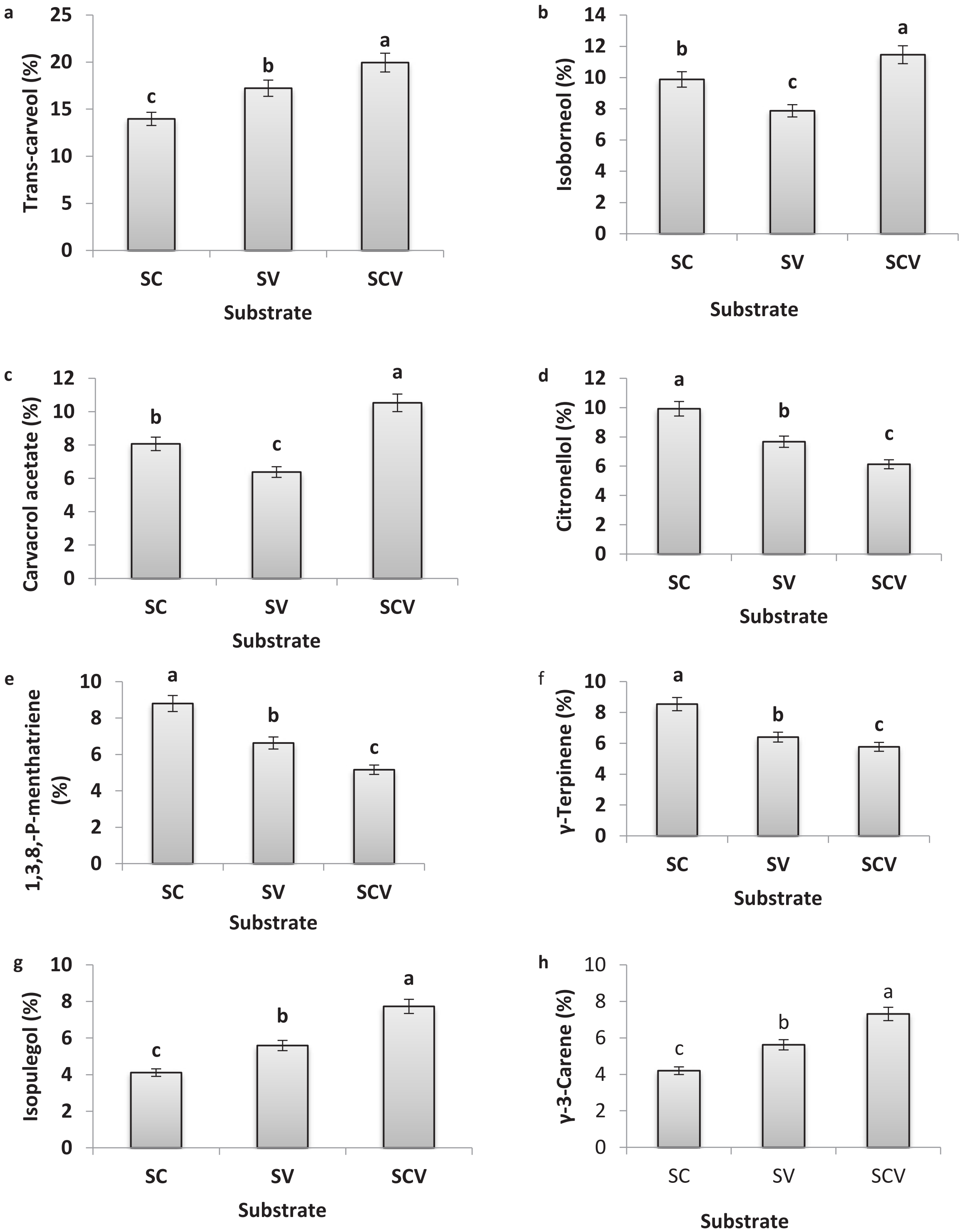

Fig. 3. Effect of substrate on essential oil compounds, Trans-carveol (a), Isoborneol (b), Carvacrol acetate (c), Citronellol (d), 1,3,8-P-menthatriene (e), γ-Terpinene (f), Isopulegol (g) and γ-3-Carene (h) of lemon balm. SC = soil + compost (1 : 1), SV = soil + vermicompost (1 : 1), SCV = soil + compost + vermicompost (1 : 1 : 1)

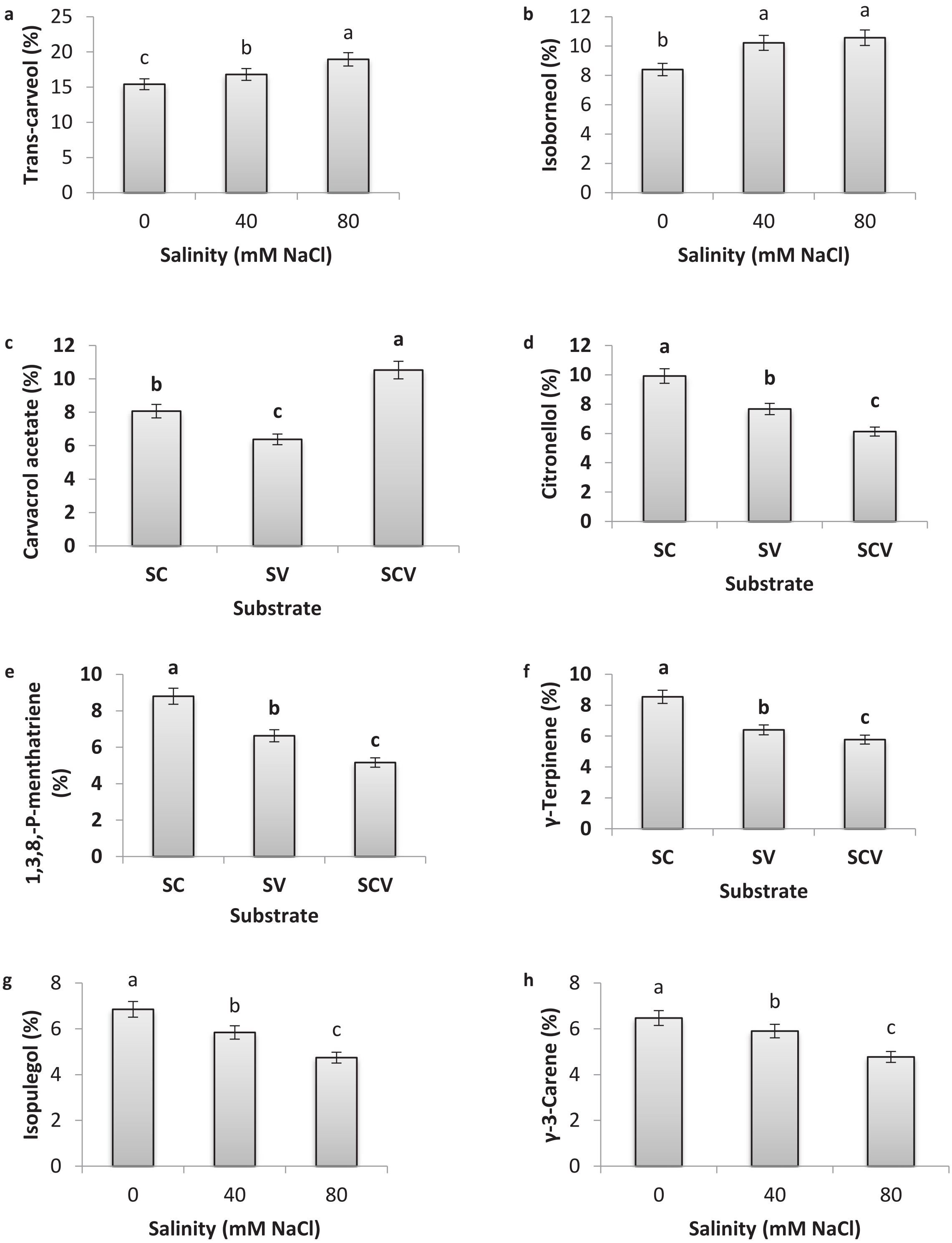

Fig. 4. Effect of salinity on essential oil compounds, Trans-carveol (a), Isoborneol (b), Carvacrol acetate (c), Citronellol (d), 1,3,8-P-menthatriene (e), γ-Terpinene (f), Isopulegol (g) and γ-3-Carene (h) of lemon balm

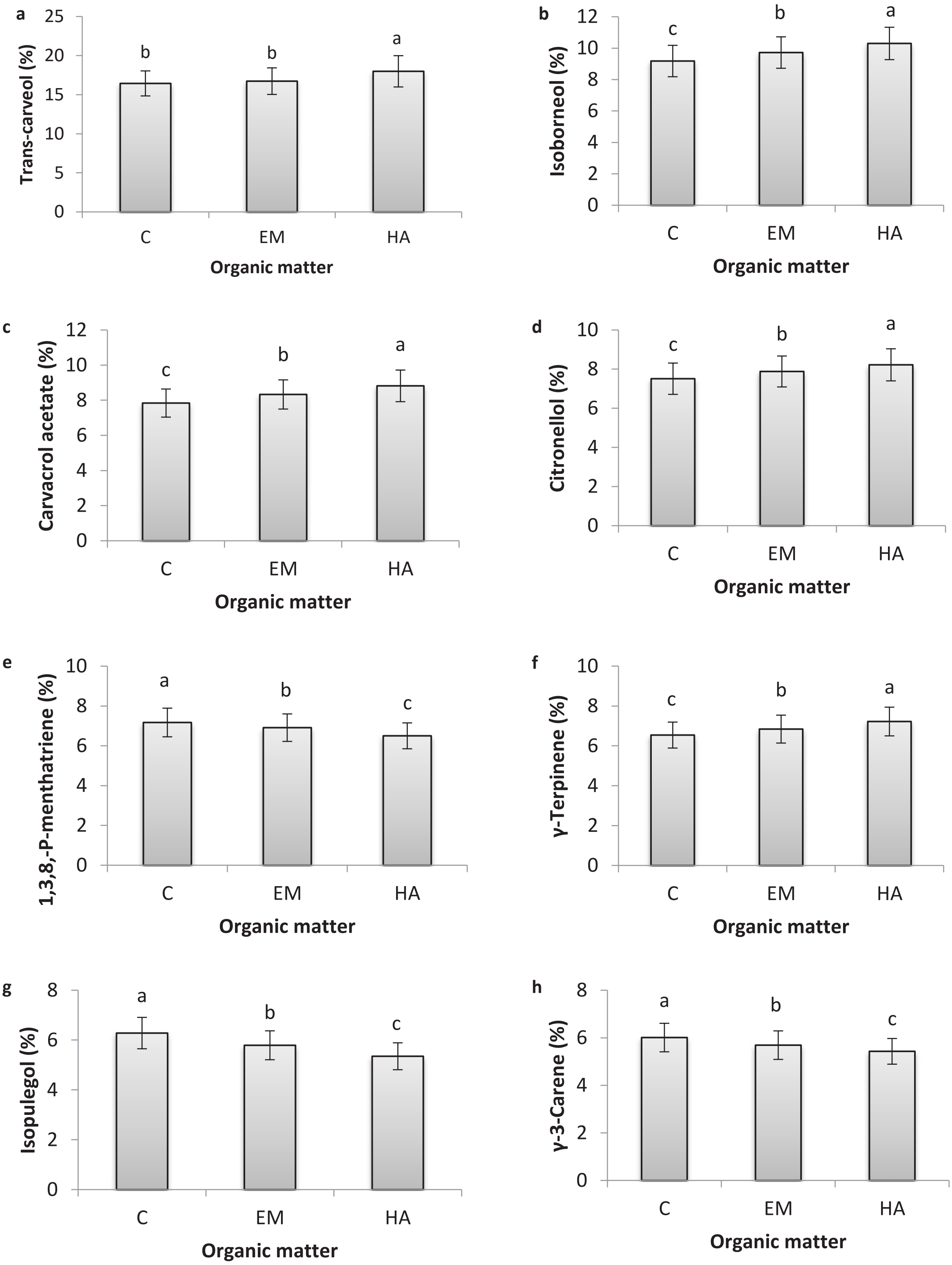

Fig. 5. Effect of organic matter on essential oil compounds, Trans-carveol (a), Isoborneol (b), Carvacrol acetate (c), Citronellol (d), 1,3,8-P-menthatriene (e), γ-Terpinene (f), Isopulegol (g) and γ-3-Carene (h) of lemon balm. C = control, EM = effective microorganism (5 g/l), HA = humic acid (5 g/l)

significantly, so that the control was 8.40%, and at the salinity levels of 40 mM and 80 mM were 10.22 and 10.57%, respectively (Fig. 4b). Interaction of substrate and salinity showed the highest amount of Isoborneol (12.90%) in substrate containing SCV at the salinity of 80 mM, and the lowest (6.70%) in substrate containing SV was observed at 40 mM salinity. In the substrate of (V and also SCV, the amount of the compound increased significantly with the severity of salinity (Tab. 2).

Carvacrol acetate. The type of substrate had a significant effect on the amount of Carvacrol acetate; so in the combined substrate of SCV was (10.5%) the highest amount. In general, the combined addition of vermicompost and compost to the arable soil was more effective than either of these two substances to the arable soil (Fig. 3c). With increasing salinity, the amount of Carvacrol acetate increased significantly, so that the control was 7.01%, and were 8.33 and 9.65% at the salinity levels of 40 mM and 80 mM, respectively (Fig. 4c). The results showed that the use of HA resulted in a significant enhancement of Carvacrol acetate content (82/8%) (Fig. 5c). Interaction of substrate and salinity showed that the highest amount of Carvacrol acetate (11.78%) was observed in substrate containing SCV at the salinity of 80 mM and the lowest (5.05%) in substrate containing SV under without salinity conditions. In all three substrates, the amount of the compound increased significantly with the severity of salinity (Tab. 2).

Citronellol. The main effects of substrates on the amount of Citronellol showed that the amount of the compound in the substrate containing SCV was (6.13%) significantly the lowest, and in the substrate containing SV was (93.9%) significantly the highest value (Fig. 3d). With increasing salinity, the amount of the compound increased significantly, so that the amount of Citronellol for the control was 6.65%, and the salinity levels of 40 mM and 80 mM were 7.88 and 9.19%, respectively (Fig. 4d). The use of HA led to a significant increase in Citronellol (8.33%) (Fig. 5d). Citronellol levels significantly increased in all three substrates with an increase in the severity of salinity. The highest (11.12%) was observed in substrate containing SC at the salinity of 80 mM and the lowest (4.82%) was observed in substrate containing SCV for the control (without salinity) – Table 2.

1,3,8-P-menthatriene. The use of HA caused a significant increase in Citronellol (8.33%) (Fig. 5d). Citronellol levels increased significantly in all three substrates with increasing the severity of salinity. The highest (11.12%) was observed in substrate containing SC at the salinity of 80 mM, and the lowest (4.82%) was observed in substrate containing SCV for the control (Tab. 2). With increasing salinity, the amount of the compound decreased significantly, so that the amount of 1,3,8-P-menthatriene for the control was 8.05%, and at the salinity levels of 40 and 80 mM were 6.81 and 72.5%, respectively (Fig. 4e). The use of organic matter led to a significant decrease in 1,3,8-P-menthatriene, so that the highest amount of 1,3,8-P-menthatriene was observed in the control (7.17%) plants (Fig. 5e). In all three substrates, the amount of 1,3,8-P-menthatriene decreased significantly with increasing the severity of salinity; so that the highest amount of the compound (9.9%) was observed in substrate containing SC under control treatment conditions (without salinity), and the lowest (4.10%) at 80 mM salinity in substrate containing SCV (Tab. 2).

γ-Terpinene. Individual effects of substrates on γ-Terpinene showed that the amount of the compound in the combined substrate of SCV (77.7%) was significantly the lowest, and in the substrate of SC (54 8.8%) was significantly the highest value (Fig. 3f). With increasing salinity, the amount of the compound increased significantly, so that the amount of γ-Terpinene for the control was 5.68%, and at salinity levels of 40 and 80 mM were 6.86 and 8.18%, respectively (Fig. 4f). The use of HA made a significant increase in γ-Terpinene (7.32%) (Fig. 5f). Interaction of substrate and salinity showed that the highest amount of γ-Terpinene (9.87%) was obtained in substrate containing SC at the salinity of 80 mM, and the lowest (4.27%) in substrate containing SCV under the control condition (without salinity). In all three substrates, the amount of γ-Terpinene increased significantly with an increase in the severity of salinity (Tab. 2).

Isopulegol. The main effect of substrate on the amount of Isopulegol showed that the amount of the compound in the substrate containing SCV (7.72%) was significantly the highest (Fig. 3g). With increasing salinity, the amount of the compound decreased significantly. The amount of the compound for the control was 6.85%, and they were 5.84 and 4.74%

Table 2. Comparison of the average percentage of compounds measured under the interaction of substrate and salinity

No.	Compound	Soil + compost			Soil + vermicompost			Soil + compost + vermicompost		
		control	40	80	control	40	80	control	40	80
		salinity (mmol NaCl)								
1	Trans-carveol	12.95^{e}	12.56^{e}	16.42^{d}	15.80^{d}	17.32^{cd}	18.58^{bc}	17.48^{cd}	20.52^{ab}	21.85^{a}
2	Isoborneol	8.47^{ef}	11.40^{b}	9.77^{cd}	8.70^{g}	7.87^{f}	9.03^{de}	10.03^{c}	11.43^{b}	12.90^{a}
3	Carvacrol acetate	6.78^{e}	7.85^{d}	9.58^{c}	5.05^{f}	6.52^{e}	7.58^{d}	9.18^{c}	10.62^{b}	11.78^{a}
4	Citronellol	8.88^{c}	9.78^{b}	11.12^{a}	6.25^{e}	7.77^{d}	8.98^{c}	4.82^{f}	6.08^{e}	7.48^{d}
5	1,3,8,-P-menthatriene	9.93^{a}	8.83^{b}	7.63^{c}	7.78^{c}	6.67^{d}	5.43^{e}	6.43^{d}	4.93^{f}	4.10^{g}
6	γ-Terpinene	7.23^{c}	8.53^{b}	9.87^{a}	5.53^{e}	6.27^{d}	7.40^{c}	4.27^{f}	5.77^{e}	7.27^{c}
7	Isopulegol	4.95^{e}	4.22^{f}	3.15^{g}	6.62^{c}	5.61^{d}	4.55^{ef}	8.98^{a}	7.68^{b}	6.52^{c}
8	γ-3-Carene	4.63^{ef}	4.30^{f}	3.67^{g}	6.30^{c}	5.77^{d}	4.80^{e}	8.47^{a}	7.63^{b}	5.83^{d}
9	Cis-Sabinene	1.32^{c}	1.10^{d}	0.77^{f}	2.10^{a}	1.83^{b}	1.47^{c}	0.98^{de}	0.82^{ef}	0.80^{ef}
10	Limonene	1.58^{bc}	1.68^{b}	1.45^{bc}	2.10^{a}	1.43^{c}	1.67^{bc}	0.73^{e}	1.05^{d}	0.78^{e}
11	Linalool	2.05^{a}	1.23^{c}	1.18^{c}	1.95^{ab}	1.78^{b}	1.70^{b}	1.18^{c}	1.08^{c}	0.82^{d}
12	Thymol	1.38^{c}	1.12^{d}	0.87^{e}	1.95^{a}	1.70^{b}	1.48^{c}	1.02^{de}	1.38^{c}	0.93^{de}
13	Citral	1.30^{b}	1.27^{b}	0.88^{c}	2.00^{a}	1.78^{a}	1.22^{b}	1.08^{bc}	0.57^{d}	0.52^{d}
14	Alpha phellandrene	1.45^{cd}	1.48^{bcd}	1.28^{de}	1.50^{bc}	1.75^{a}	1.68^{ab}	1.22^{ef}	1.02^{f}	0.70^{g}
15	Myrcene	1.53^{a}	0.92^{d}	1.05^{d}	1.60^{a}	1.50^{ab}	1.32^{bc}	1.07^{d}	1.12^{cd}	0.68^{e}
16	Alpha-pinene	1.38^{b}	1.20^{bc}	1.08^{c}	1.93^{a}	1.83^{a}	1.82^{a}	0.98^{c}	1.07^{c}	1.05^{c}
17	1,Octen-3-ol	0.88^{ab}	0.63^{c}	1.02^{a}	0.87^{ab}	1.03^{a}	1.03^{a}	0.75^{bc}	0.88^{ab}	0.92^{ab}
18	1,3-Octadiene	0.97^{abc}	0.88^{bcd}	0.87^{bcd}	0.97^{abc}	1.08^{a}	1.02^{ab}	0.83^{cd}	0.93^{abcd}	0.78^{d}
19	3-methyl-2 (methyl-2- -2 butenyl)	0.90^{cd}	0.80^{d}	0.90^{cd}	1.25^{a}	1.02^{bc}	1.08^{ab}	0.83^{d}	0.83^{d}	0.97^{bcd}
20	β-Thujone	1.60^{a}	1.32^{bc}	1.25^{bc}	1.57^{a}	1.47^{ab}	1.45^{ab}	1.12^{cd}	0.78^{e}	0.95^{de}
21	Citronellal	0.97^{cd}	0.97^{cd}	1.05^{cd}	1.72^{a}	1.33^{b}	1.02^{cd}	4.82^{f}	6.08^{e}	7.48^{d}
22	5-Hepten-1-ol	0.97^{a}	0.92^{a}	1.08^{a}	1.08^{a}	1.13^{a}	0.57^{b}	1.00^{a}	0.47^{b}	0.57^{b}
23	Bicyclo[2.2.1] heptan-2-one	0.87^{ab}	0.93^{ab}	0.60^{c}	1.07^{a}	1.02^{ab}	0.95^{ab}	0.95^{ab}	0.98^{ab}	0.82^{bc}
24	3,6,-Octadienoic acid	0.98^{bcd}	1.12^{ab}	1.27^{a}	1.03^{bc}	0.60^{e}	0.82^{cde}	0.87^{cd}	0.92^{bcd}	0.78^{de}
25	Carvacrol	1.33^{a}	0.95^{cd}	1.13^{abc}	1.20^{ab}	1.15^{abc}	1.00^{bcd}	1.05^{bcd}	0.88^{de}	0.68^{e}
26	ß-Caryophyllene	1.63^{a}	1.18^{b}	1.10^{bc}	1.07^{bcd}	1.00^{bcd}	0.93^{cde}	1.17^{b}	0.77^{e}	0.85^{de}
27	Germacrene-D	1.55^{a}	1.03^{bcd}	0.83^{cd}	1.17^{b}	1.07^{bc}	1.18^{b}	1.17^{b}	0.82^{d}	0.87^{cd}
28	ß-bisabolene	1.35^{a}	1.18^{ab}	0.80^{d}	1.03^{bc}	1.02^{bc}	1.08^{b}	0.97^{bcd}	0.85^{cd}	0.42^{e}
29	ß-Cubebene	1.33^{a}	0.60^{e}	0.80^{cde}	1.12^{ab}	1.03^{bc}	0.62^{e}	1.07^{b}	0.88^{bcd}	0.75^{de}
30	ß-Ionone	0.63^{de}	0.82^{cd}	1.12^{b}	1.48^{a}	1.18^{b}	1.08^{bc}	0.73^{de}	0.72^{de}	0.52^{e}
31	Caryophyllen epoxide	1.00^{bcd}	0.90^{d}	0.57^{e}	1.57^{a}	1.17^{b}	1.15^{bc}	0.93^{cd}	0.85^{d}	0.80^{d}
32	Eugenol	0.95^{ab}	0.88^{ab}	0.85^{b}	1.10^{a}	0.90^{ab}	0.97^{ab}	0.82^{b}	0.43^{c}	0.48^{c}
33	α-Muurolene	0.98^{ab}	0.80^{bc}	0.97^{ab}	1.02^{a}	0.45^{d}	0.97^{ab}	1.02^{a}	0.63^{cd}	0.75^{c}
34	α-Humulene	1.07^{a}	0.97^{a}	0.62^{c}	0.97^{a}	0.97^{a}	0.93^{ab}	0.53^{c}	0.53^{c}	0.70^{bc}

In each row, the means with common letters do not differ significantly at the level of one percent of Duncan's test in the salinity levels of 40 and 80 mM, respectively (Fig. 4g). The use of organic matter significantly reduced Isopulegol content, so that the highest amount (6.28%) was observed for the control (Fig. 5g). Interactions of substrate and salinity showed that the highest amount of Isopulegol (8.98%) was in substrate containing SCV under control condition (without salinity), and the lowest (3.15%) in substrate containing SC at the salinity of 80mM. In all three substrates, the amount of the compound decreased significantly with increasing the severity of salinity (Tab. 2).

γ-3-Carene. The main effects of substrates on γ-3-Carene showed that the amount of the compound in substrate containing SCV (7.31%) was significantly the highest (Fig. 3h). With increasing salinity, the amount of the compound decreased significantly. The amount of the compound for the control was 6.47% and the salinity levels of 40 and 80 mM were 5.90 and 4.77%, respectively (Fig. 4h). The use of organic matter significantly reduced γ-3-Carene, so that the highest amount of γ-3-Carene was observed for the control (6.01%) (Fig. 5h). Interactions between substrate and salinity showed that the highest amount of γ-3-Carene (8.98%) was in substrate containing SCV without salinity conditions, and the lowest (3.15%) in substrate containing SC at the salinity of 80mM. In all substrates, the amount of γ-3-Carene decreased significantly with the severity of salinity (Tab. 2).

DISCUSSION

The commercial value of lemon balm plants depends on the yield and chemical composition of their essential oils [Bonacina et al. 2017]. Based on the results obtained in the study, the simultaneous application of CV significantly increased the percentage of essential oil. The essential oils are terpenoid compounds whose constituent units (isoprenoids) such as isopentyl pyrophosphate and dimethylpatyl acyl, then they increasingly need ATP and NADPH. Considering that the presence of elements such as nitrogen and phosphorus are essential for the formation of recent compounds [Darzi 2007]; therefore, increasing the amount of compost and vermicompost increased the amount of essential oil in the vegetative part of the plant by providing more absorption of phosphorus and nitrogen, which are present in the constituents of essential oil [Anwar et al. 2005].

According to the results, the percentage of essential oil was affected by salinity stress. The lowest percentage of essential oil was obtained from plants at the salinity of 80 mM, which indicates the negative effect of high salinity on this index. The decreased essential oil may be due to the impaired photosynthesis (correlation between primary and secondary metabolites) and carbohydrate production under salinity stress, which inhibits plant growth [Flexas and Medrano 2002].

Restricting the supply of cytokinin from roots to branches and thus changing the ratio between cytokinin and leaf abscisic acid and disrupting the hormonal ratio in the plant can be another factor in reducing the amount of essential oil under salinity stress [Barrett-Lennard 2003]. Studies have shown that environmental factors and stressors always affect the chemical composition of plant essential oils and cause changes in the percentage of essential oil compounds. These differences explain the effect of plant physiological conditions on the biosynthetic pathways of compounds [Banerjee and Roychoudhury 2018]. The results showed that the number of major compounds identified as essential oils such as Trans-carveol, Isoborneol, Citronellol, Carvacrol acetate, and γ-Terpinene increased under salinity stress in lemon balm, which is consistent with the results of Khadem al-Husseini et al. [2018] on lemon balm. They also reported an increase in Caryophyllene oxide, Geraniol, Geranyl acetate, and neo-Menthol compounds with increasing salinity stress. Bonacina et al. [2017] evaluated lemon balm under salinity stress and reported that essential oil yield decreased but the number of compounds increased in all salinity treatments. Naryl acetate and granil acetate compounds were detected at salinity levels of 100, 150, and 200 mM, indicating that under salinity stress, lemon balm plants activate metabolic pathways to produce terpenoids and thus produce monoterpene. Salinity affected most of the evaluated parameters in lemon balm plants. Their results showed that lemon balm plants tolerated low salinity concentrations (up to 50 mmol). Khalid and Cai [2011] reported that the salinity of irrigation water increased the accumulation of essential oil content in lemon balm and its main components (citronella and geranyl acetate).

The increase in the composition of lemon balm essential oil in this study can be justified as follows: because salinity stress has consequences such as closing the stomata, reducing the rate of nutrient transfer in plants, reducing the water potential in plant tissues, decreased photosynthesis, growth inhibition, increased accumulation of abscisic acid and proline, free radical formation and oxidative stress, therefore, when these plants are exposed to environmental stress, they protect themselves against these conditions by producing various secondary metabolites [Akula and Ravishankar 2011]. The opposite effects of salinity stress on essential oil production have been discussed so far based on the available reports for different important medicinal plant species. It is also proposed that increasing the density of oil glands, along with many more production of glands during stress can cause the accumulation of essential oil in some plant species. Other factors that can be listed are the production of photosynthetic materials or the distribution of photosynthetic materials between growth and differentiation processes. Occasionally, a decrease in the primary metabolism of plants during stress can lead to the accumulation of some intermediate products, which occur in the form of secondary metabolites such as essential oils [Said-Al Ahl et al. 2016]. According to the results of this study, the amount of γ-3-Carene, Isopulegol, 1,3,8,-P-menthatriene, Thymol, Linalool, Limonene, Cis-Sabinene, and β-Thujon decreased with the severity of salinity, which is consistent with the results of Khadem al-Husseini et al. [2018] on lemon balm. They reported that the treatment of 1 dS/m produced the composition of menthatriene, but the treatment of 4 dS/m stopped their production by increasing the salinity stress. Ozturk et al. [2004] in their study on lemon balm and Ashraf and Akhtar [2004] on the basil plant stated that essential oil accumulatoin decreases under salinity stress. Bahcesular et al. [2020] reported a decrease in the main constituents of basil essential oil such as eugenol and methly eugenol under salinity stress. Hussein and Said-Al Ahl [2010] observed significant reductions in essential oil compounds such as carvacrol, p-Cymene, and terpinene in *Origanum vulgare* exposed to salinity stress. The decreasing effects of salinity stress on essential oil composition were more severe in lemon balm, chamomile, and basil [Said-Al Ahl and Omer 2011]. Gorgini Shabankareh et al. [2014] reported that salinity reduces the essential oil yield of lemon balm, most likely due to limited cytokinin supply from roots to branches and thus a change in the ratio between cytokines and leaf abscisic acid. Considering the contradictory results regarding the change of plant essential oil under salinity stress, it can be concluded that the production of secondary compounds in plants is not always the same and several factors can affect the production of these compounds. According to previous studies, plant species type, growth stage, special seasonal conditions, and access to mineral nutrients [Verpoorte et al. 2002], and according to the present study, stress conditions are among these factors.

The global approach in the production of medicinal plants is towards the use of sustainable agricultural systems. The use of organic fertilizers such as compost and vermicompost as a strategy in sustainable agriculture can not only increase the productivity of medicinal plants but also increase the amount of their active ingredient [Mona et al. 2008]. According to the results of this study, simultaneous use of compost and vermicompost was more effective in improving the quality traits such as amount and components of essential oil of the lemon balm than their separate uses. The highest levels of essential oils of Trans-carveol, Carvacrol acetate, Isopulegol, Isoborneol, γ-3-Carene were observed in substrate containing SCV. Therefore, the studied organic fertilizers complete each other. The results of the study are consistent with the study of Asghari et al. [2015] on the lemon beebrush. Simultaneous use of compost and vermicompost was more effective in improving the quantitative and qualitative traits of lemon beebrush than their separate uses. The results of Mirzajanial's [2019] study on lemon balm showed that the use of 10 tons per hectare of vermicompost can significantly increase the yield of lemon balm essential oil. In another study, it was found that the use of organic fertilizers and nutrients availability increased the production of essential oils in the Moldavian dragonhead plant [Fallah et al. 2018].

In this study, the use of HA could increase the main constituents of lemon balm, including Trans-carveol, γ-Terpinene, Isoborneol, Citronellol, and Carvacrol acetate. To justify this result, it can be said that HA increases the photosynthetic activity of plants by increasing the activity of the Rubisco enzyme [Delfine et al. 2005] and thus it increases the production of photosynthetic products. Because essential oils belong to the chemical group of terpenes and because glucose is a suitable precursor in the synthesis of essential oils, especially monoterpenes, photosynthesis and production of photosynthetic products are directly related to the production of essential oils [Niakan et al. 2003].

CONCLUSIONS

The results of the study showed that the highest percentage of essential oil and the amount of major essential oil compounds such as Trans-carveol, Carvacrol acetate, Isoborneol, Isopulegol, and γ-3-Carene were observed in the substrate containing arable soil + compost + vermicompost. Contradictory results were observed concerning the salinity. With increasing salinity, some compounds levels increased, and some decreased. Some major compounds content such as Trans-carveol, carochrolostat, γ-Terpinene, Isoborneol, Citronellol significantly increased with severity of salinity. The use of HA could increase the main constituents such as Trans-carveol, γ-Terpinene, Isoborneol, Citronellol, and Carvacrol acetate of lemon balm.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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