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Research Article
Citric Acid Affects *Melissa officinalis* L. Essential Oil Under Saline Soil

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Abstract

Background and Objective: Essential oil (EO) of *M. officinalis* L. used in different pharmaceutical and food industries. Salinity (SAL) stress has harmful effects on growth and EO yield. This study aimed to increase the EO composition of *M. officinalis* L. through the use of Citric Acid (CA) under SAL stress conditions. **Materials and Methods:** Plants were subjected to different levels of saline soil: 0.0, 1.6, 3.1 and 6.3 dSm^{-1} with or without CA. The experimental design followed a complete random block design. The averages of data were statistically analyzed using two-way analysis of variance (ANOVA). **Results:** The highest Fresh Mass (FM) and Dry Mass (DM) were recorded under 0.0 (SAL)×CA treatment with the values of 1776.3, 1540.2 and 300.0 g Pot^{-1} of two successive seasons. EO contents were increased with CA or salinity×CA. 1.6 dSm^{-1} (salinity)×CA treatment resulted in the highest amounts of EO with the values of 0.7 and 0.6 g $Plant^{-1}$ or 2.1-1.8 g Pot^{-1} during the 1st and 2nd seasons. The highest amounts of major constituents (citral, geranyl acetate and caryophyllene oxide) were obtained from 6.3 dSm^{-1} (SAL)×CA treatment with the values of 63.7, 11.6 and 9.2%. SAL treatment with or without CA were mostly caused highly significant $p<0.001$ changes in different chemical classes of *M. officinalis* L. EO. **Conclusion:** It was concluded that SAL×CA resulted in significant variation in FM, DM and EO composition of *M. officinalis* L.

Key words: *Melissa officinalis* L, sea salt, citric acid, citral, geranyl acetate, caryophyllene oxide

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Lemon balm or *Melissa officinalis* L. (*M. officinalis*) is an aromatic plant of the Lamiaceae family. *M. officinalis* Essential Oil (EO) use for different pharmaceutical purposes. It is used as antitumer, cancer prevention, antiviral and antioxidant against bad effects of free radicals[1,2]. *M. officinalis* EO is also used in various applications in different industries such as perfumes, cosmetics, beverages, ice creams and canned food products[3,4]. Citral, citronellal, linalool, nerol, geraniol caryophyllene and beta-caryophyllene oxide has been found as major constituents of *M. officinalis* EO cultivated in Poland[5].

Salinity (SAL) is a major environmental factor reducing plant growth characters and production[6]. Kobayashi[7] indicated that the harmful effects of high SAL stress conditions on plants can be observed at the whole plant level such as the death of plants or necrosis of plant organs and/or decreases in productivity. In aromatic plants, the yield and composition of EO varies significantly depending on environmental factors. SAL stress is a major factor affecting in the synthesis of EO[8]. SAL stress not only resulted in a significant $p<0.05$ increase in EO percentage (%) but also caused a highly significant $p<0.001$ reduction of EO yield (Plant^{-1}) of some aromatic plants[9-12]. SAL caused significant $p<0.05$ increases of the major constituents (%) of lemon balm, calendula and rosemary EOs[11-13]. Chamomile EO yields were decreased under SAL stress factor[14]. EO (%) extracted from *Nligella sativa* and its main constituents were increased under SAL conditions[15].

Citric Acid (CA) is one of the organic acids. The CA cycle in mitochondria creates cellular energy[16]. The citrate exudation of plants grows under stress factors enables these plants to absorb the nutrients (P and Fe) from such soil[17] due to the decrease in pH[18,19]. Previous investigations indicted that CA caused an increase in sweet basil (*Ocimum basilicum* L.) and dill (*Anethum graveolens*) EOs[18,19].

Increasing plant SAL stress tolerance is a focus of research and industry since SAL stress and EO yield are of major concern to maximize EO production in arid and semi-arid regions. Therefore, the present study aimed to decrease the harmful effect of SAL stress on *M. officinalis* EO by adapting *M. officinalis* plants to SAL stress conditions through the use of CA.

MATERIALS AND METHODS

Experimental: Experiments were carried out in a greenhouse at the National Research Centre (NRC), Egypt, during 2015 and 2016. *M. officinalis* seedlings were obtained from the Institute of Medicinal and Aromatic Plants (IMAP), Egypt. Uniform seedlings were transplanted into plastic pots (30 cm diameter and 50 cm height). In the first week of June during both seasons, the pots were transferred to a greenhouse adjusted to natural conditions. Each pot was filled with 10 kg of air-dried soil. Three weeks after transplanting, the seedlings were thinned to 3 plants/pot. Pots were divided into two main groups. The first group was subjected to different levels of saline soil: 0.0, 1.6, 3.1 and 6.3 dSm^{-1}. The second group was subjected to the same treatments but CA was added at 0.3 g L^{-1} as foliar spray. All agricultural practices were conducted according to the main recommendations by the Ministry of Agriculture, Egypt. The soil was salinized by sea water (highly soluble sea salts were used). Content of sea water are: Cations such as Na^+ (55%), Mg^{++} (3.7%), Ca^{++} (1.2%), K^+ (1.1%), Anions such as Cl^- (19.9%), HCO_3^- (0.4%), CO_3^{2-} (0.7%), Br^- (0.2%), SO_4^{2-} (7.7%), B^{3-} (0.1%), F^- (traces). Physical and chemical properties of the soil used in this study are: pH (7.7), EC (0.6 dsm^{-1}), organic matter (1.3%), clay (38.0%), silt (36.0%), sand (26.0%), N (0.3%), P (0.1%) and K (0.1%). Soil analyses were determined according to Margenot *et al.*[20] and Carter and Gregorich[21].

Harvesting: At full bloom, the plants were harvested twice (first and second harvests) during the growing seasons by cutting the plants 5 cm above the soil surface. Fresh Mass (FM) and Dry Mass (DM) [g plant^{-1} and g Pot^{-1}] were recorded.

EO isolation: FM (aerial part) was collected from each treatment during the 1st and 2nd harvests in both seasons, air dried and weighed to extract the EO, then 100 g from each replicate of all treatments was subjected to hydro-distillation (HD) for 3 h using a Clevenger-type apparatus[22]. The EO content was calculated as a relative percentage (v/w). In addition, total EO as g plant^{-1} and g pot^{-1} was calculated by using the DM. The EOs extracted from *M. officinalis* were collected during the 1st and 2nd harvests in both seasons from each treatment and dried over anhydrous sodium sulfate to identify the chemical constituents.

Gas chromatography-mass spectrometry (GC-MS): The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. DB-5 column (60 m×0.25 mm, 0.25 mm film thickness) was used with helium as carrier gas (0.8 mL min^{-1}). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C min that was kept constant at 220°C for 10 min and followed by elevating the temperature to 240°C at a rate of 1°C min. Split ratio was

adjusted at 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70eV. Mass range was m/z 35-450.

Gas chromatography (GC) analysis: The GC analysis was carried out using an Agilent 6890N GC system using FID detector temperature of 300°C. To obtain the same elution order with GC-MS, simultaneous auto injection was done on a duplicate of the same column at the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

Identification of components: Identification of the EO components was carried out by comparison of their relative retention times with those of authentic samples or by comparison of their Retention Index (RI) to series of n-alkanes. Computer matching against commercial (Wiley GC/MS Library, Mass Finder 3 Library)[23,24] and in-house "Başer Library of EO Constituents" built up by genuine compounds and components of known oils. Additionally, MS literature data[25,26] were also used for the identification.

Statistical analysis: In this experiment, 2 factors were considered: soil SAL (0.0, 1.6, 3.1 and 6.3 dSm^{-1}) and CA (with and without). For each treatment there were 5 replicates, each of which had 10 pots, in each pot 3 individual plants were planted. The experimental design followed a complete random block design. According to De Smith[27] the averages of data were statistically analyzed using two-way analysis of variance (ANOVA). Significant values were determined according to p values ($p<0.05$ = significant, $p<0.01$ = moderate significant and $p<0.001$ = highly significant). The applications of that technique were according to the STAT-ITCF program[28].

RESULTS

Effect of SAL, CA and their interactions on FM and DM: Responses of FM and DM to SAL, CA and their interactions during both harvests of two successive seasons are presented in Table 1 and 2. SAL×CA caused a highly significant $p<0.001$ increase in FM and DM (g Pot^{-1}) compared with SAL without CA treatments which produce highly significant $p<0.001$ reductions, CA×0.0 (SAL) treatment produced the heaviest FM with the values (941.1, 786.3, 835.2, 753.9, 1776.3, 1540.2 g Pot^{-1}) and DW with the values (185.4, 162.3, 114.6, 137.7, 300.0) during 1st and 2nd harvests and their total of both seasons.

Effect of SAL, CA and their interactions on EO content and yield: Influence of SAL, CA and SAL×CA on EO content (%) and yield is presented in Table 3. EO (%) increased at all SAL levels at both seasons. The highest accumulations of EO (%) were recorded with the highest SAL level (6.3 dSm^{-1}) with the values of 1.2 and 1.3% during 1st and 2nd seasons respectively. EO yield (g $Plant^{-1}$ or g Pot^{-1}) decreased under the highest SAL levels (3.1 and 6.3 dSm^{-1}). Lowest SAL levels (0.0 and 1.6 dSm^{-1}) resulted in significant $p<0.05$ increases of EO yield ranging from 0.5-0.6 g $Plant^{-1}$ and 1.5-1.7 g Pot^{-1} for both seasons. CA caused a significant $p<0.05$ increase in EO (%) during both seasons, the greatest percentages of EO were recorded under CA with the values of 1.0 and 0.9% at 1st and 2nd seasons. EO yield increased $p<0.05$ significantly with CA treatments, the highest values of EO yield were 0.5, 0.4 g $Plant^{-1}$ and 1.5, 1.3 g Pot^{-1} during the 1st and 2nd seasons. SAL×CA caused insignificant changes in both EO (%) and yield.

Effect of SAL, CA and their interactions on EO constituents: Twenty-three components were identified in EO extracted from *M. officinalis* aerial parts (Table 4 and 5), accounting for 95.0-99.8% of total components which belong to 4 chemical classes. Oxygenated Monoterpenes (MCHO) was the major one. The remaining fractions as Monoterpene hydrocarbons (MCH), Sesquiterpene hydrocarbons (SCH) and Oxygenated sesquiterpenes (SCHO) formed the minor classes. The main components of EO as detected by GC/MS were citral, geranyl acetate and caryophyllene oxide.

The interaction between CA and SAL (0.0 dSm^{-1}) caused a significant improve and highest amounts of citronellal (1.8%) and β-bisabolene (1.6%) in comparison with SAL without CA (Table 4). Adding CA with SAL (1.6 dSm^{-1}) resulted in positive effect in citronellol amount which recorded the highest percentage (1.9%) compared with SAL without CA treatments (Table 4). Significant $p<0.05$ increases and greatest amounts were recorded in sabinene, limonene oxide, carvone and β-humulene at CA×SAL (3.1 dSm^{-1}) with the values of 0.6, 1.1, 1.4 and 1.8%, respectively (Table 4). Applied CA with SAL (6.3 dSm^{-1}) improved and resulted the values of α-pinene, linalool, limonene oxide, menthol and geraniol compared with SAL without CA, the highest values were 0.5, 1.1, 1.1, 1.2 and 1.7% (Table 4).

Compared with SAL×CA treatments, SAL (0.0)×without CA treatment induced and obtained the greatest amounts

Table 1: Effect of salinity, CA and their interactions on FM (g Pot^{-1})

		FM (g Pot^{-1})											
		1st seasons						2nd seasons					
Treatments		1st harvest		2nd harvest		Total		1st harvest		2nd harvest		Total	
CA (0.3 g L^{-1})	SAL (dSm^{-1})	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Without CA	0.0	703.5	±3.5	638.4	±1.0	1341.9	±0.9	735.3	±0.3	694.8	±0.8	143.1	±0.3
	1.6	651.9	±1.0	585.9	±0.1	1237.8	±2.0	534.6	±1.0	412.5	±0.4	947.1	±2.0
	3.1	459.6	±0.4	350.4	±1.0	810.0	±5.0	354.9	±1.0	304.2	±0.2	659.1	±1.0
	6.3	168.9	±1.0	143.4	±0.6	312.4	±0.8	102.6	±0.2	95.4	±0.4	198.0	±2.0
Overall without CA		496.0	±2.1	429.5	±1.9	925.5	±2.1	413.9	±2.4	376.7	±2.2	790.6	±2.7
With CA	0.0	941.1	±2.5	835.2	±0.2	1776.3	±5.5	786.3	±0.5	753.9	±0.5	1540.2	±0.4
	1.6	666.3	±1.0	635.7	±0.3	1302.0	±1.0	643.5	±0.5	467.4	±0.5	1110.9	±0.4
	3.1	471.3	±0.3	404.7	±1.0	876.0	±4.0	507.3	±0.3	404.4	±0.6	911.7	±1.0
	6.3	201.6	±0.5	177.6	±0.4	379.2	±2.0	171.9	±0.1	170.7	±0.2	342.6	±0.6
Overall with CA		570.1	±2.6	513.3	±2.6	1083.4	±1.6	527.3	±2.2	449.1	±2.1	976.4	±2.2
Overall SAL	0.0	822.3	±1.9	736.8	±1.7	1559.1	±2.3	760.8	±2.7	724.4	±3.2	1385.2	±3.6
	1.6	659.1	±3.9	610.8	±2.7	1269.9	±1.9	589.1	±2.9	440.0	±3.0	1029.1	±2.9
	3.1	465.5	±2.4	377.6	±2.9	843.1	±3.1	431.1	±2.9	354.3	±2.4	785.4	±1.3
	6.3	185.3	±2.8	160.5	±1.9	354.8	±2.1	137.3	±3.7	133.1	±2.1	270.4	±2.2
F Ratio													
SAL		960.3***		853928.6***		163919.4***		1085260.4***		435036.6***		10833.8***	
CA		46.6***		58302.4***		8053.8***		149984.8***		15209.8***		876.8***	
SAL×CA		48.1***		30105. ***		7023.9***		8667.9***		9308.1***		82.7***	

***highly significant, NS, insignificant

Table 2: Effect of salinity, CA and their interactions on total DM (g Pot^{-1})

		DM (g Pot^{-1})											
		1st seasons						2nd seasons					
Treatments		1st harvest		2nd harvest		Total		1st harvest		2nd harvest		Total	
CA (0.3 g L^{-1})	SAL (dSm^{-1})	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Without CA	0.0	138.9	±0.1	101.1	±0.1	240.0	±9.0	156.3	±0.3	129.6	±0.4	285.9	±0.1
	1.6	116.4	±0.4	84.6	±0.3	201.0	±1.0	123.9	±0.1	101.1	±0.1	225.0	±0.2
	3.1	97.5	±0.5	31.5	±0.5	129.0	±1.0	60.6	±0.4	39.3	±0.3	99.9	±0.1
	6.3	33.6	±0.5	21.0	±1.0	54.6	±0.4	12.6	±0.4	12.3	±0.3	24.9	±0.5
Overall without CA		96.6	±2.1	59.6	±3.1	156.2	±2.1	88.4	±2.8	70.6	±2.8	158.9	±1.5
With													
CA	0.0	185.4	±0.4	114.6	±0.4	300.0	±5.0	162.3	±0.2	137.7	±0.1	300.0	±0.1
	1.6	139.2	±0.2	94.2	±0.2	233.4	±0.4	139.2	±0.2	117.9	±0.1	257.1	±0.1
	3.1	101.7	±0.3	34.8	±0.2	136.5	±0.5	90.3	±0.3	59.7	±0.3	150.0	±0.5
	6.3	34.2	±0.2	21.9	±0.1	56.1	±0.1	32.7	±0.3	17.4	±0.6	50.1	±0.1
Overall with CA		115.1	±2.7	66.4	±2.7	181.5	±2.0	106.1	±2.8	83.2	±2.7	189.3	±3.0
Overall													
SAL													
	0.0	162.2	±2.5	107.9	±2.3	270.0	±3.2	159.3	±3.2	133.7	±2.2	293.0	±2.3
	1.6	127.8	±1.2	89.4	±2.4	217.2	±1.7	131.6	±3.3	109.5	±2.2	241.1	±1.7
	3.1	99.6	±2.3	33.2	±1.8	132.8	±2.1	75.5	±1.1	49.5	±2.7	125.0	±2.7
	6.3	33.9	±2.0	21.5	±1.5	55.4	±1.4	22.7	±1.7	14.6	±2.8	37.5	±1.8
F Ratio													
SAL		141482.9***		53060.6***		8141.4***		218984.9***		173022.1***		4850.7***	
CA		16447.2***		1428.8***		586.6***		19402.8***		9605.6***		337.9***	
SAL×CA		662.5***		253.8***		162.6***		1596.2***		766.8***		24.5***	

***highly significant, NS: Insignificant

of trans-verbenol and β-caryophyllene with the values of 2.8% (Table 4). While geranial component recorded the greatest value (1.2%) under SAL (1.6 dSm^{-1})×without CA in regard to SAL×CA treatments (Table 4). SAL

Table 3: Effect of salinity, CA and their interactions on essential oil contents

		EO											
		Content				Yield							
		(%)				(g Plant^{-1})				(g Pot^{-1})			
		Seasons				Seasons				Seasons			
Treatments		1st		2nd		1st		2nd		1st		2nd	
CA (0.3 g L^{-1})	SAL (dSm^{-1})	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Without CA	0.0	0.5	±0.2	0.3	±0.1	0.4	±0.1	0.4	±0.1	1.2	±0.2	1.2	±0.2
	1.6	0.6	±0.2	0.4	±0.1	0.4	±0.1	0.3	±0.1	1.2	±0.2	0.9	±0.1
	3.1	0.7	±0.2	0.6	±0.1	0.3	±0.1	0.2	±0.1	0.9	±0.1	0.6	±0.1
	6.3	1.1	±0.1	1.2	±0.2	0.2	±0.1	0.1	±0.1	0.6	±0.2	0.3	±0.1
Overall without CA		0.7	±0.3	0.6	±0.4	0.3	±0.1	0.3	±0.2	1.0	±0.3	0.8	±0.2
With CA	0.0	0.6	±0.1	0.5	±0.1	0.6	±0.2	0.5	±0.1	1.8	±0.2	1.5	±0.1
	1.6	0.9	±0.1	0.7	±0.2	0.7	±0.2	0.6	±0.1	2.1	±0.2	1.8	±0.2
	3.1	1.1	±0.1	0.8	±0.1	0.5	±0.1	0.4	±0.1	1.5	±0.5	1.2	±0.2
	6.3	1.2	±0.2	1.4	±0.2	0.2	±0.1	0.2	±0.1	0.6	±0.1	0.6	±0.1
Overall with CA		1.0	±0.3	0.9	±0.4	0.5	±0.2	0.4	±0.2	1.5	±0.6	1.3	±0.2
Overall SAL	0.0	0.6	±0.2	0.4	±0.1	0.5	±0.2	0.5	±0.1	1.5	±0.4	1.4	±0.4
	1.6	0.8	±0.2	0.6	±0.2	0.6	±0.2	0.5	±0.1	1.7	±0.5	1.4	±0.5
	3.1	0.9	±0.3	0.7	±0.1	0.4	±0.1	0.3	±0.1	1.2	±0.5	0.9	±0.3
	6.3	1.2	±0.2	1.3	±0.2	0.2	±0.1	0.2	±0.1	0.6	±0.1	0.5	±0.2
F Ratio													
SAL		15.4***		43.9***		8.2*		17.1***		25.2***		18.0***	
CA		12.2***		14.3***		10.0**		15.4***		32.3***		28.5***	
SAL×CA		NS		NS		NS		NS		NS		NS	

*: Significant, **: Moderate significant, ***: Highly significant, NS: Insignificant

(3.1 dSm^{-1})×without CA resulted in greater amount of trans-carveol and neral than SAL×CA doses (Table 4), the highest values recorded under this dose were 1.4 and 1.8%. Highest level of SAL without CA caused a significant increment and reported the highest amount of germacrene D (1.5%) compared with SAL×CA levels (Table 4).

Significant changes were obtained under SAL doses of camphene, β-pinene, limonene and caryophyllene oxide according to the data revealed in Table 5. Greatest amounts of camphene and caryophyllene oxide were obtained from the treatment of 6.3 dSm^{-1} which recorded 0.5 and 9.0% while the highest amounts of β-pinene (2.2%) and limonene (0.6%) were recorded under control (0.0 dSm^{-1}) and 1.6 dSm^{-1} treatments, respectively. On the other hand, adding CA dose resulted in significant increments and highest amounts of camphene, limonene and caryophyllene oxide, the highest values were 0.4, 0.7 and 8.8% for camphene, limonene and caryophyllene oxide, respectively (Table 5).

SAL×CA recorded insignificant variation in camphene, β-pinene, limonene and caryophyllene oxide (Table 4). No significant changes in citral and geranyl acetate constituents were found under SAL, CA or SAL×CA (Table 4 and 5).

SAL doses with or without CA affected EO classes such as MCH, MCHO and SCH ($p<0.001$), 0.0 dSm^{-1} with CA produced the highest value (3.9%) of MCH while the greatest values of MCHO (85.2%) and SCH (6.0%) were obtained from the treatments of 3.1 and 6.3 dSm^{-1} without CA. SCHO was promoted significantly by SAL, or CA doses, the highest values (9.0 and 8.8%) were obtained from 6.3 dSm^{-1} or CA treatments.

DISCUSSION

In this investigation the SAL factor resulted in a significant $p<0.05$ decrease in the yield (FM and DM) and various changes in EO composition of *M. officinalis.* Plants treated with CA×SAL resulted in higher FM, DM, EO contents and major constituents of EO than those treated with SAL without CA.

The reduction of FM and DM of shoot system under SAL stress can be resulted from the exposure to SAL harmful effect which produces a reduction in turgor and decrease in

Table 4: Effect of the interactions between SAL and CA on EO components

		SAL (dSm^{-1})																
		Without CA								With CA								
		0.0		1.6		3.1		6.3		0.0		1.6		3.1		6.3		
Components	RI	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	F Ratio
α-Pinene	939	0.1	±0.0	0.2	±0.1	0.4	±0.1	0.2	±0.1	0.3	±0.2	0.3	±0.2	0.2	±0.1	0.5	±0.1	6.1**
Camphene	953	0.1	±0.0	0.1	±0.0	0.3	±0.2	0.5	±0.1	0.3	±0.2	0.2	±0.2	0.4	±0.1	0.7	±0.2	NS
Sabinene	976	0.1	±0.0	0.1	±0.0	0.2	±0.1	0.3	±0.1	0.5	±0.1	0.2	±0.1	0.6	±0.2	0.4	±0.1	4.0*
β-pinene	980	2.0	±1.0	1.6	±0.1	1.5	±0.5	1.7	±0.2	2.3	±0.3	1.6	±0.2	1.5	±0.5	1.1	±0.1	NS
Limonene	1031	0.1	±0.0	0.3	±0.1	0.2	±0.1	0.6	±0.1	0.5	±0.1	0.8	±0.2	0.5	±0.1	0.9	±0.1	NS
Linalool	1098	0.1	±0.0	0.4	±0.1	0.3	±0.1	0.7	±0.2	0.9	±0.1	0.5	±0.1	0.7	±0.2	1.1	±0.1	7.6**
Limonene oxide	1133	0.4	±0.1	0.5	±0.1	0.4	±0.1	0.8	±0.1	0.5	±0.1	0.9	±0.1	1.1	±0.1	1.1	±0.1	33.3***
Trans-Verbenol	1144	2.8	±0.3	2.4	±0.4	1.6	±0.1	0.4	±0.1	0.1	±0.0	0.4	±0.1	0.3	±0.1	1.1	±0.1	93.0***
Citronellal	1153	1.0	±0.1	1.7	±0.2	1.7	±0.2	1.3	±0.3	1.8	±0.3	0.7	±0.2	0.8	±0.1	0.4	±0.1	29.7***
Menthol	1173	0.1	±0.0	0.1	±0.0	0.3	±0.1	0.5	±0.1	0.9	±0.1	1.1	±0.1	0.4	±0.1	1.2	±0.4	20.0***
Trans-Carveol	1217	0.1	±0.0	0.4	±0.1	1.4	±0.4	1.1	±0.1	0.3	±0.1	0.7	±0.2	0.3	±0.1	1.1	±0.1	20.0***
Citronellol	1228	1.0	±0.1	1.1	±0.1	1.3	±0.3	0.7	±0.2	1.3	±0.3	1.9	±0.1	0.8	±0.1	0.2	±0.1	18.2***
Neral	1240	1.0	±0.1	1.5	±0.5	1.8	±0.2	0.1	±0.0	0.1	±0.0	0.1	±0.0	0.2	±0.1	0.3	±0.1	24.3***
Citral	1241	62.5	±2.5	62.6	±1.0	62.8	±0.8	62.8	±0.8	62.7	±0.2	62.9	±0.9	63.2	±0.2	63.7	±0.7	NS
Carvone	1242	0.1	±0.0	0.3	±0.1	0.5	±0.1	0.6	±0.1	0.8	±0.2	0.9	±0.1	1.4	±0.4	0.6	±0.1	6.2**
Geraniol	1255	0.3	±0.1	0.4	±0.1	1.3	±0.3	1.1	±0.1	0.2	±0.1	0.7	±0.2	1.1	±0.1	1.7	±0.2	30.0***
Geranial	1270	1.0	±0.1	1.2	±0.2	0.9	±0.1	0.4	±0.2	0.8	±0.1	0.5	±0.2	0.6	±0.1	0.8	±0.1	14.6***
Geranyl acetate	1383	10.6	±0.7	10.7	±0.7	10.9	±0.1	11.3	±0.3	10.9	±0.1	11.1	±0.1	11.5	±0.5	11.6	±0.6	NS
β-Caryophyllene	1418	2.8	±0.8	2.4	±0.4	0.7	±0.2	2.1	±0.1	1.8	±0.3	1.7	±0.3	1.5	±0.5	0.9	±0.1	7.5***
β-Humulene	1449	0.4	±0.1	0.3	±0.1	0.8	±0.3	1.1	±0.1	1.7	±0.2	1.3	±0.3	1.8	±0.1	0.5	±0.1	37.0***
Germacrene D	1480	0.1	±0.0	0.2	±0.1	0.5	±0.1	1.5	±0.5	0.5	±0.1	0.8	±0.2	0.4	±0.1	0.3	±0.1	22.9***
β-Bisabolene	1509	0.1	±0.0	0.1	±0.0	0.4	±0.1	1.3	±0.1	1.6	±0.6	1.5	±0.5	1.3	±0.3	0.2	±0.1	21.8***
Caryophyllene oxide	1581	8.2	±0.2	8.4	±0.4	8.5	±0.5	8.8	±0.2	8.5	±0.5	8.7	±0.2	9.1	±0.1	9.2	±0.2	NS
MCH		2.4	±0.2	2.3	±0.3	2.6	±0.1	3.3	±0.3	3.9	±0.2	3.1	±0.1	3.2	±0.2	3.6	±0.1	15.4***
MCHO		81.0	±1.0	83.3	±0.3	85.2	±0.2	81.8	±0.8	81.3	±0.3	82.4	±0.4	82.4	±0.4	84.9	±1.0	20.2***
SCH		3.4	±0.4	3.0	±0.5	2.4	±0.4	6.0	±0.1	5.6	±0.6	5.3	±0.3	5.0	±1.0	1.9	±0.1	41.5***
SCHO		8.2	±0.2	8.4	±0.4	8.5	±0.5	8.8	±0.2	8.5	±0.5	8.7	±0.2	9.1	±0.1	9.2	±0.2	NS
Total identified		95.0		97.0		98.7		99.9		99.3		99.5		99.7		99.6		

RI: Retention index, M: Mean, SD: Standard deviation, MCH: Monoterpene hydrocarbons, MCHO: Oxygenated monoterpenes, SCH: Sesquiterpene hydrocarbons, SCHO: Oxygenated sesquiterpenes, *Significant, **Moderate significant, ***Highly significant

Table 5: Effect of SAL or CA on EO components

		Overall													
						SAL (dSm^{-1})									
		Without CA		With CA		0.0		1.6		3.1		6.3		F ratio	
Components	RI	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	CA	Salinity
α-Pinene	939	0.2	±0.1	0.3	±0.2	0.2	±0.2	0.3	±0.2	0.3	±0.1	0.4	±0.2	6.7*	NS
Camphene	953	0.3	±0.2	0.4	±0.2	0.2	±0.2	0.2	±0.1	0.4	±0.1	0.5	±0.2	9.3**	14.0***
Sabinene	976	0.2	±0.1	0.4	±0.2	0.3	±0.2	0.2	±0.1	0.4	±0.3	0.3	±0.1	33.3***	6.2**
β-Pinene	980	1.7	±0.5	1.6	±0.5	2.2	±0.7	1.6	±0.1	1.5	±0.4	1.3	±0.4	NS	3.1*
Limonene	1031	0.3	±0.2	0.7	±0.5	0.3	±0.2	0.6	±0.3	0.4	±0.2	0.5	±0.2	67.5***	20.3***
Linalool	1098	0.4	±0.2	0.8	±0.3	0.5	±0.4	0.5	±0.1	0.5	±0.3	0.9	±0.3	66.7***	16.2***
Limonene oxide	1133	0.5	±0.2	0.9	±0.3	0.5	±0.1	0.7	±0.3	0.6	±0.4	1.0	±0.2	84.4***	31.4***
Trans-Verbenol	1144	1.8	±1.0	0.5	±0.4	1.5	±1.5	1.4	±1.1	1.0	±0.7	0.8	±0.4	304.4***	20.0***
Citronellal	1153	1.4	±0.3	0.9	±0.6	1.4	±0.5	1.2	±0.6	1.3	±0.5	0.9	±0.5	37.8***	8.6***
Menthol	1173	0.3	±0.2	0.9	±0.3	0.5	±0.4	0.6	±0.6	0.4	±0.1	0.9	±0.4	225.3***	23.6***
Trans-Carveol	1217	0.8	±0.5	0.6	±0.4	0.2	±0.2	0.6	±0.2	0.9	±0.7	1.1	±0.1	4.3*	24.0***
Citronellol	1228	1.0	±0.3	1.1	±0.7	1.2	±0.3	1.5	±0.4	1.1	±0.3	0.5	±0.3	NS	33.9***
Neral	1240	1.1	±0.7	0.2	±0.1	0.6	±0.5	0.8	±0.8	1.0	±0.9	0.2	±0.1	128.3***	17.8***
Citral	1241	62.6	±1.2	63.1	±0.6	62.6	±1.6	62.8	±0.9	63.0	±0.6	63.3	±0.8	NS	NS
Carvone	1242	0.4	±0.2	0.9	±0.4	0.5	±0.4	0.6	±0.1	1.0	±0.6	0.6	±0.1	68.3***	8.7***
Geraniol	1255	0.8	±0.5	0.9	±0.7	0.3	±0.1	0.6	±0.2	1.2	±0.6	1.4	±0.4	NS	51.8***
Geranial	1270	0.9	±0.3	0.7	±0.2	0.9	±0.1	0.9	±0.4	0.8	±0.2	0.6	±0.3	11.3***	4.9***
Geranyl acetate	1383	10.9	±0.5	11.3	±0.5	10.8	±0.5	10.9	±0.5	11.2	±0.5	11.5	±0.5	NS	NS
β-Caryophyllene	1418	2.0	±0.8	1.5	±0.5	2.3	±0.6	2.1	±0.5	1.1	±0.6	1.5	±0.7	8.1*	9.3***
β-Humulene	1449	0.7	±0.4	1.3	±0.6	1.1	±0.2	0.8	±0.6	1.3	±0.6	0.8	±0.3	92.1***	10.6***
Germacrene D	1480	0.6	±0.6	0.5	±0.2	0.3	±0.2	0.5	±0.4	0.5	±0.1	0.9	±0.7	NS	9.2***
β-Bisabolene	1509	0.5	±0.5	1.2	±0.7	0.9	±0.9	0.8	±0.8	0.9	±0.5	0.9	±0.6	27.0***	NS
Caryophyllene oxide	1581	8.5	±0.4	8.8	±0.4	8.4	±0.4	8.6	±0.3	8.8	±0.5	9.0	±0.3	10.0*	4.5*
MCH		2.7	±0.5	3.4	±0.4	3.2	±1.0	2.9	±0.5	3.0	±0.4	3.0	±0.3	115.1***	15.4***
MCHO		82.9	±1.8	82.8	±1.4	81.5	±0.7	83.1	±0.5	84.0	±1.6	83.7	±1.7	NS	19.0***
SCH		3.8	±1.5	4.5	±1.6	4.6	±1.2	4.2	±1.3	3.8	±1.6	4.1	±2.3	8.9*	NS
SCHO		8.5	±0.4	8.8	±0.4	8.4	±0.4	8.6	±0.3	8.8	±0.5	9.0	±0.3	10.0**	4.5*
Total identified		97.9		99.5		97.7		98.8		99.6		99.8			

RI: Retention index, M: Mean, SD: Standard deviation, MCH: Monoterpene hydrocarbons, MCHO: Oxygenated monoterpenes, SCH: Sesquiterpene hydrocarbons, SCHO: oxygenated sesquiterpenes, *Significant, **Moderate significant, ***Highly significant

cells growth and development[29]. Cell growth is the most important process affected by SAL stress, which affect plant size[30]. The capacity to trap light and the capacity of total photosynthesis depending on the leaf size, photosynthesis is restricted in SAL stress factor with a subsequent reduction in FM and DM[30].

Secondary products (EO) of aromatic plants can be altered by stress factors and saline soil is a major factor affecting the synthesis of EO[8]. Soil salinity levels caused greater effects on *M. officinalis* EO (%) and major components compared to control treatment, this may be due to the influence of soil salinity on the activities of enzymes and EO production[31]. The opposite trend was found with EO yield (Plant^{-1}), this may be due to SAL stress resulted in a significant $p<0.05$ reduction of dry matter (Table 1 and 2), so, EO yield was decreased[9]. Fresh and dry mass, EO percentage, yield and major constituents of *M. officinalis* were increased with CA and the interactions between CA and soil salinity treatments compared with salinity stress alone. Other mechanisms in addition to pH reduction appear to underline the positive effect of citric acid on mineral absorption from soil specially P and Fe[18,19]. P had a significant effect on mass and EO (qualitative and quantitative) production of aromatic plants such as some *Apiaceae* plants, *Origanum dictamnus*, *Trichosanthes cucumerina*, basil, anise, coriander and sweet fennel[32-38]. Fe resulted in a significant effect in mass production and EO composition of some aromatic plants i.e., chamomile and oregano[39-41]. Similar EO constituents of *M. officinalis* were found by Patora *et al.*[5] in Iran, Khalid *et al.*[42] in China, Khalid and Cai[12] in China. This study results are in accordance with those obtained by Misra and Srivastava[9], Khalid[10], Khalid and da Silva[11], Khalid and Cai[12], they reported that EO percentage and its major constiuents of basil, lemon balm and calendula were significantly increased $p<0.05$ with salinity stress conditions but EO yields were decreased. Jafari and Hadavi[18,19] indicated that CA has significant effects on EO isolated from basil and dill.

Adapting *M. officinalis* crop to SAL factor through the use of CA is very necessary, especially in arid and semi arid regions for increasing the natural resources of EO. *M. officinalis* EO can be used in various food industries (backed food products, confectionary, ice creams, beverages and flavors), perfumes and cosmetics. *M. officinalis* EO has various biological activities such as antioxidant, antimicrobial, anticancer and antiviral[2,43].

In the future, results of this investigation will be introduced to who benefit such as, Ministry of Agriculture, farmers and producers to help them in production yield, EO and EO major components from *M. officinalis* (important aromatic herb) under arid zones in Egypt.

CONCLUSION

It may be concluded that SAL, CA and SAL×CA resulted in significant variations of FM, DM, sabinene, linalool, limonene oxide, trans-verbenol, citronellal, menthol, trans-carveol, neral, carvone, geranial, β-caryophyllene β-humulene and MCH), while citronellol, geraniol, germacrene D and MCHO were significant for SAL or SAL×CA. CA or SAL×CA reported significant changes in the amounts of α-pinene, β-bisabolene and SCH. Separated factors (SAL or CA) resulted in significant values of EO (%), EO (g Plant^{-1} or g Pot^{-1}) camphene, limonene, caryophyllene oxide and SCHO. β-pinene was significant for SAL factor and no significant results were found in the amounts of citral and geranyl acetate SAL, CA and SAL×CA.

SIGNIFICANCE STATEMENTS

This investigation discovered that it is necessary to use CA under salinity stress condition. This study also suggested that CA×SAL improved the EO and major constituents of *M. officinalis.*

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