

## Influence of Jasmine oil and Methyl Jasmonate on Gene Expression and Menthol Production in *Mentha*

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### ABSTRACT

*Mentha* is the major source of menthol used for many important commercial and pharmaceutical purposes. For enhanced menthol production, this study aimed to assess the influence of the foliar application of Jasmine oil and Methyl Jasmonate by 0.05 and 0.1% on gene expression, essential oil, and its component especially Menthol in *Mentha piperita* L. Exogenously foliar application of Methyl Jasmonate (MeJA) and Jasmine oil (JO) treatments induced alteration of gene expression patterns which was detected by a cDNA-SCoT technique with high informative and discrimination capability. Where it targeted 33 clear transcript-derived amplicons, 21 out of them were new transcripts. Differential expression analysis of these patterns illustrated that the higher influence was by Jasmine oil with concentration 0.1% at 48 h followed by MeJA-0.1% at 24 h. On the other hand, mfs-transcripts were differentially regulated by MeJA and JO treatments. In general, the results revealed high level of volatile oil, high percentages of menthol (1.52 and 1.47-fold) with the low percentages of the menthone (0.69 and 0.77- fold), lowest level of upregulated menthofuran gene expression (-0.88 and -0.72 Log<sub>2</sub>FC). Besides, good vegetative growth relative to the control was found with exogenously foliar application of MeJA by 0.05% after 48 h and JO by 0.1% after 24 h, which increases the pharmacological quality of *Mentha* plant. Subsequently, indicating the possibility of using JO (Low-cost) after further study as a stimulant for the activity of menthol production with high quality as a cheaper alternative to exogenously foliar application of MeJA.

**KEYWORDS:** *Mentha*, Menthol, Menthofuran, Volatile oil, Methyl Jasmonate, Jasmine oil, Differential expression, cDNA-SCoT, qPCR.

### 1. INTRODUCTION

Peppermint (*Mentha piperita* L.) from the family Lamiaceae is an aromatic perennial herb that containing more than 25 species as well as many varieties obtained by natural hybridization. It is the natural hybrid (2n = 6x = 72) between *M. spicata* L. (spearmint, 2n = 48) and *M. aquatica* L. (water mint, 2n = 96) (Kokkini 1991; Banthorpe 1996; Pushpangadan and Tewari, 2006). They are of major medicinal and aromatic crops and cultivated widely for their essential oil (Inoue *et al.*, 2003) Menthol, the main component of *Mentha* oil, is found in the highest concentrations in mature leaves (Fahlen *et al.*, 1997; Rohloff 1999). *Mentha* genus plants produce a natural chemical product group known as monoterpenes and are the only source to produce menthol throughout the world (Chakraborty and Chattopadhyay, 2008). Also, some parts from *Mentha* plants are used as condiments and herbal teas in

fresh or dried form due to their featured aroma (Baser and Kurkcuoglu 1999). Menthol has wide industrial uses, where used in food additives and many medicinal applications such as oral hygiene (Eccles 1994; Patel *et al.*, 2007). Where is used to enhance the skin penetration of many medicinal compounds and it has unlimited potential to be developed into pharmaceutical compounds and synthetically modified drugs (Galeotti *et al.*, 2001; Kamatou *et al.*, 2013). Limonene synthase represents the obliged menthol biosynthesis step. In *Mentha* essential oil, limonene is a precursor to carvone. (Kjonaas and Croteau 1983). The conversion from the pathway to menthol is important to the diversion of pulegone to menthofuran catalyzed by menthofuran synthase which is considered one of the metabolites stress produced by the peppermint plant under extreme abiotic stress conditions during the leaf expansion period. It is one of the important strategies that

plants develop to increase stress tolerance (Afkar *et al.*, 2013). Nucleic acids, lipids and proteins as organic molecules are prone to be degradation damage (Galeotti *et al.*, 2001). Stress response mechanisms are activated through these conditions, either to promote or to prevent the rapid turnover the source of damaged molecules (Miranda, 2011). These strategies involve alters in the gene expression, metabolism, and some metabolites production (Ramanjulu and Bartels 2002; Staswick and Howell 1992). However, menthofuran is a highly toxic organic compound that increases in the essential oil of *Mentha* under different stress conditions. It is metabolically metabolized to produce hepatotoxic chemically reactive intermediates (Anderson *et al.*, 1996; Thomassen *et al.*, 1992). Expression reduction of Menthofuran Synthase gene (*mfs*) causes improvement of essential oil quality (Rahimi *et al.*, 2017). Plant defence responses, on the other hand, are controlled by a complex network of signaling channels that include endogenous signaling molecules, such as Saicylic acid SA and Jasmonic acid. Jasmonic acid (JA) and its methyl ester or Methyl Jasmonate (MeJA) which first isolated from jasmine oil (JO) have been reported to play an important role in the signal transportation process that regulates defense genes in plants (Farmer and Ryan 1990).

Regulation of plant growth and development is by a balance between the action of different groups of growth regulators, which promote or inhibit such processes (Prins *et al.*, 2010). Where MeJA is considered a cellular regulator that plays an important role in plant development and many physiological processes and abiotic stresses (Maciejewska *et al.*, 2004; Choi *et al.*, 2005; Warabieda and Olszak 2010; Kim *et al.*, 2009). In addition, MeJA induces the biosynthesis of many secondary metabolites that play important roles in the adaptation of plants to particular environments (Choi *et al.*, 2005). Jasmonic acid (JA) and its derivatives were collectively called Jasmonates (JAs).

The role of Jasmonates and their derivatives which collectively called Jasmonates (JAs) in controlling gene expression and secondary metabolism is so complicated (Farmer and Ryan, 1990). Choi *et al.*, 1994 reported that JAs differentially regulate  $\beta$ -Hydroxy  $\beta$ -methylglutaryl-CoA reductase genes in potato. Also in cell cultures of *Taxus canadensis*, MeJA induced the diterpene taxol accumulation and that accumulation was correlated with the induced expression of two genes coding for taxadiene synthase and downstream biosynthetic enzymes (Hefner *et al.*, 1998). Faldt *et al.*, 2003 and Martin *et al.*, 2002 showed that treatment of Norway spruce with MeJA stimulates

gene expression of monoterpene and diterpene synthases.

cDNA SCoT (cDNA starts codon-targeted) molecular technique has been proposed to be an appropriate powerful tool for identification variations of gene expression, stress tolerance and genetic stability in plants (Al-Taweel *et al.*, 2019). This method is an advantageous method compared to all other existing ones because it is cheaper, relatively more efficient, simpler to operate, faster and can be readily reproduced of the results (Luo *et al.*, 2014). cDNA-SCoT markers were used to determine the gene expression in *Saccharum officinarum*, *Phoenix dactylifera*, *Mangifera indica*, sugarcane, Olive tree and *Dendrobium officinale* (Munns and Tester, 2008; Chen, *et al.*, 2013; AL-Janabi and Al-Rawi, 2018).

More accurately, RT-PCR (reverse transcriptase-dependent polymerase chain reaction) can quantify a few molecules of a particular mRNA in extremely small amounts of starting material. Originally, this approach was created for medical purposes. Although it is commonly employed to assess the existence or absence of a certain transcript in plants, it has also been used to measure gene expression levels (Chang *et al.*, 1993 and Fleming *et al.*, 1996). Despite the higher accuracy of real-time PCR, semi-quantitative RT-PCR offers advantages in sensitivity and specificity, as well as a broad range of detection. The semi-quantitative RT-PCR technique is still vastly employed for many reasons in plant studies (Tang *et al.*, 2007). The fact that this approach combines classical PCR and real-time PCR at a reasonable cost is the fundamental reason for its widespread adoption (Marone *et al.*, 2001). Also, this technique was confirmed to generate good results compared to more advanced techniques (Souza *et al.*, 2009).

Due to inadequate data about the effect of Jasmine oil (JO) (low-cost) versus MeJA (high-cost) on *Mentha*, the current study was designed to look at the effects of exogenously foliar application of JO and MeJA on gene expression patterns, specifically expression of the menthofuran synthase gene, volatile oil percent and compositions, and vegetative traits in *Mentha*.

## 2. MATERIALS AND METHODS

Our goals were studying the effect of JO and MeJA on growth, yield, Volatile oil (VO) (quantity, quality, and composition) and expression of menthofuran gene in *Mentha* (*Mentha piperita* L.). The experiment was conducted at research farm and Biotechnology Lab. of Horticultural Research Institute (HRI), Agricultural Research Centre (ARC) and in Seed and Tissue Pathology Lab, Faculty of Agriculture, Mansoura University in season

2019/2020. Two months old seedlings were obtained from the Experimental Farm of Medicinal and Aromatic Plants Research Department, El Kanater El Khairia, Egypt. The peppermint plants initiated from 10 cm long rhizome cuttings followed by transferring into pots. Complete randomized design (CRD) was used with six replicates. Plants sprayed with Jasmine oil (JO) and Methyl Jasmonate (MeJA) as 0.05 and 0.1 in 2 percent of ethanol (v/v). The sprayed were onto peppermint plants during the early bloom stage. The treated plants were left on open air for 2 hours till JO and MeJA completely evaporated. At 0, 24, and 48 hours following treatment, leaves from untreated (control) and treated peppermint plants were randomly selected. Prior to RNA extraction, the leaves were instantly frozen in liquid nitrogen and stored at -80 °C.

### 2.1. RNA isolation and cDNA synthesis

Total RNA was isolated from leaves samples both untreated (control) and treated peppermint plants using TRIzol® reagent. Then, cDNA synthesis in Techni TC-512 PCR system using Thermo Scientific Kit (RevertAid™ First Strand cDNA Synthesis) was done as described by Abd El-Aziz *et al.* (2019).

### 2.2. cDNA-SCoT amplification and detection

The PCR reaction was performed in a total volume of 25 µl using the SCoT primers for the study of expression profiling. These primers were selected from the literature according to Collard and Mackill (2009). The cDNA concentration was about 40 ng for PCR amplification with SCoT primer (30 pmol). The PCR amplification and gel electrophoresis were carried out in Techni TC-512 Thermal Cycler and mini submarine gel BioRad respectively, as described by Abd El-Aziz *et al.* (2019). Besides, banding patterns were photographed using Bio-1D Gel Documentation system.

### 2.3. Gel reading and analysis of cDNA-SCoT expression banding patterns

cDNA-SCoT expression banding patterns were analyzed by GelAnalyzer3 (Egygene) software. Visible transcript-derived amplicons (TD-amplicons or cDNA-amplicons) identified by the cDNA-SCoT analysis were scored for each primer and entered in the form of a 0&1 binary data matrix. From this matrix, the cDNA-SCoT profile was performed according to Adhikari *et al.* (2015) with color discrimination for the three types of TD-amplicons: TD-amplicons whose enhanced their genes by one or more treatments (TDE-amplicons), TD-amplicons whose silenced their genes by one or more treatments (TDS-amplicons), and monomorphic TD-

amplicons with control (MTD-amplicons). Polymorphic information content (PIC) and resolving power (Rp) were calculated to evaluate SCoT-primers efficiency according to Gorji *et al.* (2011); Prevost and Wilkinson (1999) respectively. The band intensity of monomorphic TD-amplicons with control were measured using GelAnalyzer 2010 software for comparison on its basis among treatments and control

### 2.4. Semi-quantitative RT-PCR.

Specific primer pairs used for gene expression analysis were designed as follow:

For target gene menthofuran synthase (*mfs*) gene (Gene Bank: AF346833.1) according to Afkar *et al.* (2013) were:

*mfs* F: 5`-TCTACGCCTACATCCACCTTTC -3`  
*mfs* R: 5`-CCTCCGCTCTATCTCCATTACC -3`

For normalization of target gene expression level, actin-1 gene (*act*) (Gene Bank: KM044035.1) was used as an internal standard or reference gene according to Rahimi *et al.* (2017), the primer pairs for this gene were:

*act* F: 5`-CTACGAAGGCTACGCACTCC-3`  
*act* R: 5`-GCAATGTAGGCCAGCTTCTC-3`

These primers target specific amplicons of 341 bp for *mfs* gene and 165 bp for *act* gene and were procured from Biobasic Com. PCR was performed based on the cDNA samples with primers (5 pmol) and Master Mix (Gendirect, Germany). According to Martiansyah *et al.*, (2018), in Semi-quantitative RT-PCR analysis, the PCR product is clearly visible on an agarose gel at the 30<sup>th</sup> cycle and can be quantified in comparison to the reference gene. So, amplifications were performed with the following program: 94°C for 2 min, followed by 30 cycles of 94 °C for 45 s, 53 °C for *mfs* gene and 54 °C for *act* gene for 45 s, and 72 °C for 45 s. PCR products with 100-bp DNA ladder standards (Gendirect, Germany) were analyzed in 1.5% (w/v) agarose gel, stained with ethidium bromide, and photographed using Bio-1D Gel Documentation system. Also, according to Martiansyah *et al.* (2018), the band intensity for PCR products as expression units were measured using GelAnalyzer 2010 software.

Expression ratio (ER) was calculated by the equation:

ER= expression unit of target gene /expression unit of the reference gene.

The relative fold change (FC) can then be calculated between treated samples normalized to the reference gene by the equation:

FC= ER of treated sample/ ER of the control sample  
 Ultimately, this value was transformed to log2 according to Youssef *et al.* (2014).

### 2.5. Determination of volatile oil percentage

Volatile oil percentage (%) was estimated from all samples as described in the (British Pharmacopeia, 1963).

### 2.6. Determination of volatile oil composition:

Samples taken for the volatile oil were analyzed using DsChrom 6200 Gas Liquid Chromatography (GLC) equipped with a flame ionization detector for separation of volatile oil constituents as described by Bunzen *et al.*, 1969 and Hoftman, 1967.

### 2.7. Vegetative data:

At the end of season (after 3 months from bloom stage), data of vegetative traits were recorded on untreated and treated peppermint plants. These traits were: Plant height (cm), Fresh weight /plant (g), Dry weight/plant (g), Number of leaves/plant and Leaf area/plant (cm<sup>2</sup>)

The results of volatile oil percentage and vegetative data for treated and untreated plant were analyzed by one-way analysis of variance and comparison of means using the Tukey method and 95% confidence (at the level  $P < 0.05$ ) by Minitab 17.1.0 software.

## 3. RESULTS AND DISCUSSION

### 3.1. Differential expression analysis using cDNA-SCoT Technique:

In this study, cDNA-SCoT technique was applied for the first time to analyze the gene expression variations induced by exogenously foliar application of MeJA and JO treatments. The cDNA SCoT can be easily used to analyze the overall levels of the plant tissue transcriptions at the same time, which is especially important for differential expression analysis (Wu *et al.*, 2013). So, to

investigate whether MeJA and JO treatments (0.05 and 0.1 %) at different exposure periods (24 and 48 h) affect gene expression patterns, we used six SCoT primers that target highly expressed genes as described by Sawant *et al.* (1999) to target amplify cDNA derived from extracted RNA of treated and untreated plants.

Some molecular banding patterns and DNA profiles estimated from cDNA-SCoT technique were shown in Figures (1 and 2). It is evident from Figure 2 that different gene expression patterns between the untreated and treated plants with MeJA and JO were found. This was through targeting 33 scorable TD-amplicons with an average of 5.5 TD-amplicons/primer, with a product size ranged from 196 and 769 bp. From these TD-amplicons, twelve appeared in the untreated plants and they ranged between 10 to 28 TD-amplicons in treated plants. This agreed with Al-Qurainy *et al.* (2015), who investigated the response of Khalas cultivar of Phoenix dactylifera under different salt stress concentrations, where different amplicons of size were produced using the cDNA-SCoT markers in treated plants whereas were absent in control.

Out of the 33 TD-amplicons targeted by SCoT-primers used in this study, 21 were new transcripts (TDE-amplicons) with 63.6%. While the TDS-amplicons that expression silenced their genes in one or more treatments were 8 from 12 MTD-amplicons with 24.4% from all TD-amplicons. This indicates the high effect of MeJA and JO treatments in expression enhancing of some inactive genes in untreated plants. In addition, to their silenced effect for expression of some active genes targeted by SCoT-primers in untreated plants. Also Wu *et al.* (2013) employed the cDNA-SCoT approach to detect differential expression of gibberellin-induced genes for sugarcane stalk elongation, which indicated gene upregulation and downregulation.

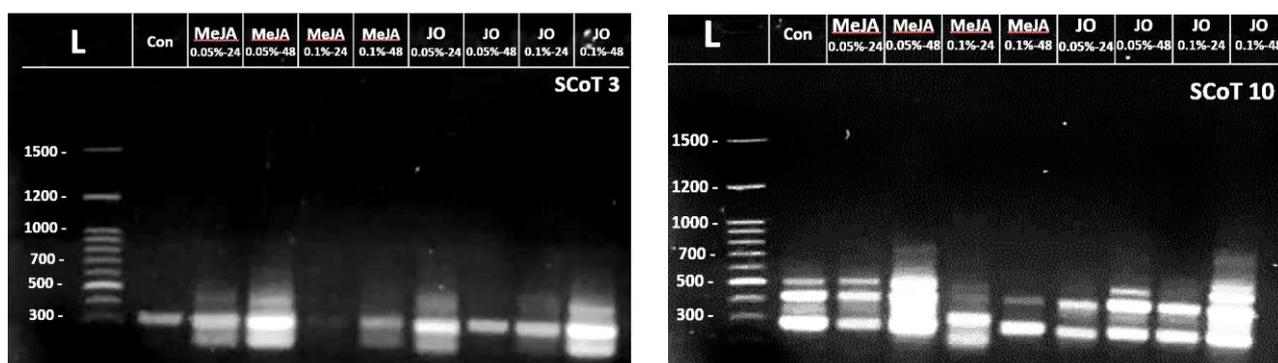
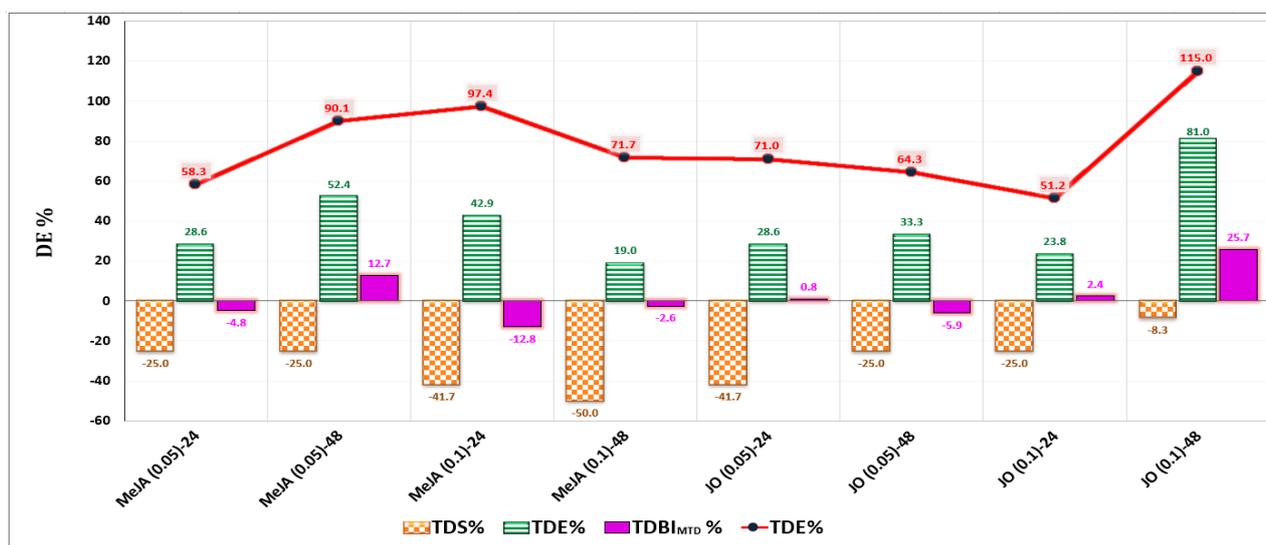


Figure 1. cDNA-SCoT amplification result of SCoT 3 and SCoT 10 after MeJA and JO treatments with two concentrations ( 0.05 % and 0.1 %) for two periods (24h and 48h) compared with control in *Mentha piprta*.





**Figure 3. Differential expression % (DE%) induced by MeJA and JO treatments attributable to control obtained by summing percentages of all types of differential TD-amplicons**

(TDS%: % of TD-amplicons whose silenced their genes by one or more treatments; TDE%:TD-amplicons whose enhanced their genes by one or more treatments; TDBI<sub>MTD</sub>%: % of total differences of MTD bands-intensity for each treatment attributing to the control).

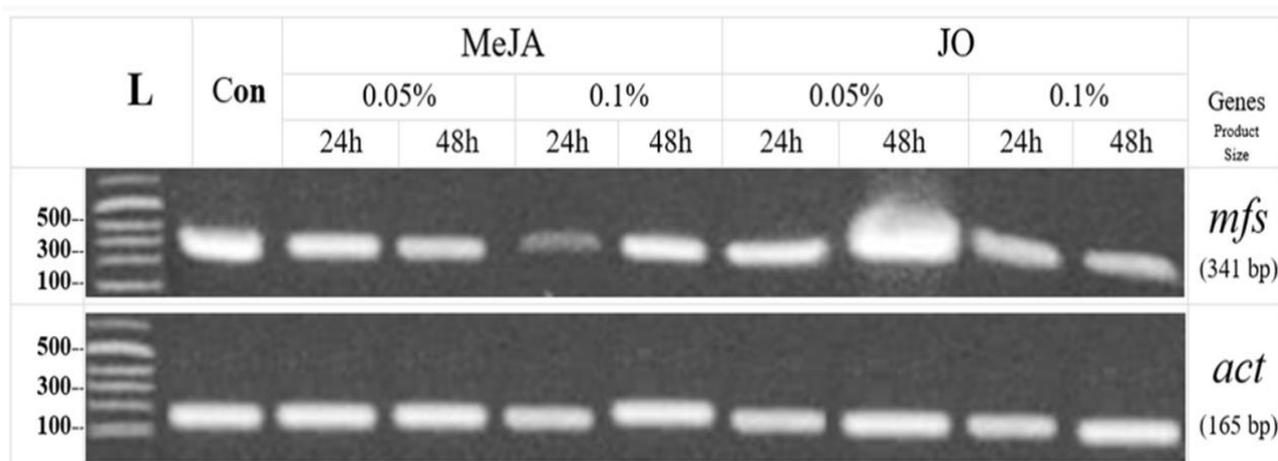
unique positive amplicons which were five (Figure 2) resulted from transcription enhancement by these two treatments only. Where three of them resulted by MeJA-0.1% at 24h, while two resulted by JO-0.1% at 48h. Indicating the higher influence of treated plants with Jasmine oil with concentration 0.1% at 48h followed by MeJA-0.1% at 24h on alteration of gene expression patterns.

Moreover, these results confirmed that cDNA-SCoT markers succeeded in detection of variable gene expression patterns between the untreated and treated *Mentha* plants with MeJA and JO as reported by HE *et al.* (2017). Who indicated that the cDNA-SCoT technique could effectively investigate the variations of gene expression in plant species. Also, Abd El-Maksoud *et al.* (2018) indicated that the cDNA-SCoT markers detected variation in gene expression patterns between untreated and treated wheat seedlings under drought stress. Likewise, Al-Taweel *et al.* (2019) found the same results between treated stevia plants with different NaCl concentrations and untreated plants.

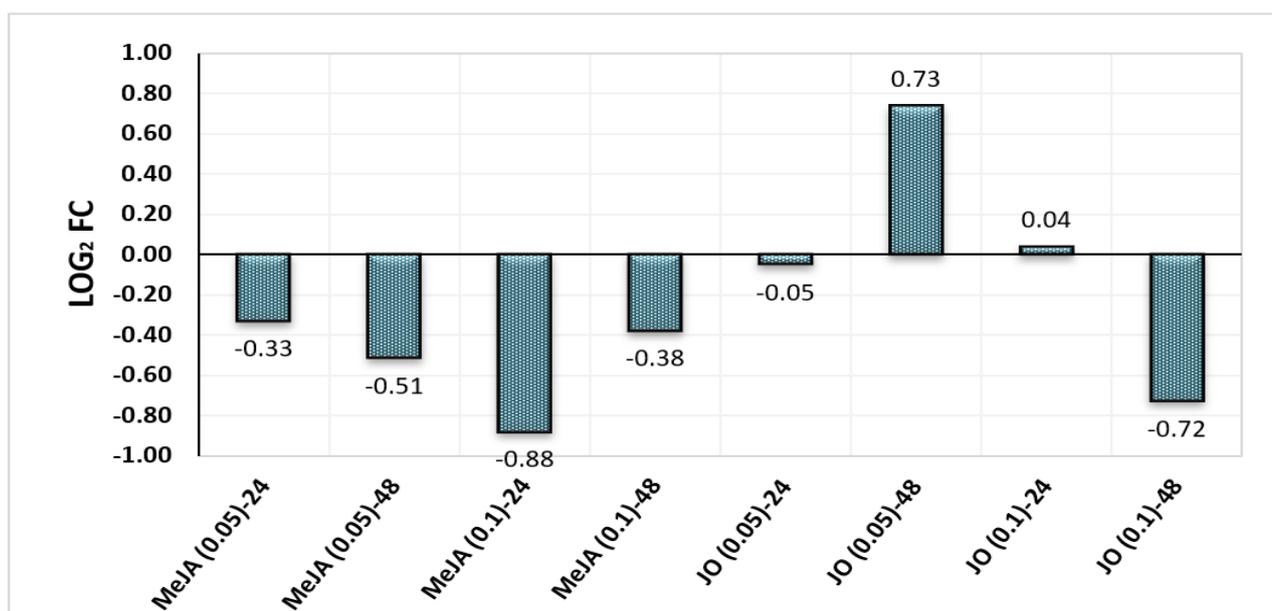
### 3.2. Semi-quantitative RT-PCR analysis for *mfs* gene

The semi-quantitative RT-PCR was successfully carried out on all cDNA samples representing untreated and treated plants. For each of the mRNA samples, 341-bp fragment of *mfs*-gene and 165-bp fragment of *act*-gene were specifically amplified. Using *act*-gene as an internal control or reference gene, the mRNA levels of the above two transcripts were determined among various cDNA samples (Figure 4). As shown in Figure 4, the band intensity of cDNA transcripts of *act-1* gene was stable throughout the cDNA-samples at different conditions which confirm the right choice of *act-1* gene as a reference gene. Meanwhile, visually varied *mfs*-transcripts throughout the various cDNA-samples can be attributed to that *mfs*-transcripts were differentially regulated by MeJA and JO treatments.

After converting the visual banding patterns of *mfs* and *act* genes into semi-quantified data using GelAnalyzer 2010 software, Figure 5 confirmed the differential expression of *mfs*-transcripts among different cDNA samples through the relative expression levels to the control as log<sub>2</sub>(FC) values. The expression of the *mfs* gene differed clearly in response to MeJA and JO under 0.05 and 0.1% concentration at 24 and 48 h after treatment induction. *mfs*-gene had the down-regulated gene expression compared with control in leaves samples treated except treated with JO 0.05 % at 48h and JO 0.1 % at 24h which recorded up-regulated gene expression with 0.73 and 0.03 log<sub>2</sub> FC, respectively. Down-regulation of the *mfs*-gene is preferred due to the menthofuran is a highly toxic organic compound in *Mentha* oil (Anderson *et al.*, 1996). Therefore, the better relative expression levels of *mfs*-gene (which more down-regulated) were recorded for treated with MeJA 0.1 % at 24h and JO 0.1 % at 48h which were -0.88 and -0.72 log<sub>2</sub> FC, respectively. Clearly, the expression of the *mfs*-gene was affected by the high percentages of differential expression that induced by treated with MeJA 0.1 % at 24h and



**Figure 4.** Semi-quantitative RT-PCR of *mfs* gene after MeJA and JO treatments with two concentrations (0.05 % and 0.1 %) for two periods (24h and 48h) compared with control in *Mentha piprta*. *act* gene served a reference gene ( internal control ) to normalize the *mfs* expression levels. L= ladder (100 bp).



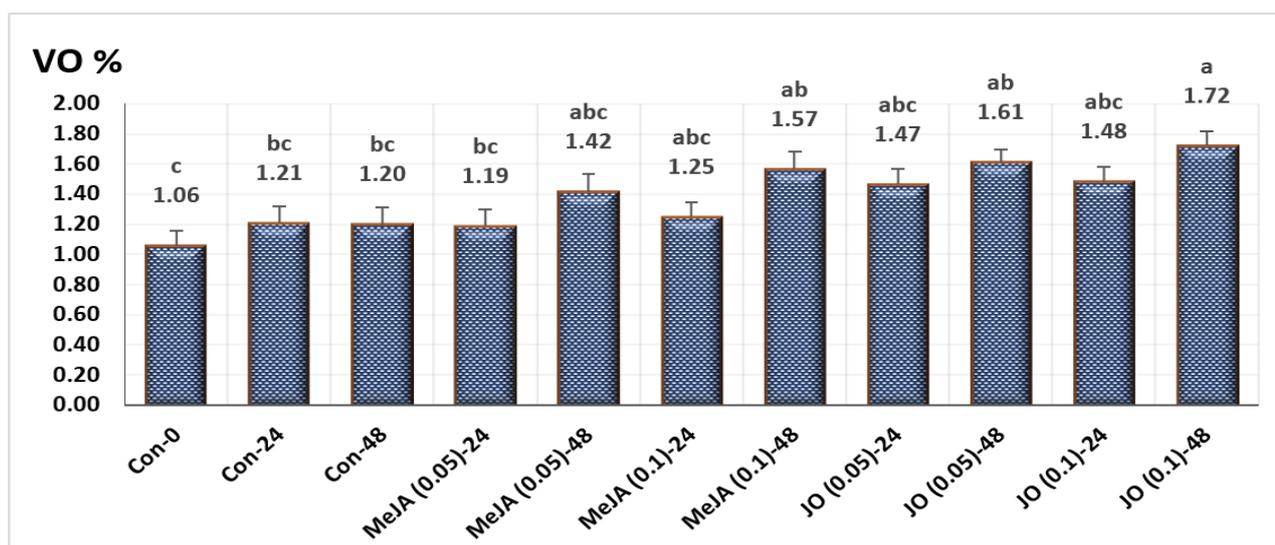
**Figure 5.** Relative expression levels to the control (Fold change log<sub>2</sub> transformed) using semi-quantitative RT-PCR for *mfs* gene normalized by *act*. Gene

JO 0.1 % at 48h. It also turns out that all MeJA treatments affected menthofuran expression, which was also happened by the treatment with low-cost jasmine oil at 0.1% concentration at 48h. Thus, we suggest that the 0.1 % jasmine oil spray onto the peppermint plants after 48h results in suppression of menthofuran expression, or the accelerated turnover of the *mfs* message like spraying with MeJA which increases the pharmacological quality of *Mentha* plant according to Afkar *et al.* (2013).

### 3.3. Volatile oil percentage and composition:

Data in Figure 6 clearly emphasized that treating *Mentha* plants with MeJA at 0.1 % and JO at 0.05 or 0.1 % after 48 h statistically increased volatile oil percentage as compared to control. The

highest volatile oil percentages were obtained from these plants applied with JO at 0.1 % after 48 h by 1.72%. Similar data had been reported for MeJA where plays a powerful role in inducing the secondary metabolites in different plants (Keinänen *et al.* 2001). The results of the GLC analysis of the volatile oils of *Mentha piperita* L were shown in Table1. The identified compounds are ranged from 92.22% at 0.05 % MeJA after 24 h to 95.76% at 0.1% MeJA after 48 h. Most compounds were from oxygenated monoterpenes type (10 compounds) and ranged from 77.53% in JO at 0.1 % after 48 h to 83.18% at 0.1% MeJA after 48 hours. While the Monoterpene hydrocarbons are represented in 6 compounds and ranged from 8.78% in JO at 0.05% after 48 h to 14.41% in JO at 0.1 % after 48 h.



**Figure 6.** MeJA and JO treatments effect on volatile oil yield percentage (%) of *Mentha piperita* L. plant. Different letters indicate significant differences according to Tukey method at  $P < 0.05$ ; Results represent mean  $\pm$  standard error (SE).

The  $\beta$ -caryophyllene was the only sesquiterpene hydrocarbons compound estimated and ranged from 0.28% in MeJA at 0.05% after 24 h to 3.69% in the control. However, there was only one alcohol compound, it was Octanol which ranged from 0.28% in the control to 1.52% in JO at 0.1% after 48 h. The main constituents of *Mentha* volatile oils in the untreated plants were Menthol (24.77%), Isomenthone (18.63%), Menthone (15.85%), Isomenthol (11.55%) and limonene (6.96%). The increase of menthol levels after the beginning of JO treatment at 0.05 and 0.1% concentrations and after 48 h for MeJA at 0.05 and 0.1% concentrations were resulted in a rapid response for plants to JO and MeJA and also for their ability of them to stimulate menthol biosynthesis. In this study, the level of the essential oil constituents varied with different concentrations of JO and MeJA, leading to increases or decreases in the various constituents percentage comparing control plants (Table 1). In this study, we measured the synthesis of volatile oil components in response to varied JO and MeJA concentrations (Table 2). The Menthol content in the oil was increased at least 1.05 to 1.49-fold at 0.05 % and 0.1 % JO treatments relative to the control (Figure 7). Menthol amount reduced after treated with MeJA at 0.05 % and 0.1 % after 24 h but after 48 h increased compared to the control especially at 0.05 % which observed the highest amount of menthol relative to the control (37.67%, 1.52-fold).

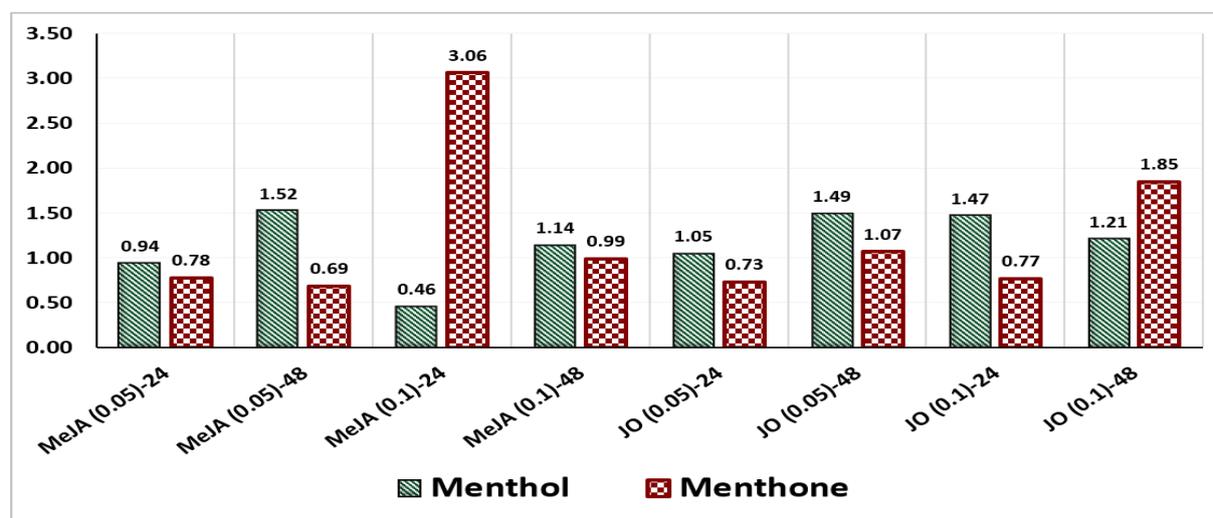
Menthol is an important major *Mentha* oil component due to its many important pharmaceutical and industry applications (Kamatou *et al.*, 2013). Besides, *Mentha* essential oil is

associated with some adverse effects (Peixoto *et al.*, 2009). Potentially toxic compounds in *Mentha* oil are pulegone and menthol. Pulegone and its metabolite menthofuran, are found in peppermint in much smaller proportions. Menthone is a constituent of the essential oils of *Mentha*. It is structurally related to menthol and industrially used in perfume and cosmetics for its characteristic aromatic and minty odor. However, the excessive inhalation of mentholated preparation may cause reversible nausea, anorexia, cardiac problems, ataxia, and other central nervous system problems (Balakrishnan, 2015). Therefore, the quality of the essential oil from peppermint is described by high menthol and low menthone levels as well as pulegone and menthofuran in very much smaller percentages (Rohloff *et al.*, 2005).

Results in Figure 7 indicated that the Menthone content in the oil was decreased compared to the control from 0.78 and 3.06-fold to 0.69 and 0.99-fold after 24h and 48h from spray for MeJA by 0.05% and 0.1%, respectively. Exactly as we expected because the menthone was a precursor of menthol, which was identified by the previous reports indicating that changes in constituents of essential oil were noticed in many aromatic plants under stress, although these changes in constituents appeared to differ among various plants and the different stresses (Baher *et al.*, 2002; Sangwan *et al.*, 1993). On the contrary, it was the response with JO treatments. Where the Menthone content in the oil was increased compared to the control after 48h at 0.05% and 0.1% JO (from 0.73 and 0.77-fold after 24h to 1.07 and 1.85-fold after 48h, respectively).

**Table 2. Effect of Methyl Jasmonate (MJ) and Jasmine oil (JO) treatments on volatile oil compositions (%) of *Mentha piperita* L. plant.**

Peak No.	Components	Retention time (min)	Con	MeJA				JO			
				0.05%		0.1%		0.05%		0.1%	
				24h	48h	24h	48h	24h	48h	24h	48h
1	$\alpha$ -pinene	2.62	0.93	1.11	1.02	1.2	1.18	0.93	1.01	0.87	0.56
2	$\beta$ -pinene	2.83	0.75	1.59	0.8	1.12	1.25	0.81	0.81	0.76	0.86
3	Phellandrene	2.87	0.53	0.76	0.69	0.84	1.14	0.76	0.68	0.6	2.98
4	Limonene	3.16	6.96	9.18	5.98	9.66	6.25	6.92	4.84	5.97	9.39
5	$\gamma$ -terpinene	3.48	0.41	0.35	0.44	0.38	0.38	0.83	0.83	0.39	0.32
6	P-cymene	3.67	0.35	0.37	1.39	0.77	0.46	0.64	0.61	0.61	0.3
7	1,8-cineol	3.21	1.79	2.15	1.73	4.58	3.19	1.39	1.37	1.14	1.88
8	L-Menthone	3.85	0.44	0.43	1.32	1.32	0.61	1.76	0.59	0.82	1.19
9	Menthyl acetate	4.24	2.13	2.77	1.09	1.88	0.95	2.3	2.69	1.35	2.44
10	Menthol	4.33	24.77	23.27	37.67	11.44	28.19	25.9	36.93	36.35	29.88
11	Isomenthone	4.44	18.63	26.62	11.54	1.41	21.35	20.97	9.71	15.36	3.61
12	Menthone	4.53	15.35	11.97	10.55	47.03	15.24	11.27	16.44	11.83	28.41
13	Isomenthol	4.59	11.55	8.28	9.93	3.56	10.26	11.79	11.73	12.53	4.69
14	Neoisomenthol	4.66	1.35	1.76	1.49	2.8	1.59	1.25	1.74	1.95	3.83
15	Linalool	4.81	1.72	0.5	0.73	1.23	1.47	1.89	1.27	1.16	1.3
16	Carvone	5.38	1.78	0.31	1.76	1.16	0.33	1.99	0.48	0.41	0.3
17	$\beta$ -caryophyllene	5.37	3.69	0.28	3.56	1.21	0.87	2.66	0.96	0.65	0.44
18	Octanol	3.97	0.28	0.52	1.1	0.89	1.05	0.95	1.4	0.6	1.52
Total Identified			93.41	92.22	92.79	92.48	95.76	95.01	94.09	93.35	93.9
<b>Unknown</b>			6.59	7.78	7.21	7.52	4.24	4.99	5.91	6.65	6.1
<b>(1:6) Monoterpene hydrocarbons</b>			9.93	13.36	10.32	13.97	10.66	10.89	8.78	9.2	14.41
<b>(7:16) Oxygenated monoterpenes</b>			79.51	78.06	77.81	76.41	83.18	80.51	82.95	82.9	77.53
<b>(17) Sesquiterpene hydrocarbons</b>			3.69	0.28	3.56	1.21	0.87	2.66	0.96	0.65	0.44
<b>(18) Alcohol</b>			0.28	0.52	1.1	0.89	1.05	0.95	1.4	0.6	1.52



**Figure 7. Menthol and Menthone content in the oil relative to the control in response to treatment with JO and MeJA.**

This indicates that the treatment with JO requires the collection of samples to oil extract within 24 h to prevent further accumulation of Menthone, which is not required by treatment with MeJA. The lowest amount of menthone was after

48h from spray for MeJA by 0.05% and after 24h from spray for JO by 0.1%.

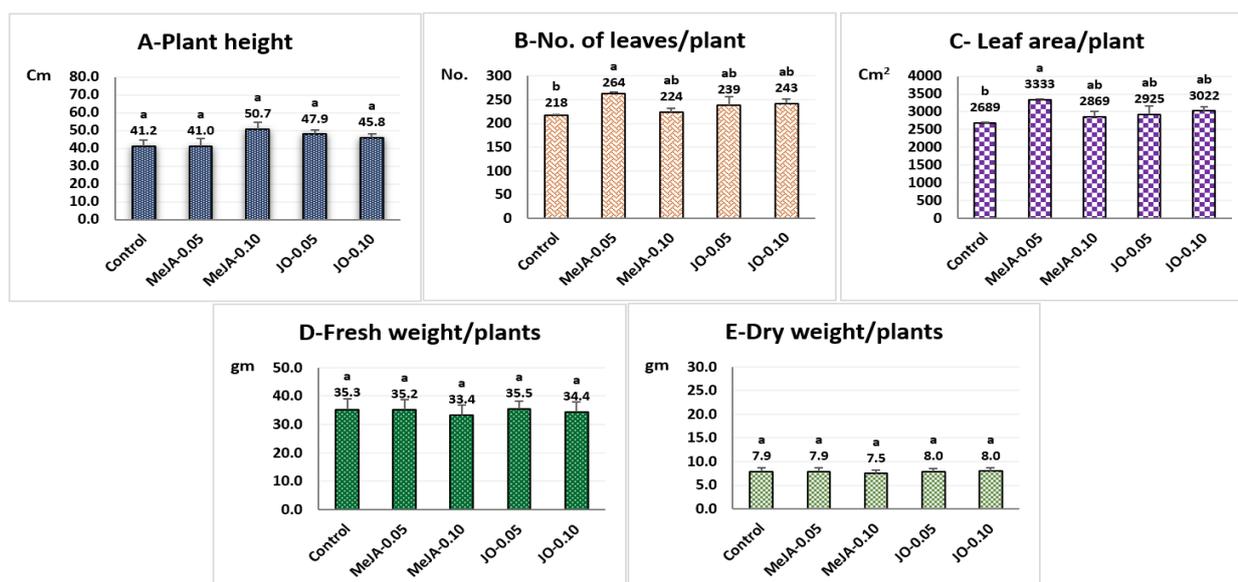
Our results represented that, application of MeJA and JO elicit menthol and as a secondary metabolite. Also, revealed that, the highest percentages of menthol with the lowest percentages

of the menthone and low level of menthofuran gene expression compared to the control, were found after 48 h from applying the spray for MeJA by 0.05% and after 24 h from applying the spray for JO by 0.1%. Previous research has shown that exogenous MeJA treatment can enhance the concentration of secondary metabolites in plants including *Ocimum basilicum*, *Nicotiana attenuata*, and *Rubus carolinianus*. (Kim *et al.*, 2007).

### 3.4. Vegetative traits:

At the end of the experiment, five vegetative traits were estimated for untreated and treated peppermint plants (Figure 8), to assess the vegetative growth under the treatment of MeJA and

JO. Where, the results presented in Figure 8 showed that there were no significant differences among all treatments in all the estimated vegetative traits. Likewise, between treated and untreated plants, except between treated plants with MeJA by 0.05% and untreated plants for leaf area/plant (cm<sup>2</sup>) and number of leaves/plant. Where the treated plants showed a significant increase compared to the control with an average of 264 leaves per plant and an average leaf area of 3333 cm<sup>2</sup>. In addition, most of the MeJA and JO treatments induced higher vegetative growth activity than the untreated plants but were not significantly.



**Figure 8. Effect of MeJA and JO treatments on Vegetative traits of *Mentha piperita* L. plant. Different letters indicate significant differences according to Tukey method at  $P < 0.05$ ; Results represent mean  $\pm$  standard error (SE).**

Finally, the vegetative traits results concluded that application of exogenous Methyl Jasmonate (MeJA) and Jasmine oil (JO) treatments enhanced the vegetative growth terms plants but were not significantly. These results are in accordance to (Rahimi *et al.*, 2013) on cumin plants reporting that all concentrations of MeJA had no significant effect on growth and yield parameters.

## 4. CONCLUSION

In general, exogenously foliar application of Methyl Jasmonate (MeJA) and Jasmine oil (JO) treatments induced alteration of gene expression patterns which was detected by cDNA-SCoT with high informative and discrimination capability. Also, mfs-transcripts were differentially regulated by MeJA and JO treatments. Our results revealed that high level of volatile oil, highest percentages of menthol with the lowest percentages of the

menthone, low level of menthofuran gene expression and good vegetative growth compared to the control were found with exogenously foliar application of MeJA by 0.05% after 48 h and JO by 0.1% after 24 h, which increases the pharmacological quality of *Mentha* plant. This indicates the possibility of using JO (Low-cost) after further study as a stimulant for the activity of menthol production with high quality as a cheaper alternative to exogenously foliar application of MeJA.

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Sukar; writing — review and editing, M. H. Abd EL-Aziz, S. Y. Mohamed and Noha A. Sukar; All authors have read and agreed to the published version of the manuscript.

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## الملخص العربي

### تأثير زيت الياسمين والميثيل جاسمونيت على التعبير الجيني وإنتاج المنثول في النعناع

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النعناع هو المصدر الرئيسي للمنثول والذي يستخدم في العديد من الأغراض التجارية والصيدلانية الهامة. لتعزيز إنتاج المنثول ، هدفت الدراسة إلى تقييم تأثير التطبيق الورقي الخارجي برش مخففات زيت الياسمين والميثيل جاسمونيت بنسبة ٠.٠٥ و ٠.٠١٪ على التعبير الجيني ، وإنتاج الزيوت الطيارة ومكوناتها خاصة المنثول في النعناع. حيث أدت معاملات الميثيل جاسمونيت وزيت الياسمين إلى تغيير أنماط التعبير الجيني التي تم تقديرها بواسطة تقنية cDNA-SCoT مع قدرة تعريف وتمييز مرتفعة للتباين على المستوى الجزيئي. حيث استهدفت البادئات المستخدمة في هذه التقنية تضخيم ٣٣ تتابع من المستسخات العكسية للحامض النووي الريبوزي الرسول ظهرت اضحة في صورة حزم على جل التفريد الكهربى ، كان ٢١ منها حزمة جديدة ومنتوعة بين المعاملات . وقد أوضح تحليل أنماط التعبير الجيني المتغير والمقارن بين المعاملات أن التأثير الأعلى لإستحداث التغيير في التعبير الجيني كان لزيت الياسمين بتركيز ٠.٠١٪ عند ٤٨ ساعة يليه الميثيل جاسمونيت بتركيز ٠.٠١٪ عند ٢٤ ساعة. ومن ناحية أخرى، بالنسبة لنشاط التعبير الجيني للجين المسئول عن تكوين المنثوفورين تم تنظيمه بشكل متغاير بواسطة معاملات الميثيل جاسمونيت وزيت الياسمين. وبشكل عام ، كشفت النتائج عن مستوى مرتفع من الزيت المتطاير ، ونسب عالية من المنثول (زيادة ١.٥٢ و ١.٤٧) مع انخفاض نسب المنثون ( بقيمة إنخفاض ٠.٦٩ و ٠.٧٧) على التوالي مقارنة بالمعاملة القياسية ، مع أدنى مستوى من التعبير الجيني المقارن للمنثوفورين بقيمة تغير نسبي لوغارتمى سالبة مقدارها (-٠.٨٨ و -٠.٧٢) مقارنة بالمعاملة القياسية . إلى جانب ذلك ، لوحظ نمو خضري جيد مع التطبيق الورقي الخارجي للميثيل جاسمونيت بنسبة ٠.٠٥٪ بعد ٤٨ ساعة و لزيت الياسمين بنسبة ٠.٠١٪ بعد ٢٤ ساعة مقارنة بالمعاملة القياسية ، مما يزيد من الجودة الصيدلانية لنبات النعناع. الأمر الذى يشير إلى إمكانية استخدام زيت الياسمين المنخفض التكلفة بعد مزيد من الدراسة كمحفز لنشاط إنتاج المنثول بجودة صيدلانية مرتفعة وكبديل منخفض التكلفة للتطبيق الورقي الخارجي للميثيل جاسمونيت.