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Influence of phytohormone on growth and active constituents ofsage *(Salvia officinalis),* parsley (*Petroselinum crispum)* and nasturtium (*Tropaeolummajus*)

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Abstract: A field experiment was conducted to study the effect of foliar application of methyl jasmonate on growth traits, essential oil content and oil composition of sage *(Salvia officinalis)* and curly parsley (*Petroselinum crispum)* plants cultivated in Egypt. Also, growth traitsand glucotropaeolincontent of nasturtium (*Tropaeolummajus*) were investigated.

Nasturtium is the most affected plant by methyl jasmonate application. Methyl jasmonate applied as foliar sprays at 100 and 200 ppm exhibited positive effects on all the growth parameters. Glucotropaeolin content in plants treated with 100 or 200 ppm methyl-jasmonate was higher than that found in control plants.

Methyl jasmonate had no significant effect with regard to oil percent % and dry matter % of sage and parsley. Parsley was less responsive to methyl jasmonate application than nasturtium. Foliar application of 200 ppm methyl jasmonate significantly improved myristicin content by 42% in the oil, but reduced the remaining components. Parsley plants sprayed with 100 ppm methyl jasmonate showed an increase in β-phellandrene content of the oil by about 79%, with a corresponding reduction in the content of 1,3menthatriene by 19%.

Methyl jasmonate had no significant effect on all growth parameters of sage plants. The lower concentration of methyl jasmonate (100 ppm) resulted in the lowest total terpene contentand the maximum value was obtained from control plants. Application of methyl jasmonate had no significant effect on the major components of sage essential oil.

Keywords: Sage, Parsley, Nasturtium, Phytohormones, Essential oil**,** Flavones, Glucotropaeolin.

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Introduction

Nasturtium is considered as an invasive species in many countries and it is recognized as a medicinal crop. Nasturtium (*Tropaeolummajus*) which is a herb indigenous to South America and it is used in mixture with other products as herbal medicine against urinary tract infections in Germany.Several pharmacological studies have demonstrated that the leaves of nasturtium were the primary site of benzyl glucosinolate synthesis and it contain high amounts of the glucosinolateglucotropaeolin that possess wide pharmacological properties such as antibacterial, antifungal, antiseptic, aperient, depurative, expectorant, purgative, vulnerary, antineoplastic, demulcent, laxative and stimulant activities[1,2]. Additionally, extracts and preparations have natriuretic and diuretic, hepatoprotective, anti-inflammatory activities[3,4,5].

The American Herb Society (http://www.herbsociety.org/herbs/) lists parsley and sage as of the ten most popular culinary herbs. Sage (*Salvia officinalis* L.) is a plant of the lamiaceae family with highly valued aromatic and medicinal properties. It is used as a culinary herb and spice and utilized in food formulations as food flavoring, preservative against food spoilage[6,7]. Furthermore, it is employed even as a fragrance in cosmetics and perfumes production.

Sage has been extensively used for remedial purposes for the treatment of various ailments as carminative, diuretic, antiheroic, analgesic, expectorant, disinfectant. Furthermore, the antioxidant and anti-inflammatory activitiesof sage leaves and its essential oil have been documented[8,9].

Sage essential oil from various geographical origins contains numerous volatile compounds, including α- thujone, β-thujone, camphor, and 1,8-cineole[10]in addition, various non-volatile phenolic content such as carnosic acid, carnosol and rosmarinic acid [11].

Parsley (*Petroselinum crispum* L.) as a medicinal plant, it is used as a carminative, diuretic, hypertensive, hypotensive, stomachic, nervine, emmenagogic, abortifacient and nutritive agent[12]. Antimicrobial and weak antioxidant activities of parsley essential oil have been reported[13].

The characteristic aroma of curly parsley leaves was initially associated with the major components, namely, myristicin, 1,3,8-p-menthatriene, β-phellandrene and myrcene and with the absence of myrcene and 1,3,8-p-menthatriene, there was a deterioration of the parsley aroma[14].

Since 1940 natural and synthetic growth regulators (GR) have been used in agriculture to control developmental processes like germination, vegetative growth, reproduction, maturation, senescence and post-harvest[15]. Jasmonic acid (JA) and its methyl ester methyl jasmonate (MeJA) which were first identified in the essential oils of jasmine and rosemary and collectively termedjasmonates. Many recent studies have demonstrated that jasmonateshave a fundamental role in the regulation of the biosynthesis of all three major classes of secondary metabolites (i.e. terpenoids, phenyl propanoids, and alkaloids) through an extensive transcriptional reprogramming of the plant metabolism[16]. Also, Jasmonates appear to involve in many critical functions, including regulate the expression of plant defense genes in response to various environmental stresses, defense against insects and pathogens by inducing phytoalexin production, plant growth and development, suggesting that they have critical roles in plant physiology[17, 18].Jasmonatesand MeJAare known to have various effects on the growth of cells, for example,they control cell division and cell elongation and promote cell expansionthereby lead to defined shapes of organs or tissues[19].

Few studies have been done to investigate the effect of foliar application of JA and/or MeJA on the accumulation of secondary metabolites in aromatic and medical plants in agricultural systems. For example, the maximum level of diterpenoidsin sage was achieved at 3 days after elicitation with 20 μMMeJA[20]. Two fold higher of rosmarinic acid amount was achieved with 50 or 100 μMMeJA on the 5th day after elicitation. Also, MeJA induced methyl chavicol production 48% higher than those in control plants at 120 h after initiation of treatment[21].

The essential oil production depends not only on genetic factors and the developmental stage of plants, but also on environmental factors which could result in biochemical and physiological alterations in plants modifying the quantity and quality of the essential oil. Among other factors influencing essential oil production are plant growth regulators or plant hormones. Endogenous levels as well exogenous application could affect essential oil production and chemical composition[15].

Therefore, the objective of this study were to evaluate the effects of various concentrations of MeJA on plant growth, essential oil contents and its composition of sage and parsley in addition to growth and glucotropaeolin content of nasturtium.

Materials and Methods

To investigate the influence of phytohormoneson growth and the concentration of secondary compounds of parsley (essential oils, flavones, terpenes), sage (terpenes) and nasturtium (glucosinolates), seeds of the three experimental plants were directly cultivated at the middle of October 2012 and 2013 seasons in pots

(40 cm diameter). Aqueous MeJAsolutions (100, 200 ppm) and triton x as control were applied three weeks before the harvest. Plants had been cultivated under well-watered conditions (100% field capacity). Phytohormones were sprayed by a hand pump as it is commonly used for pest control.

Representing samples of fresh herbs of each replicate were subjected to hydro-distillation for 3 hours using Clevenger apparatus to extract and to determine essential oil percent according to Egyptian Pharmacopoeia[22] and recorded on the basis of oil volume to herb fresh weight (ml/100g fresh herb). The essential oil was separately dehydrated over anhydrous sodium sulphate and kept in silica vials and stored at 2^{o}C till GLC analysis. Samples of each treatmentwere subsequently analyzed using GLC(Perkin Elmer Autosampler 2000).

The extracted oil has been diluted with n-hexane, injected into a GLC using an auto-sampler and the different compounds have been separated on a HP-INNOWAX (60 x 0.25 x 0.25 µ m) capillary column. Helium was used as carrier gas (flow rate 1.5 ml/min). The temperature programme was; 35 ^{o}C to 230 ^{o}C (2.5 ^{o}C/min) in course of time (92 min). Injector and flame ionization detector temperature were 250 ^{o}Cand 300 ^{o}C, respectively. Area percentages were obtained using a PC programme (Maestro chromatography data system). For identification of single compounds internal and external standard substances have been used.

HPLC analysis of glucotropaeolin was performed using a RP 18 column (250 x 4 mm)[23]. Based on the peak areas of glucotropaeolin and the internal standard, the amounts of glucotropaeolin were calculated. Total flavone concentration was also determined[24].

The collected data were subjected to the analysis of variance in Randomized Complete Block Design (RCBD) arrangement[25]using MSTAT-C V.2.1 software package[26]. Differences among means were compared for each trait by Duncan multiple range test (DNMRT)[27].

Results and Discussion

Effect of methyl jasmonate on nasturtium:

Results in Table (1) revealed that nasturtium plants are sensitive to the application of methyl jasmonate (MeJA). The growth parameters of nasturtium plants were significantly affected by the foliar sprays of MeJA at 100 and 200 ppm. Although, MeJA at 200 ppm gave the maximum values of plant height, leaves number, plant fresh and dry weights, no significant differences were observed between100 and 200 ppm. MeJAhad no significant effect with regard to dry matter content %. The positive effect of MeJAon growth may be due to that Jasmonates modulates the expression of numerous genes and influences specific aspects of plant growth, development, and responses to abiotic and biotic stresses[17].

Table (1): Effect of methyl jasmonate on the growth of nasturtium

Methyl jasmonate treatments	Plant height (cm)	Leaves number	Fresh eight g plant^{-1}	Dry weight g plant^{-1}	Dry matter (%)
		1st season			
Triton-X (control)	39.05 b	25.00 b	43.51 b	4.06 b	9.947 a
100 ppm	44.75 a	30.00 a	55.64 a	5.49 a	10.34 a
200 ppm	44.00 a	30.00 a	59.35 a	6.22 a	10.33 a
		2nd season			
Triton-X (control)	43.89 b	23.00 b	36.54 b	3.22 b	9.51 a
100 ppm	47.83 a	27.00 b	55.07 a	5.12 a	9.91 a
200 ppm	50.33 a	32.00 a	61.17 a	6.12 a	10.47 a

- Numbers with different characters statistically different at the 5% level.

MeJA applications significantly increased the glucotropaeolin content in nasturtium (Fig.1). Glucotropaeolin content in the plants which treated with 100 or 200 ppm MeJAwas highcompared to untreated plants. The lower application of MeJA (100 ppm) showed a slight increase in glucotropaeolin content contrast to the application of 200 ppm MeJA which resulted in a greater increase of about 17%.

Previous reports have shown an increase in the production of secondary products following treatment with methyl jasmonate. Exogenous jasmonic acid has been used to induce useful secondary metabolites such as alkaloids, terpenoids, and phenolics in some plants such as *Nicotina* species, *Hyoscyamusmuticus*, Norway spruce stems, and some plant cell cultures[28].

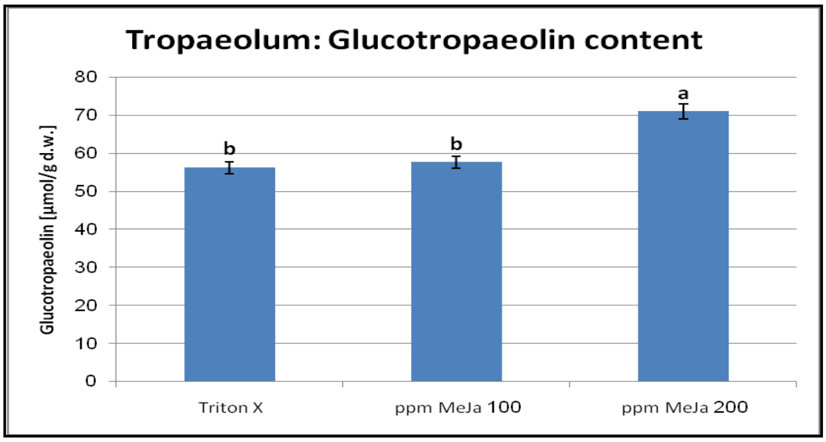

Fig. (1): Effect of methyl jasmonateon glucotropaeolincontent of nasturtium.

Effect of methyl jasmonate on parsley:

In general, parsley was a less responsive to phytohormone (MeJA) than nasturtium in this study (Table 2). MeJA at higher concentration increased plant height, leaves number, plant fresh and dry weights of parsley. The highest Plant height, Leaves number, plant fresh and dry weights were achieved by foliar spray 200 ppm MeJA yet, the values failed to be significant in some cases compared to control.

Table (2): Effect of methyl jasmonate on the growth of parsley

Methyl jasmonate treatments	Plant height (cm)	Essential oil (%)	Fresh weight g plant^{-1}	Dry weight g plant^{-1}	Dry matter (%)
		1st season			
Triton-X (control)	19.44 b	0.17 a	7.06 a	0.90 b	17.67 a
100 ppm	22.66 a	0.17 a	7.57 a	1.03 b	18.02 a
200 ppm	23.68 a	0.17 a	9.67 a	1.83 a	17.82 a
		2nd season			
Triton-X (control)	21.83 a	0.18 a	5.19 b	0.80 b	23.27 a
100 ppm	23.50 a	0.17 a	7.11 b	1.04 b	22.40 a
200 ppm	24.17 a	0.18 a	10.27 a	1.54 a	21.56 a

- Numbers with different characters statistically different at the 5% level.

The most pronounced effect of MeJA was on plant dry weight, since200 ppm MeJA resulted in the increments of 37 and 103 % in plant fresh and dry weights compared to controls respectively, in the first seasonand 98 and 37 % in the second season for the same respective parameters. MeJA had no significant effect with regard to oil percent % and dry matter content %. The flavone concentrations were also not affected by the application of MeJA (Fig.2).Similar results were obtained and revealed that the different levels of the foliar application of JA didnot have significant impacts on total dry matter or hypericin content of St. John's Wort plant[18].

The differences in plant dry weight are probably due to the differences in plant fresh weight not due to the dry matter content which was nearly the same in all treatments.

The negative effect of MeJA on oil content was contrary to another report[29] in which MeJa at concentration of 0.1 mM showed higher amount of essential oil contentafter 24 h of treatment (1.51%) and 48 h (2.42%) and 72 h (1.92%) of treatment compared with control plants of those times.

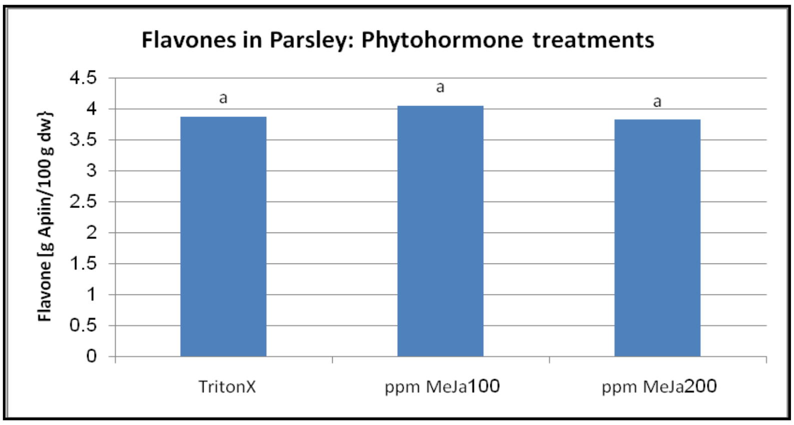

Fig (2): Effect of methyl jasmonateon flavone content of parsley.

The GLC analysis of parsley essential oil in Table (3) showed that nine hydrocarbon compounds were identified, representing about 97-98% of the total essential oil. The main components of the oil were myristicin (47.92%) followed by β-phellandrene (14.35 %) and 1,3menthatriene (12.56 %). Myrcene, cymene, terpinolene, limonene, β -elemene and sesquiphellandrene were present in smaller amounts and other components were less than 1%.

Plants treated with MeJAshowed higher percentage of some components in the parsley essential oil than untreated plants. Foliar application of 200 ppm MeJA significantly improved myristicin content by 42% in the oil, but reduced the remaining components. Parsley plants sprayed with 100 ppm MeJA showed an increase in β-phellandrene content of the oil by about 79%, with a corresponding reduction in the content of 1,3menthatriene by 19% while the amount of myristicin remained stable.

The increase in myristicin content in plants treated with 200 ppm MeJA may be due to that the increment in phenylpropanoid pathway products derived from phenylalanine ammonia-lyase (PAL), as well as the increase in the number of transcripts of the enzymes present in subsequent steps of the pathway[30].

Table (3): Effect of methyl jasmonate on chemical composition of essential oil of parsley

Compound	Triton x	100 ppm MeJA	200 ppm MeJA
Myrcene	6.22	3.96	2.545
Limonene	2.39	1.38	0.915
β-Phellandrene	14.35	25.73	11.605
Terpinolene	4.66	2.20	2.065
Cymene	4.77	1.56	2.235
1,3 Menthatriene	12.56	4.33	4.14
β -Elemene	2.03	2.33	3.02
Sesquiphellandrene	2.29	1.80	2.39
Myristicin	47.92	46.17	68.415

The results of this study are consistent with previous work on *Agastachefoeniculum*, basil and *Menth apiperita* where exogenous application of MeJA increases the amount of some componentsand reduce the amount of others [28,29,31]. In addition, other report on *Iva frutescens* indicated that application of MeJA (50 µL) resulted in an increase of 14, 5 and 8-fold on α-pinene, sabinene and limonene emission, respectively and that could be a result of terpene synthase activation and *de novo* synthesis. MeJAactivates a range of pathways in *Iva frutescens* like shykimate, octadenoid, mevalonate and methylerythritol-4 phosphate[32].

Total content of terpenes and phenylpropanoids was affected by MeJA application. There was an increase in the terpene value in plants treated with 100 ppm MeJA then the value declined in plants treated with 200 ppm MeJA but it was still superior to the control (Fig. 3).

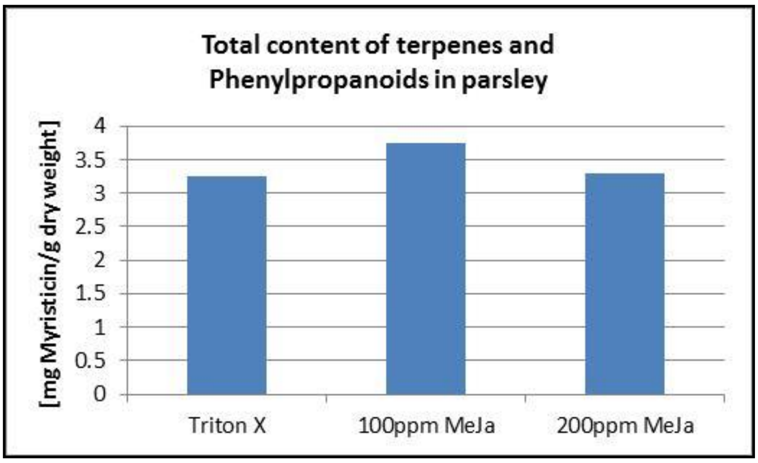

Fig (3): Effect of methyl jasmonate on total content of terpenes and phenylpropanoids of parsley.

Effect of methyl jasmonate on sage:

With regard to sage data in Table (4) the treatments had different effects from the first season to the second one since MeJA had no significant effect on all growth parameters of sage plants in the first season.The highest plant height, oil content and plant fresh weight were obtained from the application of 200 ppm MeJA in the second season.

Table (4): Effect of methyl jasmonate on the growth of sage

Methyl jasmonate treatments	Plant height (cm)	Essential oil (%)	Fresh weight g plant^{-1}	Dry weight g plant^{-1}	Dry matter (%)
		1st season			
Triton-X (control)	20.41 a	0.26 a	10.10 a	2.49 a	23.01 a
100 ppm	19.33 a	0.28 a	8.61 a	1.99 a	24.70 a
200 ppm	18.58 a	0.25 a	8.35 a	1.92 a	24.35 a
		2nd season			
Triton-X (control)	23.30 b	0.33 ab	7.26 b	1.45 a	22.55 a
100 ppm	24.20 b	0.30 b	6.87 b	1.43 a	23.35 a
200 ppm	28.40 a	0.35 a	8.45 a	1.60 a	21.99 a

- Numbers with different characters statistically different at the 5% level.

These results are in agreement with those of other authors who also demonstrated that foliar application of JA had no significant effect on growth and development of herbage dry weight of *Hyssopusofficnalis and Thymus daenensis*Celak, under greenhouse conditions[33,34].

Data in figure (4) indicated that application of MeJA has little effect on terpene content of sage. Exposure of sage plants to MeJA led to a somewhat lower concentration of terpenes in comparison with the control plants.

The lower concentration of MeJA (100 ppm) resulted in the lowest total terpene contentand the maximum value was obtained from control plants. These results disagreed with those obtained on basil where the content of terpenes in plants treated with MeJAwas higher than that found in control plants[30].

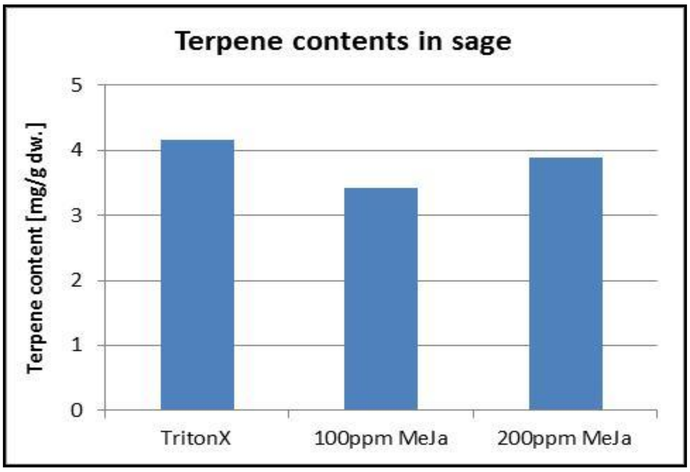

Fig. (4) : Effect of methyl jasmonate on terpene content of sage.

The GLC analysis of sage essential oil in Table (5) showed that eleven hydrocarbon compounds were identified, representing about 97 % of the total oil. The main components of the oil were α -thujone (39.88 %) followed by camphor (18.86 %),β -thujone (10.50 %) and 1,8-cineol (9.20 %). Viridiflorol, α -humulene, β-pinene, camphene, α-Pinene, β -caryophyllene and manool were present in smaller amounts and other components were less than 1%.

Results of this study indicated that the highest contents of α -thujone and β -thujone were obtained from untreated plants. Application of MeJA had no significant effect on the major components of sage essential oil. The effect of MeJA on the growth, oil content and oil constituents of sage in this study was somewhat similar to those previously reported on cuminwhere all concentrations of MeJA had no significant effect on growth and yield parameters or essential oil yield[35].

Table (5): Effect of methyl jasmonate on chemical composition of essential oil of sage

Compound	Triton x	100 ppm MeJA	200 ppm MeJA
α-Pinene	1.985	2.405	2.18
Camphene	2.56	3.175	3.065
β-Pinene	2.69	3.27	2.935
1,8-Cineol	9.195	9.39	9.705
α -Thujone	39.88	37.735	36.645
β -Thujone	10.5	7.76	10.24
Camphor	18.855	19.135	17.375
β -Caryophyllene	1.665	1.635	1.76
α -Humulene	3.95	4.135	4.435
Viridiflorol	4.82	5.46	5.685
Manool	1.01	2.71	3.09

Few studies have been conducted on the effect of phytohormones on plant growth and active ingredients of medicinal and aromatic plants, and the results of these studies varied depending on the type of the plants and its components.The above data is supported by reports on *S. hortensis* where different levels of JA had no significant effects on oil yield and dry herbage whileit had only significant impacts on chemical components of the oils[36]. On the other hand, with thyme speciesJA significantly improved plant height, root length, antioxidant activity and carvacrol and thymol contents in the oils but reduced the essential oil yield and amount of γ-terpinene in the oils[33].

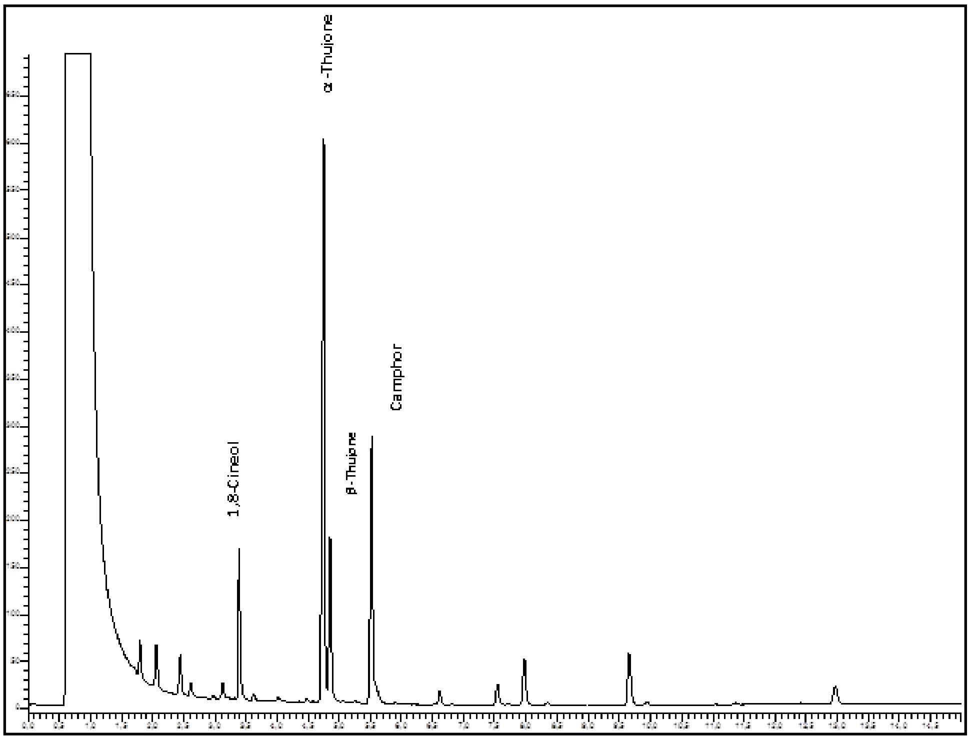

Fig. (5): GC chromatogram of the main components in sage essential oil

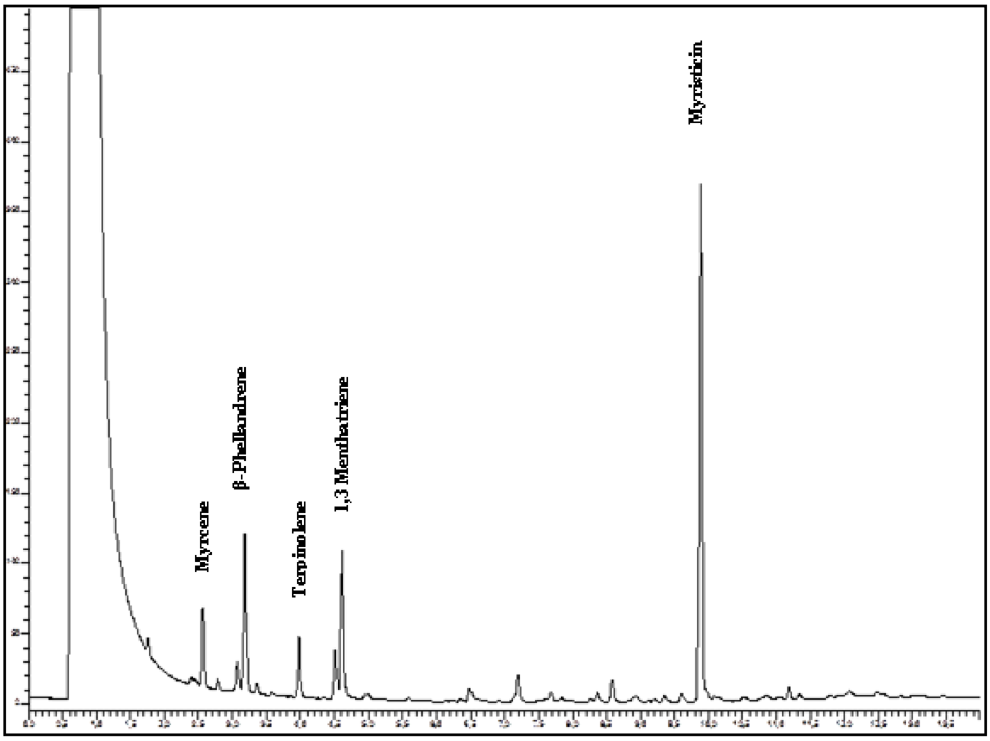

Fig. (6): GC chromatogram of the main components in parsley essential oil

References

1. Lykkesfeldt, J. and Moller, B.L. (1993). Synthesis of benzyl glucosinolate in *Tropaeolummajus* L. (Isothiocyanates as potent enzyme inhibitors). Plant Physiol 102(2):609–613.
2. Kleinwächter, M.; Schnug, E. and Selmar, D. (2008). The glucosinolate- myrosinase system in nasturtium (*Tropaeolummajus*L.): variability of biochemical parameters and screening for clones feasible for pharmaceutical utilization. J. Agric. Food Chem. 56 (23):11165–70.

3. Gasparotto, A. J.; Prando, T.B.; Leme, T.S.; Gasparotto, F.M.; Lourenço, E.L.; Rattmann, Y.D.; Da Silva-Santos, J.E., Kassuya, C.A. and Marques, M.C. (2012). Mechanisms underlying the diuretic effects of *Tropaeolummajus* L. extracts and its main component iso-quercitrin. J.Ethnopharmacol 141(1):501–509.
4. Koriem, K.M.; Arbid, M.S. and El-Gendy, N.F. (2010).The protective role of *Tropaeolummajus*on blood and liver toxicity induced by diethyl maleate in rats. Toxicol. Mech. Methods 20(9):579–586.
5. Butnariu, M. and Bostan, C. (2011). Antimicrobial and anti-inflammatory activities of the volatile oil compounds from *Tropaeolummajus* L. (nasturtium). Afr. J. Biotechnol., 10 (31): 5900-5909.
6. Grieve, M. (1971). A Modern Herbal: The Medicinal, Culinary, Cosmetic and Economic Properties, Cultivation and Folk-lore of Herbs, Grasses, Fungi, Shrubs, & Trees with All Their Modern Scientific Uses, Volume 2.
7. Hay, R.K.M. and Waterman, P.G. 1993. Volatile oil crops: Their biology, biochemistry and production. In: R. K. M., Hay & P. G. Waterman. Longman Scientific and Technical, Harlow, England, pp.1–2.
8. Dweck, A. C. (2000). Salvia,The Genus Salvia. Singapore: Overseas Publishing Group.
9. Perry, N.; Bollen, C.; Perry, E. and Ballard, C. (2003). Salvia for dementia therapy: review of pharmacological activity and pilot tolerability clinical trial." Pharmacology, Biochemistry and Behavior" 75: (2003): 651-659.
10. Länger, R.;Mechtler,C.H.; Tanzler,H. and JurenitschJ. (1993). Differences in the composition of the essential oil within an individual of *Salviaofficinalis*. Planta Medica, 59, 635–636.
11. Cuvelier, M. E.;Berset, C. and Richard, H. (1994).Antioxidant Constituents in Sage (*Salvia officinalis*).J. Agric. Food Chem., *42* (3), pp. 665–669.
12. Robbers, J. E. and Tyler, V.E. (1999). Tylers' Herbs of Choice. The Therapeutic Use of Phytochemicals. Haworth Herbal Press.,New York.
13. Gazzani, G. (1994). Anti and pro-oxidant activity of some dietary vegetables. Rivista di Scienzadell' Alimentazione, 23(3), 413–420.
14. Masanetz, C. and Grosch, W. (1998). Hay-like off-flavour of dry parsley. Z LebensmUntersFor 206(2):114–120.
15. Prins, C. L.; Vieira, I. J. C. and Freitas, S. P. (2010).Growth regulators and essential oil production. Braz. J. Plant Physiol., 22(2): 91-102.
16. Avanci, N.C.;Luche, D.D.; Goldman, G.H. and Goldman, M.H.S. (2010). Jasmonates are phytohormones with multiple functions, including plant defense and reproduction. Genet. Mol. Res. 9 (1): 484-505.
17. Creelman, R. A. and Mullet, J. E. (1997). Biosynthesis and action of jasmonates in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 48:355–81.
18. Hamedi, B.; Pirbalouti, A. G. andMoradi, P. (2014). Effect of foliar application of jasmonic acid on hypericin content of St. John's Wort (*Hypericumperforatum*L.).Electronic Journal of Biology, Vol. 10(2): 35-39
19. Koda, Y. (1997). Possible involvement of jasmonates in various morphogenic events. Physiol. Plant. 100: 639-646.
20. Grzegorczyk, I. and Wysokinska, H. (2009). The effect of methyl jasmonate on production of antioxidant compounds in shoot cultures of *Salvia officinalis* L. Herbapolonica, Vol. 55 No 3 2009.
21. Deschamps,C.; Raskiny, I. and Simonz, J. E. (2008). Regulation of essential oil accumulation in Basil. Int. J. Plant Sci. 169(8):981–986.
22. Egyptian Pharmacopoeia (1984): General Organization for Governmental Printing Affairs, Cairo.
23. Matallana, L.;Kleinwächter, M. and Selmar, D. (2006). Sulfur is limiting the glucosinolate accumulation in nasturtium *in vitro* plants (*Tropaeolummajus* L.) Journal of Applied Botany and Food Quality 80, 1 – 5.
24. Kleinwächter, M.; Paulsen, J.; Bloem, E.; Schnug, E. and Selmar, D. (2015). Moderate drought and signal transducer induced biosynthesis of relevant secondary metabolites in thyme (*Thymus vulgaris*), greater celandine (*Chelidoniummajus*) and parsley (*Petroselinum crispum*). Industrial Crops and Products 64, 158–166.
25. Snedecor, G.W. and Chochran, W.G. (1990). Statistical Methods. 11th. Ed. Iowa state College Press. Ames, Iowa, U.S., pp. 369-375.
26. Steel, R.G.D.; Torrie, G.H. and Dickey, D.A. (1997). Principles and Procedures of Statistics: A Biometrical Approach.3rd ed. McGraw-Hill, New York.
27. Duncan D.B., 1955. Multiple range and multiple F test. Biometrics., 11: 1-42.

28. Malekpoor, F.; Salimi, A. and Pirbalouti, A. G. (2015). Effects of Jasmonic acid on essential oil yield and chemical compositions of two Iranian landraces of basil (*Ocimumbasilicum*) under reduced irrigation.Journal of Herbal Drugs, Vol. 6, No. 1: 13-22.
29. Fard, F. R.; Omidbaigi, R.; Sharifi, M.; Sefidkon, F. and Behmanesh, M. (2012). Effect of methyl jasmonate on essential oil content and composition of *Agastachefoeniculum*. Journal of Medicinal Plants Research Vol. 6(45), pp. 5701-5705.
30. Kim, H.J.; Chen, F. and Rajapakse, N.C. (2006). Effect of methyl jasmonate on secondary metabolites of sweet basil (*Ocimumbasilicum*L.). J Agric Food Chem 54: 2327-2332.
31. Afkar, S. and Karimzadeh, G. (2014). Changing in the chemical composition of the essential oils of *Menthapiperita* after MeJA treatment. Int. J. Agri. Crop Sci. Vol., 7 (15), 1493-1496.
32. Degenhardt, D.C. and Lincoln, D.E. (2006) Volatile emissions from an odorous plant in response to herbivory and methyl jasmonate exposure. J. Chem. Ecol., 32: 725-743.
33. Alavi-Samani, S. M.; Kachouei, M. A. and Pirbalouti, A. G. (2015). Growth, yield, chemical composition, and antioxidant activity of essential oils from two thyme species under foliar application of jasmonic acid and water deficit conditions. Hortic. Environ. Biotechnol. 56(4):411-420.
34. Pirbalouti, A. G.; Gorgij, A. and Rahimmalek M. (2014).Phytochemical response of hyssop (*Hyssopus officinalis* L.) to foliar application of jasmonic acid. Journal of Herbal Drugs, 4 (1): 7-14.
35. Rahimi, A. R.; Rokhzadi, A.; Amini, S. and Karami, E. (2013). Effect of salicylic acid and methyl jasmonate on growth and secondary metabolites in *Cuminumcyminum*L. J. Bio.andEnv. Sci. Vol. 3, No. 12, p. 140-149.
36. Pirbalouti, A. G.;Rahimmalek, M.; Elikaei-Nejhad,L. and Hamedi, B. (2014). Essential oil compositions of summer savory under foliar application of jasmonic acid and salicylic acid. Journal of Essential Oil Research,Volume 26, Issue 5.

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