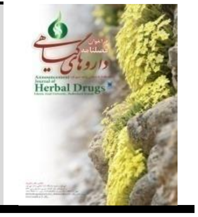
Journal of Herbal Drug
journal homepage: www.jhd.iaushk.ac.ir



Effects of IAA and BAP on chemical composition and essential oil content of lemon verbena (*Lippia citriodora* H.B.K.)

Hassan Nourafcan[1], Fatemeh Sefidkon*[2], Ahmad Khalighi[1], Amir Mousavi[3], Mozaffar Sharifi[4]

[1]Department of Horticulture, College of Agriculture, Tehran Science and Research branch, Islamic Azad University, Tehran, Iran;

[2]Research Institute of Forests and Rangelands, Tehran, Iran;
***Email:** Sefidkon@rifr-ac.ir*

[3]National Institute of Genetic Engineering and Biotechnology, Tehran, Iran;

[4]Department of Plant Sciences, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran;

ARTICLE INFO

Type: *Original Article*
Topic: *Plant Physiology*
Received *10th June 2014*
Accepted *24th June 2014*

Key words:
- ✓ *Essential Oil*
- ✓ *Citral*
- ✓ *Iaa And Bap*
- ✓ *1,8 Cineole*
- ✓ *Lemon Verbena*

ABSTRACT

Background & Aim: The world production and consumption of essential oils and perfumes are increasing very fast. *Lippia citriodora* H.B.K. is an herbal species mainly used as a spice and medicinal plant. Endogenous levels as well exogenous application of plant growth regulators (PGR) could affect essential oil production and chemical compositions.

Experimental: To study the effect of exogenous application of pgr on chemical composition and volatile oil content of lemon verbena (*Lippia citriodora* H.B.K.), an experiment was conducted based on Randomized Complete Block Design (RCBD) with three replications. Experimental treatments included distillated water (positive Control), without treatment (negative Control), indole-3-acetic acid (IAA) ($2mgL^{-1}$), 6-benzyl amino purine (BAP) ($2mgL^{-1}$) and IAA + BAP ($2mgL^{-1}$). The hydro-distillated essential oil was analyzed by GC and GC/MS.

Results: More than 23 volatile components have been characterized as constituents of *L. citriodora* oil. Neral, geranial, 1,8-cineole and limonene were the major compounds of the essential oil from lemon verbena. Neral significantly increased by exogenous application of IAA + BAP ($2mgL^{-1}$), whereas 1,8-cineole significantly increased by IAA ($2mgL^{-1}$).

Recommended applications/industries: Citral (geranial + neral) is a valuable flavor and scent reagent that is used in food and perfume industries. Generally, exogenous application of IAA, IAA+BAP and distilled water can increase medicinal and nutritional values of *L. citriodora*.

1. Introduction

Medicinal plants are well-known natural sources of remedies for the treatment of various diseases since antiquity. According to a report by World Health Organization (WHO), nearly 20000 plant species are currently being used for medicinal purposes (Zare *et al.*, 2011). Lemon verbena, *Lippia citriodora* (Ort.)H.B.K. (Verbenaceae) is an herbal species mainly used as a spice and medicinal plant (Pastorelli *et al.*, 2012; Mosavi, 2012). *L. citriodora*is found at South Africa and some parts of Asia , which is planted in some area for plant essence production (Mohammadi *et al.*, 2013).

Generally, the leaves are being used for flavoring beverages, desserts, fruit salads and jellies and for seasoning food. A decoction made from the leaves and flowers is given as febrifuge, sedative and anti-flatulent. In addition, the herb has antispasmodic and antibacterial and anti-*Candida* (Rao *et al*., 2013).

Essential oils are a diverse group of natural products that are important sources of aromatic and flavoring chemicals in food, industrial and pharmaceutical products (Charles and Simon, 1990). The essential oil production depends to genetic factors, the developmental stage of plants, environmental factors and techniques of agronomical management. Plant growth regulators (PGR) or plant hormones influence on essential oil production. Endogenous levels as well exogenous application could affect essential oil production and chemical composition. The environmental factors can influence biochemical pathways and physiological processes that alter plant metabolism such as essential oil biosynthesis. Plant Growth Regulators (PGR_S) have been defined as one of the main factors influence plants growth and their primary and secondary metabolites pool. The PGRs have been shown to improve herb yield in basil and coriander and fenugreek (Rohamare *et al*., 2013). Researchers reported that application of growth regulators (gibberellic acid or indole acetic acid) did not increase herb and oil yields in *Mentha piperita*. Foliar application of mepiquat chloride (MC) under field conditions reduced excessive vegetative growth of plants. It decreased plant height, leaf area and main stem node number (Abbas and El-Saeid, 2012). Essential oil of chamomile was increased by foliar spray of benzyl adenine (Rada *et al*., 2010).

Another research showed that α-bisabolol oxide A, increased in chamomile with application of 100 ppm IAA (Reda *et al*., 2010). Growth and essential oil yield of Mentha piperita were improved by the application of polyamines (Youssef *et al*., 2002). Silva et al., (2005) reported that auxin and cytokinin increased some components of the lemon balm oil. Povh and Ono (2007) showed that application of gibberellic acid influenced the chemical composition of Salvia oil. A report revealed that sodium salt of NAA and IAA increased the essential oil of Mentha piperita (Koseva-kovacheva and Staev, 1978).

From those bibliographic data, we are interested in studying the influence of two plant growth regulators (IAA and BAP) on the yield and chemical composition of essential oils of lemon verbena in order to provide useful information to pharmaceutical or cosmetic industries by changing these compositions.

2. Materials and Methods

2.1. Plant material

2.2. Lemon verbena, *Lippia citriodora* was field-grown at the Medicinal Plant Research Field (Miyaneh, located in East Azerbaijan province, Iran) during summer 2013. The experiment was arranged in Randomized Complete Block Design (RCBD) with three replicates. The plants were sprayed at pre-flowering stage for three times within ten days with distilled water (positive control), without treatment (negative control), indole-3-acetic acid (IAA) ($2mgL^{-1}$), 6-benzyl amino purine (BAP) ($2mgL^{-1}$) and combination of IAA ($2mgl^{-1}$) and BAP ($2mgL^{-1}$) (Sigma-Aldrich). Solutions were sprayed to the point of runoff. At flowering stage (one week after the last application), the aerial parts of *L. citriodora* were harvested. The leaves were dried naturally on laboratory benches at room temperature (23-27°C) until crisp. The dried leaves were subjected to hydro-distillation using an all glass Clevenger-type apparatus, to extract essential oils, The essential oils were separated from the aqueous layer, dried over anhydrous sodium sulfate and stored in sealed vials at low temperature (4°C) before gas chromatography (GC) and gas chromatography-mass spectrometric (GC/MS) analysis (Alavi *et al*., 2011; Khani *et al*., 2012; Agah and Najafian, 2012).

2.3. GC and GC/MS analysis

GC analyses were performed using a ultra-fast gas chromatograph (UFGC) (Thermo-UFM). The GC column was PH-5, 10m, 0.1mm (ID), 0.4 (FT), Oven: 60-285 °C/min, Rate: 80 °C/min, Hold time: 3 min, Run Time: 5.8min. Detector: FID, 280 °C, Injector: 280 °C, Carrier Gas: He, 0.5ml/min With Chrom-card software in Research Institute of Forests and Rangelands, Tehran.

GC-MS analyses were done on a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (30 m x 0.25 mm i.d.); Oven temperature was 40°C to 250°C at a rate of 4°C, transfer line temperature 260° C, carrier gas helium with a linear velocity of 31.5 cm/s, split ratio 1/60, Ionization energy 70 eV; scan time 1s and mass range of 40-300 *amu*. The percentages of compounds were calculated by the area normalization method, without considering response factors. The components of the oil were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices either with those of authentic compounds or with data published in the literature (Shibamoto, 1987; Adams, 1995). The retention indices were calculated for all volatile constituents using a homologous series of *n*-alkanes

2.3. Statistical analysis

The data were analyzed by using SAS ver. 9.1 statistical software. Means were compared using Duncan's Multiple Range Test (DMRT) at 5% probability level.

3. Results and Discussion

More than 23 volatile components have been characterized as constituents of *L.citriodora* oil. The identified constituents with their RIsare summarized in Table 1.

Essential oils are a diverse group of natural products that are important sources of aromatic and flavoring chemicals in food, industrial and pharmaceutical products. Essential oils are largely composed of terpenes and aromatic polypropanoid compounds derived from the acetate-mevalonic acid and the shikimic acid pathways, respectively. Essential oil composition of plants varies and is due to genetic and environmental factors that influence genetic expression (Charles and Simon, 1990). Citral is a valuable flavor and scent reagent that is used in the food and perfume industries. Previous investigations have reported that citral is synthesized from geraniol or nerol by an alcohol dehydrogenase or alcohol oxidase (Iijima *et al*., 2006).

The quality and quantity of essential oil isolated from the leaves of *L. citriodora* that were affected by PGRs are shown in Table 3. The analysis of *L. citriodora* essential oil revealed that neral, geranial, limonene and 1,8-cineole were the main components of the oil and each of these compounds is 10 to 25%.

Table 1. Chemical composition of the essential oil from *L.citriodora* identified by GC/MS

Row	Compounds	RI
1	α-Pinene	940
2	Sabinene	976
3	β-Pinene	980
4	Limonene	1039
5	1,8-Cineole	1031
6	ɣ-Terpinene	1060
7	Terpinolene	1090
8	*cis*Limonene oxide	1138
9	*trans*-Pinocarveol	1141
10	*Cis*-Sabinol	1145
11	Citronellal	1155
12	α-Terpineol	1191
13	Neral	1240
14	Geranial	1269
15	α-Terpinylacetate	1352
16	Geranylacetate	1380
17	β-caryophyllene	1420
18	ɣ-Elemene	1438
19	α-Humulene	1456
20	Cubenol	1517
21	Spathulenol	1580
22	Globulol	1587
23	*epi*-α-Cadinol	1640

The analysis of variance indicated that all measured compounds except Transpinocarveol and Cissabinol were significantly different at 5% of probability (Table 2).

Table 2. Analysis of variance for volatile oil compositions of *Lippia citriodora* H.B.K. affected by plant growth regulators.

S.O.V.	D.F.	Mean of squares									
		α-Pinene	Sabinene	β-Pinene	Limonene	1,8-Cineole	ɣ-Terpinene	Terpinolene	*cis*Limonene oxide	Citronellal	α-Terpineol
Block	2	0.002^{ns}	0.0001^{ns}	**0.25	0.1 ^{ns}	**1.004	**0.052	**0.07	**0.035	**0.04	**0.03
Treatment	4	**0.01	*0.01	**0.9	**5.09	**1.1003	**0.044	**0.03	**0.02	**0.02	**0.232
Error	8	0.001	0.002	0.003	0.03	0.035	0.0023	0.0004	0.001	0.0006	0.001
C.V. (%)		4.98	14.05	1.2	1.125	1.26	3.91	1.76	6.2	3.72	1.96

ns: non-significant, * and ** : significant at 5% and 1% of probability levels, respectively.

Table 2 Continued

S.O.V.	D.F.	Mean of squares										
		Neral	Geranial	α-Terpinyl acetate	Geranylacetate	β-caryophyllene	γ-Elemene	α-Humulene	Cubenol	Spathulenol	Globulol	*epi*-α-Cadinol
Block	2	0.036^{**}	0.066^{**}	0.05^{**}	0.064^{**}	0.06^{**}	0.04^{**}	0.03^{**}	0.063^{**}	0.04^{**}	0.025^{**}	0.045^{**}
Treatment	4	6.89^{**}	16^{**}	0.01^{**}	0.01^{**}	0.256^{**}	1.36^{**}	0.012^{**}	0.05^{**}	0.33^{**}	1.178^{**}	0.02^{**}
Error	8	0.0005	0.002	0.00002	0.0002	0.001	0.0006	0.0008	0.001	0.0024	0.001	0.001
C.V. (%)		0.14	0.17	0.49	3.52	1.83	0.46	5.18	2.78	1.3	0.7	5.09

ns: non-significant, * and ** : significant at 5% and 1% of probability levels, respectively.

Furthermore, lesser amounts of the other components include α-pinene, sabinene, *trans*pinocarveol, geranyl acetate, α-humulene and *epi*-α-cadinol were existed in essential oil of the plant. According to research findings, the main components in the essential oils of *L. citriodora* leaves were geranial, neral, limonene, 1,8-cineole, spathulenol, geraniol, β-caryophyllene, nerol and sabinene. The main constituents of the essential oil extracted from fresh leaves of *L. citriodora*, were geranial, neral and limonene. Also, GC/MS analysis of essential oils revealed that 1, 8-Cineole, α-curcumene, geranial, limonene and caryophyllene oxide were the main components of essential oils of *L. citriodora* leaves, respectively (Khani *et al*., 2012).

Table 3. Effect of foliar sprays plant growth regulators on essential oil components (%) on *Lcitriodora*

Component Treatment	α-Pinene	Sabinene	β-Pinene	Limonene	1,8-Cineole	γ-Terpinene	Terpinolene
Control	0.55^{b}	0.36^{a}	4.83^{b}	15.23^{b}	14.09^{d}	1.08^{c}	1.23^{b}
Distilled water	0.59^{ab}	0.36^{a}	4.48^{c}	14.23^{d}	14.96^{b}	1.34^{ab}	1.13^{c}
IAA (2mgL^{-1})	0.49^{c}	0.29^{ab}	5.07^{a}	17.04^{a}	15.72^{a}	1.36^{a}	1.35^{a}
BAP (2mgL^{-1})	0.62^{a}	0.25^{b}	4.45^{c}	14.68^{c}	15.12^{b}	1.15^{c}	1.2^{b}
IAA + BAP (2mgL^{-1})	0.48^{c}	0.24^{b}	3.63^{d}	13.63^{e}	14.6^{c}	1.25^{b}	1.1^{c}

Means in each column with the same letters are not significantly different at 5% level of probability DMRT.

Table 3. Continued

Component Treatment	*Cis*-Limonene oxide	Citronellal	α-Terpineol	Neral	Geranial	α-Terpinylacetate	Geranylacetate
Control	0.5^{bc}	0.71^{a}	1.17^{d}	15.9^{d}	23.34^{d}	0.9^{a}	0.4^{b}
Distilled water	0.52^{b}	0.61^{b}	1.11^{e}	16.95^{b}	26.75^{a}	0.79^{b}	0.3^{c}
IAA (2mgL^{-1})	0.65^{a}	0.55^{c}	1.8^{a}	13.33^{e}	20.93^{e}	0.9^{a}	0.42^{a}
BAP (2mgL^{-1})	0.45^{c}	0.7^{a}	1.25^{c}	16.4^{c}	25.99^{b}	0.8^{b}	0.32^{c}
IAA + BAP (2mgL^{-1})	0.52^{b}	0.7^{a}	1.35^{b}	17^{a}	24.74^{c}	0.8^{b}	0.43^{a}

Means in each column with the same letters are not significantly different at 5% level of probability DMRT.

Table 3. Continued

Component Treatment	β-Caryophyllene	γ-Elemene	α-Humulene	Cubenol	Spathulenol	Globulol	*epi*-α-Cadinol
Control	1.79^{a}	5.65^{b}	0.55^{ab}	1.12^{a}	4.1^{a}	4.25^{b}	0.62^{a}
Distilled water	1.07^{e}	4.41^{e}	0.45^{c}	0.85^{c}	3.44^{c}	3.85^{d}	0.45^{b}
IAA (2mgL^{-1})	1.7^{b}	5.89^{a}	0.61^{a}	1.01^{b}	3.95^{b}	4.21^{b}	0.6^{a}
BAP (2mgL^{-1})	1.39^{d}	4.6^{d}	0.5^{bc}	0.81^{c}	3.4^{c}	4.04^{c}	0.49^{b}
IAA + BAP (2mgL^{-1})	1.64^{c}	5.57^{c}	0.59^{a}	1^{b}	4.02^{ab}	4.5^{a}	0.6^{a}

Means in each column with the same letter(s) are not significantly different at 5% level of probability using DMRT.

The antimicrobial activity of 1,8-cineole has been reported (Folashade and Omoregie, 2012). The main use of 1,8-cineoleextracted from *Eucalyptus* oils is for the pharmaceutical industry, and citronellaluses for the perfumery and piperitone and α-phellandreneare used for industrial use (Fathi and Sefidkon, 2012). According to the literature, geranial, neral and limonene were the component found to occur in higher quantities in essential oils of the *L. citriodora* (Khani*et al*., 2012) In this study, neral, geranial, and 1,8-cineole significantly increased by exogenous application of IAA + BAP, distilled water, and by IAA, respectively. Mahmoud (1996) studied the influence of GA_3, IAA and kinetin on yield and chemical composition of essential oil of *O. basilicum*. He reported that the GA leads to the decrease in essential oil yield, while kinetin and IAA increased the yield oil. This change is accompanied by a decrease in levels of the main compound (methyl chavicol) for all treatments. This variation in yield directly to the number of glandular hairs: GA leads to a decrease in the number of these structures, whereas treatment with BAP increases the number of glands in the leaves in several species (*Lavanduladentata*, *Thymus mastichina* and *Piceaabies*) (Prins *et al*., 2010). IAA leads to an increase in neither yield of *O. gratissimum* L. essential oils which passes from 0.22% to 0.3%, with no significant change neither in the content nor the main compounds composition of essential oils. These results are the same as those found in the literature (Hazzoumi *et al*., 2014).

Unexpectedly, in essence performance, were not significantly different between treatments. The reports of Hazzoumi *et al.* (2014) showed that the regarding various medicinal plants as influenced by different plant growth regulators. They stated that application of IAA increases slightly the yield of *O. basilicum* oil; this variation goes with small change in methyl chavicol. The same observation was made on lemon balm (*Melissa officinalis* L.) and thyme (*Thymus vulgaris* L.) (Shukla and Farooqi, 1990; Affonso *et al*., 2009). Some scientists reported that monoterpenes *O. baslicum* and *Lavendula dentata* are highly influenced by plant growth substance treatments due to the genes regulation, which cause an increase in enzyme numbers related to the metabolic pathways of these compounds They suggested that cytokinins stimulate the metabolism and accumulation of essential oils, specifically monoterpenes in *M .piperita*, and *Salvia officinalis* L. (Kim *et al*., 2006; Li *et al*., 2007; Hazzoumi*et al*., 2014). The application of growth regulators may affect essential oils due to their effects on enzymatic pathways of terpenoid biosynthesis (Amiri*et al*., 2014).

It is well known that essential oil is derived from mevalonic acid via the isoprene pathway in a manner similar to that for other terpenes. Thus, PGRs that exert their effect at the level of gibberellins metabolism, might increase the accumulation of essential oil in plants. Growth retardants such as phosphone-D and chlormequat chloride (CCC) which influence gibberellins metabolism have been shown to increase terpene formation resulting in increased essential oil content of peppermint and sage (Abbas and El-Saeid, 2012).

4. Conclusion

Essential oils synthesis is basically controlled by genetically processes but their synthesis is strongly affected by environmental parameters. Lemon balm growth and essential oil production were stimulated with exogenously applied plant growth regulators.

5. References

Abbas, S.M. and El-Saeid, H.M. 2012. Effects of some growth regulators on oil yield, growth and hormonal content of lemon grass (*Cymbopogon citrates*). *BotanicaSerbica*., 36 (2): 97-101.

Adams, R.P. 1995. *Identification of essential oil components by gas chromatography/mass spectroscopy*, Carol Stream, IL: Allured Publishing Co. Pp.230.

Affonso, V.R., Bizzo, H.R., Lage, C.L.S. and Sato, A. 2009. Influence of growth regulators in biomass production and volatile profile of *in vitro* Plantlets of *Thymus vulgaris* L. Journal of Agriculture and Food Chemistry. 57: 6392–6395.

Agah, M. and Najafian, S. 2012.Essential oil content and composition of *Lippia citriodora* as affected by drying method before flowering stages. *European Journal of Experimental Biology*., 2 (5):1771-1777.

Alavi, L., Barzegar, M., Jabbari, A. and Naghdibadi, H. 2011.The effect of heat treatment on chemical composition and antioxidant property of *Lippia citriodora* essential oil. *Journal of Medicinal Plants*., 10(39): 65-75.

Amiri, S. Sharafzadeh, S. and Ordookhani, K. 2014. The effect of gibberellic acid and benzyl adenine on growth and essential oils of German chamomile. *Indian Journal of Fundamental and Applied Life Sciences*., 4(1):186-188.

Charles, D.J. and Simon, J.E. 1990. Comparison of extraction methods for the rapid determination of essential oil content and composition of Basil. *Journal of American Society Horticultural Sciences*., 115(3): 458-462.

Fathi, E. and Sefidkon, F. 2012. Influence of drying and extraction methods on yield and chemical composition of the essential oil of *Eucalyptus sargentii*. *Journal of Agriculture and Sciences Technology.*, 14: 1035-1042.

Folashade, K.O. and Omoregie, E.H. 2012. Essential oil of *Lippiamultiflora*Moldenke: A review.*Journal of Applied Pharmaceutical Science*., 2 (1): 15-23.

Hazzoumi, Z., Moustakime, Y. and Joutei, K.A. 2014. Effect of gibberellic acid (GA_3), indole acetic acid (IAA) and benzylaminopurine (BAP) on the synthesis of essential oils and the isomerization of methyl chavicol and *trans*-anethole in *Ocimum gratissimum* L. *Springer Plus*., 3(321): 1-7.

Iijima, Y., Wang, G., Fridman, E. and Pichersky, E. 2006.Analysis of the enzymatic formation of citral in the glands of sweet basil. *Archives of Biochemistry and Biophysics*., 448: 141–149.

Khani, A., Basavand, F. and Rakhshani, E. 2012.Chemical composition and insecticide activity of lemon verbena essential oil. *Journal of Crop Protection.*,1(4): 313-320.

Kim, H.J., Chen, F., Chen, F. and Rajapakse, N.C. 2006. Effect of methyl jasmonate on secondary metabolites of sweet basil (*Ocimum basilicum* L.). Journal of Agriculture and Food Chemistry. 54: 2327–2332.

Li, Z., Wang, X., Chen, F. and Kim, H.J. 2007. Chemical changes and over expressed genes in sweet basil (*Ocimum basilicum* L.) upon methyl jasmonate treatment. Journal of Agriculture and Food Chemistry. 55: 706–713.

Mahmoud, S.E.D. 1996. Response of growth and essential oil content of sweet basil (*Ocimum basilicum* L.) to some natural hormones. International Symposium on Medicinal and Aromatic Plants. ISHS Acta Horticulturae 426.

Mohammadi, M. Tobeh, A., Fakhari, R., Naghdi, H. and Ochi, M. 2013. The quantitative yield of lemon verbena (*Lippia citriodora*) as influenced by different biological Fertilizers.*International Journal of Agronomy and Plant Production*., 4(9): 2126-2129.

Mosavi, A.A. 2012. The optimization of lemon verbena (*Lippia citriodora*) medicinal plant tissue culture. *International Journal of Agronomy and Plant Production*., 3(11): 561-565.

Pastorelli, G. Rossi, R. and Corino, C. 2012. Influence of *Lippia citriodora* verbascoside on growth performance, antioxidant status, and serum immunoglobulins content in piglets. *Czech Journal of Animal Sciences*, 57(7): 312–322.

Prins, C.L., Vieira, I.J.C. and Freitas, B.S.P. 2010. Growth regulators and essential oil production. Journal Plant Physiology. 22: 91–102.

Rada, F., Abd El-Wahed, M.S.A. and Gamal El Din, K.M. 2010.Effect of Indole acetic acid, gibberellic acid and kinetin on vegetative growth, flowering, essential oil pattern on a Chamomile plant (*Chamomile recutita* L. Rausch). *World Journal of Agricultural Sciences*, 6(5): 595-600.

Rao, G.V., Gopalakrishnan, M. and Mukhopadhyay, T. 2013. Secondary metabolites from the leaves of *Lippia citriodora* H.B.K. *Der Pharmacia Lettre*. 5(3): 492-495.

Rohamare, Y., Nikam, T.D. and Dhumal, K.N. 2013.Effect of foliar application of plant growth regulators on growth, yield and essential oil components of Ajwain (*Trachyspermumammi* L.). *International Journal of Seed Spices*., 3(2): 34-41.

Shibamoto, T. 1987. *Retention indices in essential oil analysis.* (eds) Sandra P, Bicchi, C. HuethigVerlag, New York, 259-274.

Shukla, A. and Farooqi, A.H.A. 1990. Utilization of plant growth regulators in aromatic plant production. Current Research Medicinal and Aromatic Plants.12: 152–157.

Zare, Z., Majd, A., NejadSattari, T., Iranbakhsh, A. and Mehrabian, S. 2011. The comparative study of an antimicrobial activity of leaves and flowers methanolic extracts of *Lippia citriodora* H.B.K. (Verbenaceae). *American-Eurasian Journal of Agriculture and Environmental Science*, 10(5): 901-905.

Reda, F., Abd El Wahed, M.S.A. and Gamal El Din, K.M. 2010. Effect of indole acetic acid, gibberellic acid and kinetin on vegetative growth, flowering and essential oil pattern of chamomile plant. World Journal of Agricultural Sciences, 6:595-600.

Youssef, A.A., Aly, M.S., Abou Zied, E.N., Iliey, L. and Titiana, S. 2002. Effect of some growth substances on mass production and volatile oil yield of Mentha piperita. Egyptian Journal of Applied Sciences, 17:610-623.

Povh, J.A. and Ono, E.O. 2007. Efeito do acido giberelico na composicao do oleo essencial de Salvia officinalis L. Public UEPG Biological and Health Sciences, 13:7-10.

Koseva-kovacheva, D. and Staev, D. 1978. Effect of some growth regulators and hydrogen peroxide on the content and quality of peppermint oil. Resteniedni dni u Nauki, 15:21-25.