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ORIGINAL ARTICLE

Changes in essential oil content of *Lippia citriodora* in response to induction of bio-active compounds and plant growth regulators

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Background & aim: Bioactive compounds and plant growth regulators could change a plant's essential oil content and composition. In this study, the effect of bioactive compounds and plant growth regulators on *lippia citriodora* was investigated.
Objective: This experiment was done with three replications on the basis of factorial experiment in randomized complete block design. The bio-regulators were sprayed in four levels, i.e. distilled water, 50 ppm gibberellic acid (GA_3) + 50 ppm indole-3-butyric acid (IBA), 50 ppm GA_3 + 100 ppm IBA, and 100 ppm GA_3 + 50 ppm IBA. The chitosan was sprayed in two levels: distilled water and 400 ppm chitosan. The methanol was sprayed in two levels: distilled water and 5% v/v methanol. The essential oil was extracted by hydrodistillation method and analyzed by GC and GC/MS.
Results: Thirty-two compounds were identified, representing 95.08% of the total essential oil. The main components were *E*-citral (geranial) (25.02%), *Z*-citral (neral) (16.04%), *ar*-curcumene (10.37%), caryophyllene oxide (9.56%), spathulenol (6.83%), limonene (4.99%) and *(E)*-caryophyllene (2.29%). The studied traits were significantly affected ($p < 0.01$) by the interaction of bioregulators, methanol and chitosan application.
Conclusion: Foliar application of IBA, GA_3, chitosan and methanol could increase aromatic values of *L. citriodora* essential oil.
Key words: chitosan; gibberellic acid; indole-3-butyric acid; Lemon verbena; methanol.

Abbreviations

ANOVA= analysis of variance, Ch=400ppm chitosan, FID=Flame Ionization Detector, G1=50ppm GA_3, G2=100ppmGA_3, GA =Gibberellic acid**,** GC=gas chromatography, GC-MS**=** gas chromatography-mass spectrometry, GRAS= generally regarded as safe, I1=50ppm IBA,I2=100ppm IBA, IBA**=**Indole-3-butyric acid**,** L.S.D= least significant difference, M5=0%v/v methanol, RCBD**=** Randomized complete blocks design,

Introduction

Lemon verbena, scientifically known as *lippia citriodora* H.B.K, is a deciduous shrub of the *verbenaceae* family, which grows to a height of 3-5 m (Mozafarian, 2010). The long light green leaves of this plant are located on the stem in stacks of three. It has small and white flowers. The plant is woody at the bottom and near the soil surface. The genus *lippia* has over 200 species, among which *L. citriodora* is especially important. *Lippia citriodora* H.B.K. synonyms are *aloysia citriodora* palauand *aloysia triphylla* (L'Herit.) Britton and *Verbena citriodora* cav is a native species in Argentina, Chile and Peru and grows throughout Latin America as well as North Africa (Morocco), Southern Europe and parts of Asia (Carnat *et al.*, 1999; Botta, 1979; Rotman and Mulgura Romero, 1999). At present, this species is cultivated in large-scale in Iran. The leaves and vegetative organs of this plant are antipyretic, analgesic, carminative, digestive and calming. They can be used for cold and headaches, too. The lemon verbena tea is highly calming and soothing (valention et al., 2002). The essence of this plant is that it has antimicrobial properties against dental micro flora (Torrent Martia, 1976). It is a sedative, antipyretic and inhibits histamine-inducing effects (Nakamura et al., 1997). In addition to the essence, its leaf contains alkaloids, flavonoids, mucilage, tannin and phenolic acids (Shammas, 1998; Marita, 1979). The essence of lemon verbena is that it has different compounds, most important of which are geraniol, myrcene, limonene, geranial and citronelle (Montes et al., 1973). The essential oils and leaves are used in the perfume industry and for producing drinks with different flavours, respectively and for food preparation (Pascual *et al.*, 2001). In the United States, *Lippia citriodora* is has the tag generally regarded as safe (GRAS) for human consumption in alcoholic beverages (Gomes *et al.*, 2006). Its extracts and essential oils of leaves are also used extensively in the cosmetics and flavouring industries. This study was to evaluate phytochemical changes of lemon verbena (*lippiacitriodora* H.B.K) in response to induction of bio-active compounds

and plant growth regulators. Although the biosynthesis of secondary metabolites was controlled genetically, it was affected markedly by agricultural factors. Since bio-active compounds and plant growth regulators are applied for improvement of quantitative and qualitative characteristics of some medicinal and aromatic plants, this experiment to study the effects of gibberellic acid (GA_3), indole-3-butyric acid (IBA), chitosan and methanol on lemon verbena was conducted.

The commercial uses of gibberellins, especially GA_3, are that they help stimulate growth in fruits, give a fillip to the production process of malted barley as well as in brewery industries and increases sugar yield in sugarcane. Also, gibberellins help regulate transition from adolescence to puberty and are used in grape grouping (Taiz and Zieger, 2006).

IBA is among synthetic auxins, which are more resistant to light than natural auxins. IBA helps regulate apical dominance, stimulates and helps form adventitious roots, prevents leaves from falling and causes vascular differentiation (Taiz and Zieger, 2006).

Among PGRs, auxin and gibberellin play a vital role in regulating developmental processes within plant bodies (Gou et al., 2010). IBA is a synthetic auxin. Auxins are used commercially for enhancing crop production and regulating plant growth. They also help in the rapid growth of shoot tissue, young leaves and developing seeds as well as for their elongation and promote lateral root development (Nagel., 2001). Gibberellin helps in cell growth of the stem, leaves and other aerial parts by causing cell elongation and increasing inter-nodal length. Chitosan is a naturally-occurring compound that helps in agriculture by controlling plant diseases. This molecule was shown to display toxicity and inhibit fungal growth and development (Hadrami *et al.*, 2010; Zhang *et al.*, 2011). Moreover, chitosan is polysaccharides produced through Chitindeacetylation that can be used to form an edible semi-permeable film on the outside surface of the fruits to extend storage life and reduce some forms of decay caused by fungi during storage (Bautista-Banos *et al.,* 2006). The first condition to achieve high performance per unit area is to produce large amounts of dry matter because around 90% of a plant's dry weight is due to CO_2 assimilation by photosynthesis. As a consequence, speeding up CO_2 fixation is useful for raising production capacityof crops (Downie et al, 2004; Ramberg et al., 2002; Hanson and Roje, 2001).

One of these solutions is to use chemicals such as methanol, ethanol, propanol and butanol as well as amino acids like glycine and glutamate to increase tricarbonic plants. The most important benefit of using such chemicals is prevention and reduction of stress-induced photorespiration on crops (Gout et al., 2000).

The most important role proposed for methanol in tricarbonic plants is its inhibition of photorespiration probably due to the increased concentration of CO_2 inside leaves. This is because CO_2 concentration in the leaves causes ribulose 5'-phosphate to react with CO_2 rather than with O_2 and be carboxylated**.** Hence, tricarbonic plants are increasingly being treated with methanol because methanol is a direct carbonic source for biosynthesis and reduces carbon losses through photorespiration (McGriffen and Manthery, 1996; Fall and Bensan; 1996).

Natural methanol, which is produced in leaves as a result of pectin methylesterase activity in the process of cell wall expansion, can increase cytokine production and stimulate plant growth (Holland et al., 1997). Methanol-spraying solution on aerial parts reduces drought stress and water requirements (Nemecek et al., 1995).

Material and method

Experimental

To investigate the effects of foliar application of bio-active compounds and bio-regulators on phytochemical changes of lemon verbena, a three-factor factorial experiment based on randomized complete blocks design (RCBD) with 16 treatments and three replications was conducted at a research greenhouse in 2015. The first factor included the application of bio-regulators in four levels. Namely: controlled distilled water, 50 ppm GA_3 + 50 ppm IBA, 50 ppm GA_3 + 100 ppm IBA and 100 ppm GA_3 + 50 ppm IBA. The second factor, chitosan induction, was considered at two levels: controlled distilled water and 400 ppm chitosan. The third factor was methanol induction in two levels: distilled water and 5% v/v methanol (table 1). The solutions were sprayed four times during the growth stages with 15-day intervals on the aerial parts of the plant.

Essential oil extraction and analysis

The fresh biomass samples were submitted to hydro distillation in a Clevenger type apparatus over 1 hour, using volumes of 1.0 ml of n-hexane for retention of the hydro distillate components. The hydro distillates from all samples were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS).

GC analysis was carried out on a Younglin Instrument Acme 6000M gas chromatograph equipped with Flame Ionization Detector (FID) and a HP-5 capillary column (30 m×0.25 mm; 0.25 µm film thicknesses). The oven temperature was kept at 50ºC for 5 minutes and then programmed at 3ºC min-1 to 240ºC and after that programmed at 15ºC min-1 to 300ºC (held for 3 minutes). Other operating conditions were: carrier gas He with a flow rate of 0.8 mL min-1; injector and detector temperatures was 290ºC and split ratio, 1:10.GC/MS analysis was performed on a GC mentioned above coupled with an Agilent Technologies 5973 Mass system. The other operating conditions were the same as described above and mass spectra was taken at 70 eV. Mass range was from m/z 35–375 amu. Quantitative data was obtained from the electronic integration of the FID peak areas. The gamma-terpinene was identified by comparing their mass spectra and retention indices with those published in the literature (Adams., 1995; Swigar and Silverstein., 1981) and presented in the MS computer library. Each analysis was performed in triplicate.

Statistical analysis

The averages of data were statistically analyzed using analysis of variance (ANOVA) and values of least significant difference (L.S.D) were around 5% of the probability level.

Table 1. Treatments used in the experiment and abbreviations code

	Treatment	GA_3(ppm)	IBA(ppm)	methanol (% V/V)	Chitosan(ppm)
T1	No Spray	0	0	0	0
T2	M5	0	0	5	0
T3	Ch	0	0	0	400
T4	ChM5	0	0	5	400
T5	I1G1	50	50	0	0
T6	I1G1M5	50	50	5	0
T7	I1G1Ch	50	50	0	400
T8	I1G1ChM5	50	50	5	400
T9	I2G1	50	100	0	0
T10	I2G1M5	50	100	5	0
T11	I2G1Ch	50	100	0	400
T12	I2G1ChM5	50	100	5	400
T13	I1G2	100	50	0	0
T14	I1G2M5	100	50	5	0
T15	I1G2Ch	100	50	0	400
T16	I1G2ChM5	100	50	5	400

Abbreviations code

GA_3; Gibberellic acid — M5=0%v/v methanol — Ch=400ppm chitosan — pm IBA
IBA;Indole-3-butyric acid — I2=100ppm IBA — G1=50ppm GA_3 — G2=100ppm GA_3

Results

In this study, more than 16 volatile components have been characterized as constituents of *L.citriodora* oil. E-citral, Z-citral, R-curcumene, caryophyllene oxide, spathulenol and limonene were the main components of the essential oil. The analysis of variance indicated that the bio-active compounds and bio-regulators and their interaction had significant effects ($p \leq 0.01$) on essential oil percentage and its composition of *L. citriodora* (Table 2, 3).

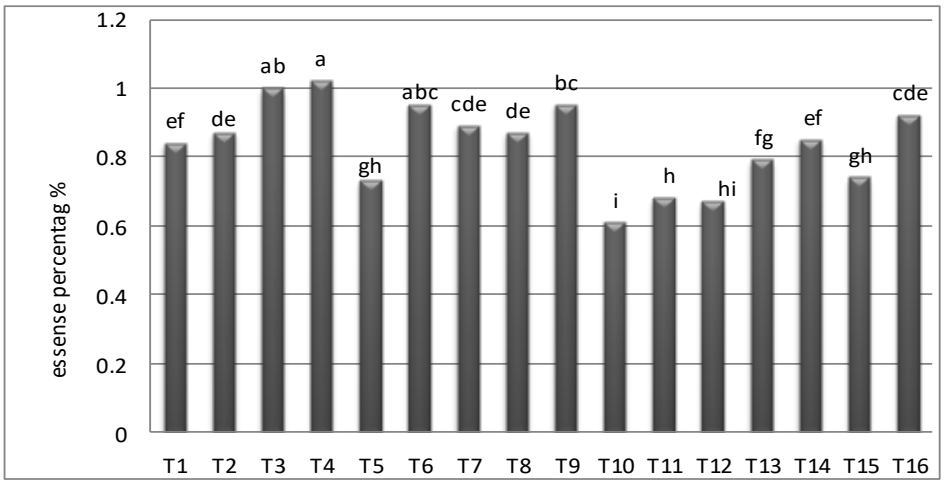

Fig. 1. Mean comparison of Effect of foliar sprays plant growth regulators and bio-active compounds on essential oil percentage (%) on *L. citriodora*

The highest essential oil percentage was observed in the treatment with abbreviation code (ChM5) (Fig. 1, Table 1). According to our research findings, the main components in the essential oils of *L. citriodora* leaves were E-citral, Z-citral, r-curcumene, caryophyllene oxide, spathulenol and limonene (Table 3,4).

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

S.O.V.	D.F.	Mean of squares								
		Limonene	1,8-Cineole	Borneol	α-Terpineol	Z-Citral	E- Citral	Bornylacetate	Carvacrol	Geranylacetate
Block	2	27.30**	1.57**	0.00003ns	0.0032 ns	0.07 ns	0.25 ns	0.02 *	0.0021 ns	0.0001 ns
hormone	3	25.92**	2.17**	0.0856**	0.0341 ns	35.07**	68.75**	0.47**	0.2148**	0.0061 ns
chitosan	1	22.85**	0.86**	0.0257 ns	0.0261 ns	0.25 ns	1.53 ns	0.01 ns	0.5874**	0.0698**
methanol	1	31.69**	0.0001 ns	0.0032 ns	0.1474**	2.64 ns	2.46 ns	2.91**	0.5874**	0.2147**
chitosan × hormone	3	27.04**	6.08**	0.3057**	0.6942**	54.50**	11.45**	0.97**	0.2148**	0.1415**
methanol × hormone	3	11.94**	1.18**	0.2661**	0.9264**	70.93**	42.06**	0.47**	0.2148**	0.0155 ns
chitosan × methanol	1	20.59**	2.82**	0.6143**	1.0502**	20.48**	0.28 ns	0.01 ns	0.5874**	0.0347 *
methanol × chitosan × hormone	3	22.86**	3.18**	0.1156**	0.2551**	58.24**	30.87**	0.97**	0.2148**	0.2073**
Error	30	0.08	0.02	0.0164	0.0161	2.24	1.93	0.01	0.0010	0.0077
C.V. (%)		3.80	5.58	17.98	12.20	7.79	5.05	8.14	11.97	10.76

S.O.V.	D.F.	Mean of squares								
		Caryophyllene	Curcumene	Muurolene	Nerolidol	Spathulenol	Caryophylleneoxide	Cadinol	Terpen hydrocarbon	Terpen Oxigenated
Block	2	0.0018 ns	0.02 ns	0.0042 ns	0.000002 ns	0.04 ns	0.00008 ns	0.00106 ns	27.30**	0.07 ns
hormone	3	1.8583**	37.32**	0.1313**	0.3647**	6.10**	22.26**	0.75**	25.92**	227.03**
chitosan	1	0.2930 *	2.03 ns	0.2269**	1.1939**	2.59 *	12.31**	0.01 ns	22.85**	0.42 ns
methanol	1	0.4389**	10.63**	0.1519**	0.0624 ns	0.43 ns	6.01**	0.08 ns	31.69**	5.90 ns
chitosan × hormone	3	0.3985**	17.99**	0.7723**	1.1279**	11.85**	43.66**	0.94**	27.04**	188.30**
methanol × hormone	3	2.4725**	57.30**	0.1689**	0.5089**	9.95**	11.40**	0.62**	11.94**	276.41**
chitosan × methanol	1	0.4163**	16.07**	0.0588*	0.0391 ns	9.99**	19.67**	0.42**	20.59**	55.79 *
methanol × chitosan × hormone	3	1.0376**	33.44**	0.2022**	0.7852**	13.31**	31.36**	0.75**	22.86**	213.52**
Error	30	0.01	0.02	0.46	0.45	0.03	0.08	7.93	1.22	2.28
C.V. (%)		12.62	10.00	11.73	8.90	11.97	3.80	5.40	10.75	9.43

S.O.V.	D.F.	Mean of squares			
		Sesquiterpene Hydrocarbons	Oxygenated Sesquiterpenes	Essential oil percentage	Leaf dry weight
Block	2	0.06 ns	0.06 ns	0.07**	0.15 ns
hormone	3	60.69**	65.77**	0.09**	1.75**
chitosan	1	2.21 ns	37.54**	0.01 *	0.18 ns
methanol	1	18.60**	3.11 ns	0.004 ns	4.78**
chitosan × hormone	3	30.80**	144.84**	0.03**	11.62**
methanol × hormone	3	91.03**	59.71**	0.05**	5.42**
chitosan × methanol	1	23.97**	71.22**	0.01 ns	5.27 *
methanol × chitosan × hormone	3	52.38**	116.34**	0.04**	1.55**
Error	30	1.22	2.28	0.002	0.11
C.V. (%)		10.75	9.43	4.93	10.82

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

Table 3. Effect of foliar sprays plant growth regulators and bio-active compounds on essential oil components (%) on *L. citriodora*

Component / Treatments	Limonene	1,8-Cineole	Borneol	α-Terpineol	Z- Citral	E- Citral	Bornyl acetate	Carvacrol	Geranyl acetate
T1	5.49 g	1.63 f	0.78 bcd	0.80 ef	18.32 fg	28.83cd	1.31 b	0 c	1.21 bc
T2	9.05 cd	1.61f	0.70 bcd	1.07 c	19.79 ef	28.97bcd	0 e	0c	0.94 fghi
T3	5.35 gh	2.72 c	0.58 de	0.99 cde	22.87 bc	32.99 a	0 e	1.08 a	0.95 efghi
T4	9.44 c	2.91 c	0.91 b	1.50 b	22.37 bcd	30.63 bc	0 e	0 c	1.13 cd
T5	4.24 i	1.35 g	0.65 cde	0.97 cde	15.76 hi	25.16 fgh	0.24 d	0 c	1.33 ab
T6	8.79 d	2.33 d	0.58 de	0.96 cde	22.14 bcde	31.19 ab	0 e	0 c	1.00 defgh
T7	8.25 e	2.26 de	0.63 cde	1.03 cd	20.67 cdef	28.90 bcd	0 e	0 c	1.08 cdef
T8	8.94 d	1.70 f	0.73 bcd	1.13 c	20.42 cdef	28.38 cd	0 e	0 c	0.98 efghi
T9	9.42 c	4.00 a	1.34 a	2.02 a	26.48 a	30.55 bc	0 e	0 c	0.84 i
T10	6.31 f	2.07 e	0.44 ef	0.68 fg	13.40 i	20.85 i	0 e	0 c	0.98 efghi
T11	2.64 j	0.74 h	0.33 f	0.56 g	13.50 i	24.52 gh	0.44 c	0 c	1.46 a
T12	4.39 l	0.91 h	0.45 ef	0.63 fg	13.88 hi	23.27 h	0 e	0 c	1.09 cde
T13	12.84 a	3.40 b	0.59 cde	0.83 def	18.32 fg	26.06 efg	0.28 d	0 c	0.93 ghi
T14	9.10 cd	2.44 d	0.80 bc	1.17 c	20.20 def	27.17 def	0 e	0 c	1.07 cdefg
T15	4.99 h	1.65f	0.73 bcd	0.67 fg	16.04 gh	25.01 fgh	1.67 a	0.69 b	1.34 ab
T16	10.20 b	3.80 a	1.15 a	1.62 b	23.51 b	27.94 de	0 e	0 c	0.88 hi

Component / Treatments	(E)- Caryophyllene	ar-Curcumene	α-Muurolene	E-Nerolidol	Spathulenol	Caryophyllene oxide	epi-α-Cadinol	Monoterpene Hydrocarbons	Oxygenated Monoterpenes
T1	1.89 def	8.04 d	0.81 cd	1.37de	5.41de	8.72cd	1.46c	5.49g	52.88d
T2	1.63 efgh	6.95 de	0.79 cde	0.92g	5.87cd	7.62de	1.40c	9.05cd	53.08d
T3	1.49 gh	6.12 ef	0.73 def	1.06fg	4.73ef	6.06fg	1.07def	5.35gh	62.18ab
T4	0.97 ij	3.58 h	0.59 fg	1.16ef	4.72ef	6.08fg	0.86f	9.44c	59.45b
T5	3.00 a	12.79 ab	1.24 b	1.95bc	7.72ab	8.69cd	1.78b	4.24i	45.46fg
T6	1.60 fgh	5.79 ef	0.64 ef	1.06fg	4.77def	5.34g	1.07def	8.79d	58.20bc

T7	1.97 cde	7.07 de	0.76 de	1.47d	5.38de	6.03fg	1.27cde	8.25e	54.57cd
T8	1.82 defg	6.75de	0.74 def	1.50d	5.87cd	6.82ef	1.33cd	8.94d	53.34d
T9	1.32 hi	4.03gh	0.23 h	0.45h	2.14g	2.79i	0.50g	9.42c	65.23a
T10	3.09 a	13.79a	0.74 def	1.30de	7.93ab	9.78c	2.05a	6.31f	38.42h
T11	2.06 cd	10.39 c	1.42 a	2.01ab	8.57a	14.50a	2.12a	2.64j	41.55gh
T12	2.58 b	11.58 bc	1.33 ab	2.20a	8.28a	11.18b	2.01ab	4.39i	40.23h
T13	1.49 gh	6.11ef	0.74 def	1.06fg	4.73ef	6.78ef	1.10de	12.84a	50.41de
T14	1.36 h	5.30fg	0.68 def	1.49d	5.84cde	6.53ef	1.39c	9.10cd	52.85d
T15	2.30 bc	10.37 c	0.94 c	1.78c	6.83bc	9.56c	1.03ef	4.99h	47.80ef
T16	0.94 j	3.65 h	0.46 g	0.94fg	3.75f	4.12h	0.87f	10.20b	58.90bc

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

Table 4. Effect of foliar sprays plant growth regulators and bio-active compunds on essential oil components (%) on *L. citriodora*

Component / Treatments	Sesquiterpene Hydrocarbons	Oxygenated Sesquiterpenes	Leaf dry weight(g)
T1	10.74 e	16.96 de	1.70 f
T2	9.37 ef	15.81 ef	3.07 cd
T3	8.34 fg	12.92 gh	3.44 bc
T4	5.14 i	12.82 gh	5.70 a
T5	17.03 ab	20.14 c	2.17 ef
T6	8.03 fg	12.24 h	3.05 cd
T7	9.80 ef	14.15 fgh	3.03 cd
T8	9.31 ef	15.52 ef	3.88 b
T9	5.58 hi	5.88 j	5.87 a
T10	17.62 a	21.06 c	2.90 cd
T11	13.87 cd	27.20 a	1.70 f
T12	15.49 bc	23.67 b	2.08 ef
T13	8.34 fg	13.67 fgh	2.28 e
T14	7.34 gh	15.25 efg	2.87 d
T15	13.61 d	19.20 cd	1.68 f
T16	5.05 i	9.68 i	3.37 bcd

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

The maximum content of E-citral was related to (ChM5) and the lowest content was observed at (I2G1M5). The maximum content of Z-citral was observed at (I2G1) and the lowest content at (I2G1M5) was achieved. The maximum amount of r-curcumene by (I2G1M5) and the lowest was observed at (ChM5). The maximum content of caryophyllene oxide was achieved by (I2G1Ch) and its minimum was related to (I2G1). The maximum content of spathulenol was achieved at (I2G1Ch) and its minimum was observed at (I2G1). The maximum content of limonene was observed at (I1G2) and the minimum content was related to (I2G1Ch) (Tables 1, 3, 4).

However, the maximum value of essential oil component was related to oxygenated mono trepans with value of 47.80% of the total component, which was observed at (I2G1). The maximum yield of oxygenated sesquiterpenes, with value of 19.20% of the total component, was observed at (I2G1Ch) with the content of 27.70%. Also, sesquiterpene hydrocarbons with value of 13.61% of the total component with the maximum yield 17.62% was observed at (I2G1M5). The maximum yield of monoterpene hydrocarbons with value of 4.99% of the total component was observed at (I1G2) with the content of 12.84% (Tables 1 & 3). The maximum leaf dry weight was achieved by (I2G1) (Tables 1, 3, 4).

Discussion

Essential oils are various groups of natural products that are largely composed of terpenes and aromatic polypropanoid compounds derived from the acetate-mevalonic acid and the shikimic acid pathways, respectively. Plant essential oil content and compositionare highly affected by genetic and environmental factors via genetic expression influence (Charles and Simon, 1990).

In this experiment, E-citral, Z-citral, r-curcumene, caryophyllene oxide, spathulenol and limonene were the main components of the oil. Previously, Khani *et al*. (2012) revealed that 1, 8-cineole, α-curcumene, geranial, limonene and caryophyllene oxide were the main components of essential oils of *L. citriodora* leaves. Previous studies indicated that citral is synthesized from geraniol or nerol by an alcohol dehydrogenase or alcohol oxidase (Iijima *et al*., 2006). Ismaelzadeh behabadi and Sharifi (2013) showed that the use of biological elicitor might increase or change the production of secondary metabolites in lemon verbena. Citral is a valuable flavour and scent component that is used in the food and perfume industries. The highest amount of E-citral was observed in a treatment where only chitosan was there. Khan et al. (2002) reported that using leaf spray of chitosan and chitin on corn and soybean might stimulate physiological activity. Changes in phytochemical have been the result of morphophysiological changes through induction of bio-active compounds and plant growth regulators.

In this experiment, the maximum value of essential oil component was related to oxygenated mono trepans with value of 47.80% of the total component. This was observed at (I2G1). Bideshki et al. (2012) on *Alliumsativum* L. indicated that using IBA

could increase allicin content by 25%. Hassanpour Aghdam et al. (2011) on *Lavendula officinalis* observed that by increasing gibberellic acid concentration to 300 mg, the essential oil yield and content increased. Plant growth regulators (PGRs) have crucial impact on primary and secondary metabolism of plants (Hassanpouraghdam et al., 2011). Among PGRS, there is strong evidence that GA_3 had effects on plants growth and development and consequently in their active principle content and yield. Application of 100 mg L^{-1} GA_3 resulted in higher essential oil content of *Salvia officinalis* L. compared to control (Povh and Ono. 2006). Sesquiterpene hydrocarbons with value of 13.61% of the total component with the maximum yield of 17.62% were observed at (I2G1M5) .According to previous experiment, spraying of methanol (Downie et al., 2004) and the application of sucrose as a carbon source and bio-stimulator in the culture medium could increase the secondary products and also plant growth and yield (Tabatabai and Omidi., 2011). These results are in accordance with our studies. In this study, use of such materials could increase production and operation of lemon verbena. In our research, the highest content the sesquiterpene hydrocarbon was observed in treatments where methanol was present with gibberellic acid and indole butyric acid. The results are in accordance with other studies. In our experiment on lemon verbena, it was found that different concentrations of gibberellic acid and indole butyric acid could increase leaf dry weight. Midan et al. (1982) indicated that spraying onion seedlings with solution IBA after transferring the main land significantly increased yield and growth factors. There were also quantitative and qualitative improvements in the properties of onion. Amal et al. (2009) indicated that using 100 ppm of IBA significantly increased number of leaves, fresh and dry weight of peas plant, which agreed with our studies.

Conclusion

In this experiment, the formulation of bio-active compounds and plant growth regulators had positive effects on growth and phytochemical traits of *L. citriodora*. The E-citral compound, which is a valuable flavour and scent reagent used in food and perfume industries, was increased by chitosan application. The maximum value of components was related to oxygenated mono trepans with value 47.80% of the components. This was observed in the treatment of plant growth regulators. The highest essential oil percentage was achieved by methanol and chitosan. Generally, foliar application of GA_3, IBA, methanol and chitosan could increase medicinal and nutritional values of *L. citriodora*.

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Author contributions

The following declarations about author contributions to the research have been made: M.R. implemented the project and wrote the paper; A.M. designed the research; A.M., H.N., F.KH. Conducted the research; A.M., H.N. were Advisors and F.KH. was Consulting Advisor of the research.

Conflict of interest

Authors declare no conflict of interest.

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