INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 5, No. 2, p. 56-63, 2014

RESEARCH PAPER OPEN ACCESS

Effect of planting density and sowing date on the essential oil content and composition of lemon verbena (*Lippia citriodora)*

Ali Nematian[1*], Gholam Reza Dalvandi[1], Mohammad Ali Shariati[2, 3]

[1]*Department of Agronomy, Islamic Azad University, Borujerd, Iran*

[2]*Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran*

[3]*Rahpouyan Kamyar , Quality Control Lab , Food Inspection Laboratory , Khansar, Iran*

Key words: *Lippia citriodora,* sowing date, planting density, essential oil.

http://dx.doi.org/10.12692/ijb/5.2.56-63 Article published on July 25, 2014

Abstract

Lemon verbena leaves are used as the plant for injection, aromatic, digestive and antispasmodic properties. Lemon verbena leaves has a mild sedative and reputation for relieving abdominal discomfort. In order to examine the influence of planting density and sowing date on the essential oil Neral, Geranial and Limonene in *Lippia citriodora*, an experiment was performed in Rasht experimental farm, Gilan province, 2011. The experiment was done as a factorial on the basis of randomized complete blocks design in three replications. Density treatments at three levels: 2, 4, 6 plants /m^2 and sowing treatment of March 24th, April 8th and April 24th. The chemical composition was analyzed by Gas chromatography (GC) and Gas chromatography/mass spectrometry (GC/MS). 37 compounds were identified in the Essential oil of *Lippia citriodora*. The most important compounds in plant lemon verbena were, Neral, Geranial and Limonene. Regarding the obtained results, planting density and sowing date treatments had a significant effect on the rate of essential oil and the highest amounts of Neral, Geranial and Limonene were measured as 23.25, 33.68 and 5.84 in the 4 plant/m2 and planting on March 24th.

* **Corresponding Author:** Ali Nematian ✉ alinematian1982@gmail.com

Introduction

The genus Lippia (*Verbenaceae*) brings together about 200 species which are native to South and Central America and Africa. These compounds have widespread uses in foods, cosmetics and household products (Catalan and Lampasona, 2002; Santos-Gomes *et al*., 2005). The essential oils and leaves are used in perfume industry and producing drinks with different flavors, respectively and for food preparations(Pascual *et al*., 2001).In the United States, *Lippia citriodora* is listed in Generally Regarded As Safe (GRAS) for human consumption in alcoholic beverages(Gomes *et al*., 2006). *Lippia citriodora* synonyms are *Aloysia citriodora* Palau (*Aloysia triphylla*) and *Verbenaceae* which 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). *Lippia citriodora* is an aromatic perennial shrub that is valued for its medicinal use (as a tea or tincture) as a digestive aid, antimicrobial, antispasmodic, analgesic, diuretic plant; besides, it is used as a treatment for cold, insomnia and anxiety (Botta, 1979; Carnat *et al*., 1999; Rotman and Mulgura Romero, 1999). Antibacterial and antioxidant activities of *Lippia citriodora* have been found in essential oils, tea and tinctures (Bilia *et al*., 2008; Cowan, 1999; Pereira and Meireles, 2007; Bully *et al*., 2002; Ohno *et al*., 2003; Sartoratto *et al*., 2004). Extracts of leaf essential oils are also used extensively in cosmetics and flavoring industries.

Today, due to revealing the lateral complication of chemical drugs, the use of herbal drugs is increasing. Whereas very low researches have been applied on enhancement of medicinal plants production, presenting the appropriate planting methods is very important for increasing the quantity and quality of medicinal plants(Farooqi *et al*., 2001). The planting product of a medicinal plant is cost-effective when its secondary metabolites measure would be reached to an appropriate amount. The purpose of trading production of medicinal plants is obtaining further amount of biomass in area containing high rate of active substances (Default *et al*., 2003). One of the most important peripheral factors for growth of medicinal plants that have a major effect on the quantity and quality of active substances may refer to planting density and sowing date.

In the study applied on the effect of sowing date on the chicory root yield, the researcher concluded that the earlier sowing date will increase the desiccated root and fructose yield (Schittenhem, 2001). Circella *et al*(1993) performed a study on Ajowan, observed that the planting rows up to 50cm are suitable for branching and growing growth. While studying the influence of different densities on the yield and quality of Artemisia essential oil, it was observed that the density had significant effect on the yield and essential oil of this plant and within the density of 15 plant/m^2, the highest amount of Artemisia essential oil and compound (Ayanoglu & Mert, 2002). Experiments with spacing *Thymus vulgaris* have shown that, while wider spacing led to the highest level of biomass and yield of essential oil plant cultivation dense significantly increased the yield of essential oil per unit area(Clark and Menary,1979;Shalaby Razin, 1992).

The density was shown to have a global effect on *Mentha piperita* essential oil yield per hectare, giving higher yields in the mid-range 10 to 30 cm densities, deserved simply to the increase in biomass, as the concentration of essential oil did not differ significantly between spacings(Rissanen *et al*., 2002). The quality of essential oil of lemon verbena is largely determined by the merger of the two isomers of citral (Neral and Geranial) and Limonene. Studies have shown that these compounds are present in all chemotypes of *Lippa citriodora*, but their concentrations vary considerably according to source of origin, parts of the plant sampled, and stage of plant development (Argyropoulou *et al*.,2007; Gil *et al*., 2007). Production of high quality aromatic herbs requires an excellent raw material, which implies good agricultural procedures, collection date correct, appropriate industrial processing and storage conditions, all measures that could affect the sensory quality of the final product (Rodrigo *et al*., 2009).

Perusing the references implies that so far no study has been applied on the effect of different climatic factors such as planting density and sowing date on lemon verbena in Iran. Considering the importance of this plant in the society health and pharmaceutical, food, cosmetic and hygienic industries, as well as studying the factors affecting essential oil upraising, this research has been implemented in Gilan Province, Rasht County.

Materials and methods

Planting

This study has been applied with the objective of measuring the lemon verbena essential oil rate in different sowing dates and planting densities as the factorial on the basis of randomized complete blocks design in 3 replications, in Gilan Province, Rasht County on a experimental farm 450 square meters in area, each patches including 5 planting lines 4m in length, the interval between two planting lines 50cm (the area of each patch is 10 square meters and patch length is 4m and patch width is 2.5m). The climatic specifications and testing area soil have been provided in tables 1 and 2. The test treatments included sowing date for three dates of March 24th, April 8th and April 24th, and planting density for 2, 4 and 6 plant/m^2; in density 2, the interval between 2 cuttings is 100cm, in density 4, the interval between 2 cuttings is 50cm, and in density 6, the interval between 2 cuttings is 33.3cm. At first, the field is plowed then was flattened and patched. Firstly, some health, juicy and same-sized cuttings 30cm in length and 5mm in diameter were provided fro the lower parts of the plant. The cuttings have been placed in greenhouse mode after treating by Indole Butyric Acid hormone (concentration of 100 ppm) in sand bed. They were rooted after about one month. The rooted cuttings were cultivated with relative densities on the related dates. The weeds were controlled manually in several times. During the test, no pests and diseases were observed.

Collecting

Collections had been made early in the morning of days of sunshine 120 days after cultivation. Two leaves were collected from the middle node through five stems of two plants in each replicate(20 sheets in total). The leaves were placed into packages with aluminum foil and stored in a refrigerator for transport to the laboratory for analysis. The sheets were utilized to measure surface area and were dried for two days at 60 ° C.

Extracting

Essential oil extraction from growing body is performed via distillation by water and Clevenger. For this purpose, 40gr of desiccated root of each treatment (3 replicates) is weighted and grinded thoroughly and put in Clevenger for 4 hours in 500ml of water in order to extract its essential oil (European Pharmacopoeia, 2005).

GC/GC MAS Chromatography

For identification and measurement of lemon verbena essential oil constituents, gas chromatograph and gas chromatograph connected to the mass spectroscope with the following specifications were used:

GC: Gas chromatograph Shimadzu model A column DB-5 30m in length and 0.25mm in diameter, stationary phase layer thickness 0.25µm, column thermal planning from 50 to 280°c with the temperature upraise of 3°c per minute, detector FID with 270°c temperature, helium carrying gas with the pressure of 3kg/cm^2.

GC/MS

Gas chromatograph connected to the mass spectroscope Saturan model 3400 column DB-5 30m in length and 0.25mm, stationary phase layer thickness 0.25µm, column thermal planning from 50 to 250°c with the temperature upraise of 4°c per minute, injection chamber temperature of 360°c, ionization energy of 170 electron volt , helium carrying gas. Identification of spectrums aiding their inhibition index and upon injection of normal hydrocarbons (C9-C27) under equal mode was calculated by injecting the essential oils and aiding a program in Basic language(Bos *et al.*, 2000). The quantitative calculation (determination of composition rate) was performed via Area

normalization method and response factor related to the spectrums.

The data of this study was analyzed by statistical software MSTAT-C software and the mean values were compared by Duncan method.

Results

Planting density

The summary of analysis of variance indicated that the effect of different ranges of density on the essential oil rate is significant within 0.01(table 3). As well as, the result of comparing the mean values by Duncan test showed that the highest rate of Neral with the mean value of 21.58 and highest rate of Geranial with the mean value of 30.6, and highest rate of Limonene with the mean value of 4.9 have been obtained for 4 plant/m² (table 4). No significant difference observed between the density D1 and D2 in both tested traits, and have been placed in a statistical group.

Table 1. Climatic characteristics of experimental farm.

Longitude	Latitude	Mean precipitation	Mean humidity	Maximum temperature	Minimum temperature
Eastern 49˚,36'	Northern 37˚,18'	1357 mm	81.9%	20.5	11.3

Table 2 . Chemical and physical characteristics for soil of experimental farm (at the soil depth of 0 - 30 cm).

Soil texture	pH	EC (ds/m)	Water absorption %	Organic Carbon %	Total Nitrogen %	Absorptive Phosphorus % (p.p.m)	Absorptive Potassium % (p.p.m)	Clay %	Sand %	Silt %
Loamy- clay	7.2	1.05	59	4.26	0.13	19.73	289	33	37	25

Table 3. Variance analysis results of plant density and sowing date on the characteristics of lemon verbena.

S.O.V	df	Square Means			
		Essensiol oil	Neral	Geranial	Limonene
Replication	2	0.008ns	78.5ns	84.6ns	312.8ns
Density	2	**1.512	165413.2**	62573.6**	40368.2**
Sowing date	2	**1.573	**179353	**52848.9	**411154.3
Density*Sawing date	4	**0.027	**3571.6	**490304.8	**1839.5
Error	16	115.5	102.6	117.5	167.4
C.V%		4.2	5.2	4.8	5.7

ns,* and **: Non significant ,and significant at the 5% and 1% levels of probability, respectively.

Sowing date

The summary of analysis of variance indicated that the effect of sowing date ranges on the essential oil rate is significant within 0.01(table 3). Upon comparing the mean values by Duncan test, it was concluded that the highest rate of Neral, Geranial, Limonene with the mean value respectively equal to 22.35, 32.7 and 5.53 was observed in sowing date treatment S_1(April 24th) (table 4).

Interaction between planting density and sowing date

The summary of ANOVA table implies the significance of interaction between planting density and sowing date affecting all tested traits within 0.01(table 3). Comparison of mean values by Duncan

test showed that the highest rate of Neral, Geranial, Limonene was obtained in treatment D_2S_1 (density of 4 plant/m² and sowing date April 24th)(table 5).

Discussion

Upon reviewing the references, it is concluded that there are rather reliable information on the pharmaceutical-medical and health effects of lemon verbena is available but the just finite information on the planting and agricultural management of this plant is available (Bandoni *et al.*, 2008).

Table 4. Mean comparisons of various density and sowing date levels on Essensioal oil, Neral, Geranial, Limonene of lemon verbena.

Density (plant/m²)	Measured Features			
	Essensiol oil	Neral	Geranial	Limonene
D_1	1.85a	a58.21	a62.30	a9.4
D_2	1.31a	a72.20	a8.29	a1.4
D_3	1.08b	b2.18	b85.22	b21.3
Sowing date				
S_1	1.73a	a22/35	a7.32	a53.5
S_2	1.42b	b93.18	b94.29	b91.4
S_3	1.02c	c82.14	c8.17	c12.3

-Means with the same letters in each column are not significantly different.

-D_1: 2(Plant/m²) , D_2: 4(Plant/m²) , D_3: 6(Plant/m²), S_1 = March 24th, S_2 = April 8th and S_3 = April 24th.

Table 5. Mean comparison of density and sowing date interaction for the features of Essensioal oil, Neral, Geranial, Limonene.

Plant density*Sowing date	Measured Features			
	Essensioal oil %	Neral %	Geranial %	Limonene%
D_1S_3	de 2	e 19.32	e 24.6	de 3.96
D_2S_3	e 1.3	ef 19.76	e 25.4	e 3.81
D_3S_3	f 1.1	g 18.5	g 18.3	f 3.1
D_1S_2	bc 2.7	b 21.34	b 30.41	b 4.93
D_2S_2	cd 2.5	b 20.96	c 29.83	b 4.85
D_3S_2	d 2.3	d 19.6	f 20.42	d 4.12
D_1S_1	b 2.9	ab 22.23	a 32.3	ab 5.21
D_2S_1	a 3.2	a 23.25	a 33.63	a 5.84
D_3S_1	c 2.5	c 20.42	dc 29.2	c 4.34

The peripheral factors are effective on the quality and quantity of active substances of pharmaceuticals such as planting density and sowing date. The biosynthesis of active substances of lemon verbena is dependent on the light regimes and plant breathing (Letchamo *et al.*, 2004). Therefore, in density D_1 and D_2, due to lower ghosting and higher light absorption by the lower parts of Canopy, the maximum active substance was obtained. In density D_1 and D_2, more light is received by the plant and this light increasing has positive effect on the essential oil value, and in density D_3, due to the ghosting the essential oil value is reduced. These results are in compliance with the observations(petrropoulos, 2004) of parsley and research applied by Schittenhelm(2001) on chicory. For optimum use of peripheral factors such as water, foodstuffs and prevention from emerging the intensive competition, the number of shrubs in the area unit must be appropriate. The appropriate density of shrub is a density on account of which all peripheral factors are used completely by the lemon verbena, yet the competition inside and outside of shrub to be minimum, in order to obtain the maximum value of essential oil with the high quality.

Due to the quality and climatic status appropriateness for increasing the biosynthesis of active substance, sowing date April 24th is the better sowing date for this trait. So that the thermal mode of this sowing date comparing to the warmer days, at the time of harvest intends to increase the active substance. Upon increasing the temperature during the plant growth and growing period shortening, reduction of leaf area and reduction of photosynthesis area will reduce the active substance. Timely cultivation of lemon verbena increases the growth period so that leads to maximum leaf production and ultimately increasing the active substance (Rocha *et al.*, 2011). At this date, due to the plant's whole use of raining thoroughly, better competition with weed, less infection by pests and diseases and suitable climatic status at the season beginning, the rate of above essential oil has been obtained higher than other sowing dates. The delay in planting results in germination delay and reduction of plant growth speed (Zehtab Salmasi *et al.*, 2002). This is consistent with the results found by Argyropoulou *et al.* (2007). Similar results have been shown to enhance the production of essential oil of thyme, lavender, mint Australian and Hungarian perppermint and Tasmania(Clark and Menary 1979; Shalaby and Razin 1992; Topalov and Zheljazkov, 1996; Rissanen *et al.*, 2002; Arabaci *et al.*, 2007).

Table 6. Lemon verbena essential oil compounds.

Row	Compounds	RI[a]	Area,%
1	5-hepten-2-one-6-methyl	993	1.5
2	1,8-cineol	1034	1.8
3	β-pinene oxide	1152	0.3
4	ð-Elemene	1337	0.6
5	(E)-caryophyllene	1424	3.2
6	Geraniol	1263	0.9
7	τ-cadinol	1657	0.2
8	α-humulene	1454	0.2
9	δ-cadinene	1522	0.6
10	α-zingiberene	1497	0.9
11	Geranyl propanoate	1479	0.3
12	trans-p-mentha 1(7),8-dien-2-ol	1186	0.3
13	Linalool	1104	0.4
14	Nerol	1238	0.7
15	Neral	1255	23.5
16	Geranial	1285	33.2
17	iso-Isopulegol	1165	0.2
18	-pinene α	932	0.2
19	trans-β-ocimene	1048	3.2
20	cis-sabinene hydrate	1073	0.2
21	caryophyllene oxide	1586	2.6
22	Bicyclogermacrene	1502	4.3
23	3-octanol	998	0.2
24	Limonene	1031	5.3
25	(E)-Nerolidol	1567	1.7
26	Spathulenol	1582	0.8
27	α-curcumene	1514	4.1
28	Sabinene	973	0.6
29	1- Octen-3-ol	982	0.2
30	Rosefuran epoxide	1177	0.2
31	α-terpineol	1197	0.8
32	Eugenol	1360	0.2
33	Germacrene D	1484	5.3
34	Geranyl acetate	1386	1.1
35	allo-Aromadendrene	1462	0.3
36	α-cedrene	1413	0.3
37	α-copaene	1375	0.3

[a] Retention indices on BP-20 column; (experimental).

Based on the results of our study should be planted lemon verbena (4 plant/m² & March 24th) in order to optimize the percentage of essential oils from leaves.

Acknowledgements

Thanks for Dr.ghooshchi and Dr.ariapour for supporting me in duration of this research.

Reference

Argyropoulou C, Daferera D, Tarantilis PA, Fasseas C, Polissiou M. 2007. Chemical composition of the essential oil from leaves of Lippia citriodora HBK (Verbenaceae) at two developmental stages. Biochemical Systematic and Ecology **35,** 831-837.
http://dx.doi.org/10.1016/j.bse.2007.07.001

Arabaci O, Bayram E, Baydar H, Savran AF, Karadogan T, Ozay N. 2007. Chemical composition, yield and contents of essential oil of Lavandula hybrida reverchon grown under different nitrogen fertilizer, plant density and location. Asian Journal of Chemistry **19(21),** 84-2192.

Bandoni AL, Lira PD, van Baren CM, Retta D, Gil A, Gattuso M, Gattuso S. 2008.Characterization of lemon verbena (Aloysia citriodora Paláu) from Argentina by the essential oil. Journal of Essential Oil Research **20,** 350-353.
http://dx.doi.org/10.1080/10412905.2008.9700028

Bilia AR, Giomi M, Innocenti M, Gallori S, Vincieri FF. 2008. HPLC-DAD-ESI-MS analysis of the constituents of aqueous preparations of verbena and lemon verbena and evaluation of the antioxidant activity. Journal of Pharmaceutical and Biomedical Analysis **46,** 463-470.
http://dx.doi.org/10.1016/j.jpba.2007.11007

Botta S. 1979. Las especies argentinas del genero Aloysia (Verbenaceae). Darwiniana **22,** 67-108.

Carnat A, Carnat AP, Fraisse D, Lamaison JL. 1999. The aromatic and polyphenolic composition of lemon verbena tea. Fitoterapia **70,** 44-49.
http://dx.doi.org/10.1016/s0367-326x(98)00016-1

Cowan MM. 1999. Plant products as antimicrobial agents. Clinical Microbiology Reviews **12,** 564-582.

Clark RJ, Menary RC. 1979. Importance of Harvest date and plant-density on the yield and quantity of Tasmanian peppermint oil. Journal of the American Society for Horticultural Science **104,**702-706.

Catalan CAN, PDe Lampasona ME. 2002. The chemistry of the genus Lippia (Verbenaceae). In S.E. Kintzios, (ed.) Oregano: The genera Origanum and Lippia, Taylor and Francis; London 127-149.

European Pharmacopoeia. 2005.5th ed. Council of Europe, Strasbourg **2,** 2667-2668.

Farooqi AA, Sreeramu BS. 2001. Book on cultivation of medicinal and aromatic crops. Universities press (india) Lth 115-120.

Gomes PCS, Oliveira HRC, Vicente AMS, Ferreira MF. 2006. Production, transformation and essential oils composition of leaves and stems of lemon verbena [Aloysia triphylla (L'Herit.) Britton] grown in Portugal. Rev. Bras. Pl. Med., Botucatu **8,** 130-135.

Gil A, Van Baren CM, Lira P, Bandoni AL. 2007. Identification of the genotype from the content and composition of the essential oil of lemon verbena (Aloysia citriodora Paláu). Journal of Agricultural and Food Chemistry **55,** 8664-8669.
http://dx.doi.org/10.1021/jf0708387

Letchamo W, Ward W, Heard B, Heard D. 2004. Essential oil of Valeriana officinalis cultivars and their antimicrobial activity as influenced by harvesting time under commercial organic cultivation. Journal of Agricultural and Food Chemistry **52,** 3915-3919.
http://dx.doi.org/10.1021/jf0353990

Mert A, Ayanoglu F. 2002. The effect of different plant density on yield, yield component and quality of Artemisia annua. Journal of Haworth Press **48(2),** 413-418.

Ohno T, Kita M, Yamaoka Y, Imamura S, Yamamoto T, Mitsufuji S, Kodama T, Kashima K, Imanishi J. 2003.Antimicrobial activity of essential oils against Helicobacter pylori. Helicobacter **8,** 207-215. http://dx.doi.org/10.1046/j.1523-5378.2003.00146.x

Pereira CG, Meireles MAA. 2007. Evaluation of global yield, composition, antioxidant activity and cost of manufacturing of extracts from lemon verbena (Aloysia triphylla [L'herit.] Britton) and mango (Mangifera indica L.) leaves. Journal of Food Process Engineering **30,** 150-173. http://dx.doi.org/10.1111/j.1745-4530.2007.00100.x

Rodrigo Infante, Pia Rubio, Loreto Contador, Violeta Moreno. 2009. Effect of drying process on lemon verbena (Lippia citrodora Kunth) aroma and infusion sensory quality. International Journal of Food Science and Technology **45,** 75–80. http://dx.doi.org/10.1111/j.1365-2621.2009.02105.x

Rissanen KS, Aflatuni A, Tomperi PH, Jalonen JE, Laine KM. 2002. Herbage and essential oil yield and composition of Mentha piperita L. in different plant densities in northern latitudes. Journal of essential oil research **14,** 243-246. http://dx.doi.org/10.1080/10412905.2002.9699841

Rotman AD, Mulgura de Romero ME. 1999. Verbenaceae. Flora del Valle de Lerma **5,** 1-37.

Schittenhelm S. 2001.Effect of sowing date on performance of root chicory. European Journal of Agronomy **15(3),** 209-220. http://dx.doi.org/10.1016/s1161-0301(01)00105-8

Sartoratto A, Machado ALM, Delarmelina C, Figueira GM, Duarte MCT, Rehder VLG. 2004. Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. Brazilian Journal of Microbiology **35,** 275-280. http://dx.doi.org/10.1590/s151783822004000300001

Shalaby AS, Razin AM. 1992. Dense cultivation and fertilization for higher yield of thme (Thymus vulgaris L). Journal of Agronomy and Crop Science-Zeitschrift Fur Acker Und Pflanzenbau **168,** 243-248. http://dx.doi.org/10.1111/j.1439037x.1992.tb01005.x

Santos-Gomes PC, Fernandes-Ferreira M, Vicente AMS. 2005. Journal of Essential Oil Research **17,** 73.

Valentao P, Fernandes E, Carvalho F, Andrade PB, Seabra RM, Bastos MD. 2002. Studies on the antioxidant activity of Lippia citriodora infusion: Scavenging effect on superoxide radical, hydroxyl radical and hypochlorous acid. Biological & Pharmaceutical Bulletin **25,** 1324-1327. http://dx.doi.org/10.1248/bpb.25.1324

Zheljazkov V, Topalov V. 1996. Effect of planting time and density on yields from rooted mint cuttings. Journal of herbs, spices & medicinal plants **4,** 15-24. http://dx.doi.org/10.1300/j044v04n03_03