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Comparison of morphological traits and essential oils content in *Lippia citriodora* L. cultivated in greenhouse and outdoor

Heidar Meftahizade*[1], Hojat Moradkhani[2], Seyed Hossein Rajab Nejad[3], Atefeh Fayazi Barjin[4]

[1]*Department of Horticulture, Faculty of Agriculture, University of Guilan, Guilan, Iran*

[2]*National Academy of Science of the Republic of Armenia*

[3]*Research Institute of Food Science and Technology, Mashhad, Iran*

[4]*University of Tehran, Tehran, Iran*

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Abstract

Lippia citriodora L., Verbenaceae family, is cultivated due to useful secondary metabolites like essential oil compound, which apply in the food, cosmetics industries. This plant is used as decreasing blood sugar, anti-blood releasing of nose and enteral. There are a lot previous studied which investigated essential oil compound in *Lippia citriodora* in greenhouse or field alone. Because *L. citriodora* were cultivated extensively in greenhouse and field in Yazd province. Knowing more about yield (morphological and essential content) in both conditions is very necessary. This study was designed with objective to evaluate the performance of *L. citriodora* for morphological and essential oil (yield and content) under different conditions of cultivation (greenhouse and field) in Yazd conditions. Our results showed Location exerted a highly significant influence ($P<0.01$) on all investigated traits. Year showed a highly significant influence ($P < 0.01$) on all the traits. Fresh leaf yield per plant, dry leaf yield per plant in *L. citriodora* is considered as economic traits. Location, year and interaction of Location × Year were significantly difference for these both parameters. GC-Mass chromatogram showed that the main compounds were D-limonene (7.85vs 6.68), Alpha-citral (6.57vs 5.52), Beta-Citral (5.68vs 4.65), spathulenol (4.58 vs 3.04), Caryophyllene oxide (6.54vs 4.96), in field and greenhouse respectively. As a results, cultivation of L. *citriodora* in greenhouse cause to increase plant biomass, while essential oil content were increased in field.

***Corresponding Author:** Heidar Meftahizade ✉ hmeftahi@yahoo.com

Introduction

Lippia citriodora L. is a green shrub from Verbenaceae family. The genus *Lippia* has approximately 200 species indigenous to southern and Central America and Africa. *Lippia* compounds have widespread applications in the food and cosmetics industries. This plant is used as decreasing blood sugar, anti-blood releasing of nose and enteral. The leaves of this plant are also useful for stomache pain, heartbeat, the feeling of doubtful sounds in ear and mental disturbances. (Santos-Gomes *et al.*, 2005). *Lippia citriodora* L. is cultivated mainly due to the lemon-like aroma spread from its leaves that are utilized for the preparation of herbal tea, which is reputed to have antispasmodic, antipyretic, sedative and digestive propertied. (Carnat, A, *et al.*, 1999). There are several previous studied, which investigated phytochemical analysis in greenhouse and field alone. yousefzadeh and meshkatalsadat, 2013 identify 55 compound in essential oils extracted from aerial parts *of Lippia citriodora* with essential oil yields 0.70%. Linde *et al.*, 2009 reported the main essential oils components in *Lippia citriodora* were include: E-citral, geranial, neral, limonene, caryophillene oxide, spathulenol, curcumen, borneol, neryl acetate, camphor, carvacrol, beta-caryophillene and para-cymene).

Gudaityte and Venskutonis, 2007 indicated that chemical differentiation of *lippia* essential oils might be correlated with environmental conditions, geographic, climatic, and genetic, plant age, soil, phase of vegetation, anatomical part of plant and harvesting season. Due to extension of *lippia* cultivation in Yazd province- Iran, investigation of morphological and phytochemical compositions in both conditions of greenhouse and Field (outdoor) is necessary. For access to the best condition for *lippia* cultivation with high yield, it is necessary to perform a research plan. Many previous studies have been concentrated on *lippia* cultivation in greenhouse or outdoor alone, whereas compare between these two condition has not been done by now. So, this study was designed with objective to compare the performance of *L. citriodora* for morphological and essential oil (yield and content) under different conditions of cultivation (greenhouse and field).

Material and method

The experiment was performed in two locations (greenhouse and Field: outdoor) at Yazd city, Iran for three years in 2013/2014, 2014/2015 and 2015/2016 cropping season. Hard stem bottom cuttings with 20 cm were taken from two year old disease free mother plants of Yazd research Station for seedling preparation. Seedlings were raised in the nursery for 75 days before being transplanted to the field experimental plots in three replications. A row and plant spacing of 80 cm was used. No chemical or fertilizer was applied during experimentation. Harvesting was made 3months after transplanting.

some morphological parameters were measured, such as: Plant height (cm), number of branches/plant, number of leaves/plant, dry leaf weight/plant (g), fresh leaf weight/plant (g), leaf yield/ha/year (kg), essential oil (EO) were noted. EO content was determined on a fresh weight basis from 100 g of leaves harvested. Leave were dried at shade. In greenhouse, controlled environment temperature hold at 30/18° C day/night temperature, and 72% relative humidity.

Essential oil extraction

The dried samples of *Lippia citriodora* were prepared to hydro-distillation using a Clevenger apparatus. Extraction times were performed (3:30 hour) with three replications. The essential oils were separated from the aqueous layer, dried over anhydrous sodium sulfate and calculated average of essential oil yield. The extracted essential oils were dried over anhydrous sodium sulphate and stored in sealed vials at low temperature (4°C) before gas chromatography-mass spectrometric (GC-MS) analysis. Essential oil content was defined as followed: R (%) = (mass essential oil/mass of the dried leaves) x 100.

Gas chromatography-mass spectrometry analysis of essential oil

The essential oil was analyzed in RIFST (Research institute of food science and Technology) Mashhad, Iran. Two μL aliquots of the concentrated dichloromethane extract were analyzed by gas chromatography-mass spectrometry (GC-MS), using an Agilent 6890N gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) attached to a JMS-600W mass spectrometer (JEOL Ltd., Tokyo, Japan).Phenyl Methyl Siloxane capillary column (30 m×0.25 mm, film thickness 0.25 μm) in the split mode (1:50) at 250°C. The oven temperature was set at 50°C for 1 min, then raised to 300°C at 2°C minG1 and finally held at this temperature for 10 min.

For statistical analysis, five samples were taken from each plot. Experimental data was statistically analyzed by analysis of variance (ANOVA) using SAS at $P< 0.01$. Differences between means were assessed using the Duncan's test at $P< 0.01$.

Result and discussion

Anova analysis for different parameters are listed in Table 1. As seen, Location exerted a highly significant influence ($P < 0.01$) on all the parameters considered in the study (Table 1). This indicates these parameters were influenced by changing in the environment. These significance was expected, because all conditions in green house and field was different. In green house most changes will be controlled, but in field, due to high evaporation and temperature it could not be controlled and environmental changes were occured. This result is consistent with Gudaityte and Venskutonis, 2007, which mentioned that chemical differentiation of *lippia* essential oils might be correlated with environmental conditions, geographic, climatic, and genetic, plant age, soil, phase of vegetation, anatomical part of plant and harvesting season. Fehr, 1991 reported, that any factor that is a part of the environment of a plant has the potential to cause differential performance. The performance of *lippia* was affected by year. Year showed a highly significant influence ($P < 0.01$) on all the parameters (Table 1). The main time for high performance of *Lippia* is second and third year, because in the first year, establishment and adaptation with environment is considered. But during second and third year, yield will be increased. So significance difference in aspect of year was expected.

Table 1. Anova analysis of morphological parameters in *Lippia citriodora* L.

Source	df	PH	NB/P	NL/P	FLW	DLW	LY/ha	EOC
Location	1	85.65**	192.14**	163.49**	438.29**	316.29**	1889.54**	171.751**
Year	2	93.14**	45.01**	26.77**	52.37**	29.70**	20.90**	9.911**
Location × Year	2	2.75	2.17	7.60**	13.67**	9.25**	0.759	19.313**
R	2	1.033	3.014	0.714	0.775	0.708	0.266	0.510

PH: Plant height, NBP: Number of branch/plant, NLP: Number of leave/plant, FLW: Fresh weight leaf, DLW: Dry weight leaf, LY/ha: Leaf Yield /hectare, EOC: essential oil Content.

Plant height was found statistically different over the testing. It varied form 35-120 cm over the testing location during three year (data not shown). 120 com were observed in green house in third year, but 35 com were seen in field in first year. According to growth stages of plant, this is acceptable, because in green house, relative humidity, which is necessary for high growth of *lippia*, was high and on contrary, temperature and evaporation was lower than outside (field) condition. Number of branches was influenced with location and years. The highest value of branches number were recorded in third year in greenhouse and the lowest amount were noted in first year in field.

Number of leaves produced was influenced with location, years and interaction of Location × Year (Table 1). As shown in Table 2, the highest amount of Number of leaves per plant were recorded in greenhouse (L_1) and second year (Y_2). On contrary cultivation in L2 (field) in first year has produced the least amount of Number of leaves (Table 2). A relative lower range of leaf number per plant from185-1290 was reported by Azarmi *et al.*, 2012, under condition in Iran.

Fresh leaf yield per plant, dry leaf yield per plant in *Lippia* is considered as economic traits. Location, years and interaction of Location × Year were significantly difference for both parameters.

Biomass yield of *lippia* varied during years and location. The highest value of dry leaf yield per plant was recorded in green house in third year (68.54 g), while the lowest were observed in field in first year (16.7 g) (Table 2).

Beemnet Mengesha Kassahun *et al.*, 2013 reported that, the values for fresh leaf yield per plant and dry leaf yield per plant were increased with elevating testing years up to second Year and starts to decline then after.

Table 2. Interaction of location × Year on many traits.

L×Y	L1×Y1	L1×Y2	L1×Y3	L2×Y1	L2×Y2	L2×Y3
NLP	438.16[c]	758.66[a]	623.66[ab]	189.66[e]	289.66[d]	398.25[c]
FLW	168[c]	187[b]	300[a]	101.3[e]	103.5[e]	143[d]
DLW	28.5[c]	32.8[b]	68.54[a]	16.7 [de]	18.5[e]	24.6[d]
EOC	0.65[bc]	0.63[c]	0.62[c]	0.69[b]	0.81[a]	0.82[a]

L: Location, Y:Year, NLP: Number of leave/plant, FLW: Fresh weight leaf, DLW: Dry weight leaf, EOC: essential oil Content.

An increasing trend of leaf yield from first year to second year was also reported by Karik and Azkan, 2011. Beemnet Mengesha Kassahun *et al.*, 2013 reported that, average fresh leaf yield per plant and dry leaf yield per plant were 73.42 g and 10.50 g. Fresh and dry leaf yield per plant obtained in the our study are consistent with Azarmi *et al.*, 2012, who reported a range of values from 18.1-250.3 g for fresh leaf yield per plant and 4.9-58 g for dry leaf yield per plant tested under different production system in Iran.

The variation in the performance of *lippia* (fresh leaf yield per plant and dry leaf yield per plant) is due to hereditary differences in the plants, difference in the environments in which the plants are grown, or a combination of both (Allard, 1960).

The content of essential oil in investigated condition in different year showed that. *Lippia* cultivated in field (out of greenhouse) in second and third year has the highest amount of essential oil (0.81-0.82 % respectively), while the lowest value is in greenhouse in third year (0.62). Beemnet Mengesha Kassahun *et al.*, 2013 reported that, the essential oil yield range obtained in their study was within the range of essential oil content from 0.08 to 0.8%. Another Study conducted in Brazil demonstrated an essential oil content range between 0.58 and 1.49% on dry weight basis (Pereira, C.G. and Meireles, 2007).

Essential oil composition: The chemical compositions of essential oils were analyzed by GC/MS. The chemical compositions are reported in Table 3 (e.g. Fig. 2 and e.g. Fig. 4). The main compounds are D-limonene (7.85vs 6.68), Alpha-citral (6.57vs 5.52), Beta-Citral (5.68vs 4.65), spathulenol (4.58 vs 3.04), Caryophyllene oxide (6.54vs 4.96), in field and greenhouse respectively.

Table 3. GC-Mass analysis of *Lippia citriodora* L.

Number	Name of compound	RT (min)	Formula	Area% (field)	Area% (greenhouse)
1	Alpha-Pinnene	5.91	$C_{10}H_{16}$	0.84	0.38
2	Sabinene	6.76	$C_{10}H_{16}$	1.78	1.08
3	D-limonene	8.07	$C_{10}H_{16}$	7.85	6.68
4	Cineol	8.14	$C_{10}H_{18}O$	3.54	2.72
5	Pipperitone	9.56	$C_{10}H_{16}O$	0.58	--
6	linalol	9.81	$C_{10}H_{16}O$	3.04	2.7
7	Iso - geraniol	11.22	$C_{10}H_{16}O$	0.25	0.58

Number	Name of compound	RT (min)	Formula	Area% (field)	Area% (greenhouse)
8	Levomenthol	11.83	$C_{10}H_{20}O$	2.17	1.83
9	Iso-neral	12.01	$C_{10}H_{16}O$	2.54	1.22
10	Alpha- terpineol	12.34	$C_{10}H_{18}O$	1.08	1.02
11	Beta-Citral	13.63	$C_{10}H_{16}O$	5.68	4.65
12	Alpha-citral	14.41	$C_{10}H_{16}O$	6.57	5.52
13	Citraldiethyle acetate	16.86	$C_{14}H_{26}O_2$	0.38	0.38
14	Alpha- Curcumine	18.26	$C_{15}H_{22}$	1.85	2.35
15	spathulenol	21.125	$C_{15}H_{24}O$	4.58	3.04
16	Caryophyllene oxide	22.385	$C_{15}H_{24}O$	6.54	4.96
17	delta.-Cadinene	23.623	$C_{15}H_{24}$	1.26	0.7
Total				50.53	39.81

Our results indicate that, essential oil compound in field (outside greenhouse) are higher than essential oil compounds in greenhouse. These results are contrary with morphological traits. However, the amount of investigated morphological traits in greenhouse circumstance are high, but essential oil compound (quality traits) in greenhouse are lower than field test. Many other studies have been reported essential oil compounds, for instance: Taheri Aziz Abadi *et al.*, 2014, reported, The main constituents of the *lippia* essential oil in field were sabinene (1.34%), 6 methyl-5heptene-2one (3.46%), D- limonene (5.81%), 1,8-cineole (2.51%), trans-beta ocimene (1.17%), alpha terpineol (1.75%), neral (12.6%), geranial (15.07%), geranial acetate (1.15%), caryophyllene (4.02%), D- germacrene (3.52), alpha-curcumene (4.17%), bicyclogermacrene (3.42%), nerolidol (1.59%), spathulenol (4.40%) caryophyllene oxide (2.04%), alpha-cadinol (1.06%).

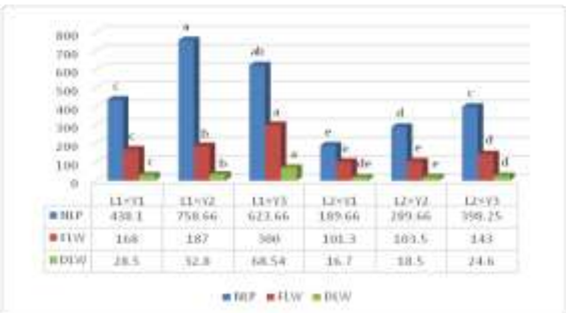

Fig. 1. Interaction of Location × Year on many parameters.

NLP: Number of leave/plant, FLW: Fresh weight leaf, DLW: Dry weight leaf

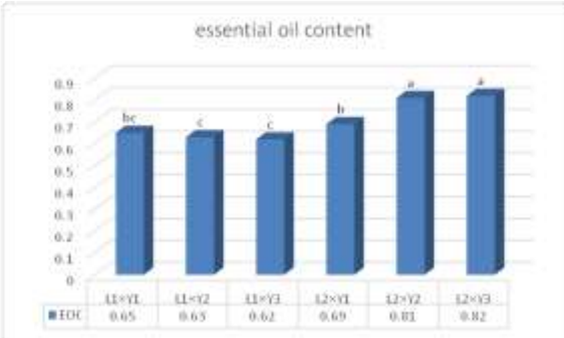

Fig. 2. Interaction of location × year in essential oil content.

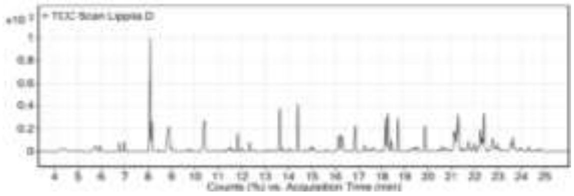

Fig. 3. GC-Mass analysis of *Lippia citriodora* L. in field.

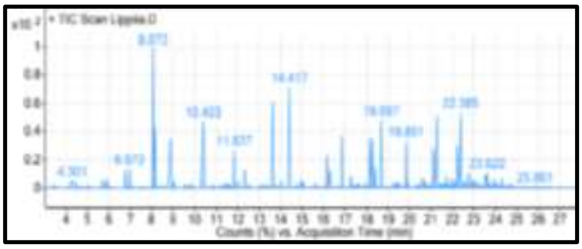

Fig. 4. GC-Mass analysis of *Lippia citriodora* L. in greenhouse.

Conclusion

The comparison of these two conditions (green house and field) showed that the essential oil content in the field was more than in the greenhouse condition, but in aspect of morphological traits, our results indicated that, *Lippia citriodora* L. cultivated in greenhouse were more than in field. This may due to changes in environmental like as temperature, relative humidity and so on. Due to the growing commercial importance of *L. citriodora* secondary metabolites, there is great interest in enhancing their production via greenhouse.

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