Effect of nitrogen fertilization on essential oil yield and composition in different species and accessions of *Lippia*

Efecto de la fertilización nitrogenada en el rendimiento y la composición de los aceites esenciales de especies y accesiones de Lippia

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Abstract

Some species of *Lippia* genus have acquired importance due to its potential to yield essential oils with multiple uses and therefore requires research aspects related to the crop managing. In experiments conducted at the Experimental Center of Universidad Nacional de Colombia, Palmira (CEUNP), the effect of two sources of Nitrogen fertilization (hen manure and urea (50 and 100 kg of N/ha), two accessions of *Lippia alba* (Cítrica and Típica) and three of *Lippia origanoides* (Patía, Típica and Cítrica) were evaluated. A randomized complete block experimental design with five treatments and three repetitions was used. Yield of fresh and dry biomass and water vapor extraction of the essential oils were assessed and, chemical composition of essential oils was made. The highest dry matter yield and essential oils obtained (3.382 kg/ha and 82,9 l/ha respectively) were for *L. origanoides* accessions. The highest essential oil concentration (4,0 ml/100g) was obtained from *L. origanoides* Patía treated with 100 kg of N (urea)/ha. There were not significant differences ($P < 0.05$) in chemical composition of the essential oils and the chemical differences founded between the essential oils were more associated to specific accessions than to the nitrogen supply.

Key words: Accessions, concentration, essential oils, fertilization, *Lippia sp.*

Resumen

En el Centro Experimental de la Universidad Nacional de Colombia sede Palmira (CEUNP) se evaluó el efecto de la aplicación de nitrógeno (N) en la producción de materia seca y aceites esenciales de dos especies de *Lippia*. Se utilizaron las accesiones Cítrica y Típica de *Lippia alba* (Miller) N.E.Brown ex Britton & Wilson y Patía, Típica y Cítrica de *L. origanoides* H.B.K. Como fuentes de N se aplicaron gallinaza y urea en dosis equivalentes de 50 y 100 kg/ha de N. Se utilizó un diseño experimental de bloques completos al azar, con cinco tratamientos y tres repeticiones para cada accesión. Se evaluaron los rendimientos de biomasa fresca y seca (MS) y de los aceites esenciales y su composición por arrastre de vapor en hojas secas. Los mayores rendimientos de MS y de aceite esencial (3.318 kg/ha y 82.9 lt/ha, respectivamente) se obtuvieron con las accesiones de *L origanoides.* La mayor concentración de aceite esencial (4 ml/100 g) se registró en *L. origanoides* Patía con la aplicación de 100 kg/ha de N como urea. Las diferencias en la composición química de los aceites estuvieron más asociadas con la especie y accesión que con la aplicación de N.

Palabras clave: Accesiones, aceites esenciales, calidad, fertilización, *Lippia* spp.

Introduction

Colombia, as some tropical and subtropical regions, has an outstanding ancestral experience in the management of health problems using the biodiversity of medicinal plants. These plamts are the most important resource and, sometimes, the only one that is accessible to several communities. Nowadays, social pressures to use the natural and organic have stimulated and increased the interest of industries that use alternatives from biodiversity as primary sources to produce food, aromatics, cosmetics, agrobiological and herbal medicines, among others (Katewa *et al.*, 2004).

The genus *Lippia* (Verbenaceae) comprises approximately 200 species of herbs and bushes distributed in several countries in Central and South America, which indicates its high botanical diversity, abundance, wide distribution and variety of uses (Verdcourt, 1992). Many of them are traditionally used in treatments for gastrointestinal and breathing conditions and as spices in food preparation (Pascual *et al.*, 2001).

Lippia alba (Miller) N.E. Brown ex Britton & Wilson, known also with the popular name 'prontoalivio' is native of America were it is widely distributed and adapted to different climates (Cáceres, 1996; García, 1992; Gupta, 1995). It is a shrub characterized for its intense aroma and content of essential oils (0.2 to 0.6%) based on fresh weight (Mejía *et al.*, 2007); additionally is known for its high content of carvone, a compound widely used in the cosmetic, food and pharmaceutical industries (Hernández *et al.*, 2004).

Lippia origanoides H.B.K. known as 'oregano de monte' is a wild shrub from the northeast of South America and some countries of Central America and The Antilles. In Colombia is found on the departments of Guajira, Magdalena, Cauca, Cundinamarca, Norte de Santander and Santander (Vicuña *et al.*, 2010). Its leaves in infusion are used as expectorant and to treat bronchial and lung conditions; besides asthma, cough and spams (García, 1992). Due to its high content of carvacrol and thymol, the essential oil of this species has antiviral (Meneses *et al.*, 2009), antifungal and bactericide activities (Duarte *et al.*, 2005).

The content and chemical composition of the essential oils is variable and depends on multiple genetic factors and on the agroecological conditions of the production places, crop management, extraction method, phenological state, tissue type and plant origin (Sangwan *et al.*, 2011). Studies performed to evaluate the essential oils in several places in Brazil and Colombia suggest the existence of many chemotypes in both *L. origanoides* and *L. alba* (Matos *et al.,* 1996).

Fertilizer applications, specially nitrogenized ones, affect the yield of essential oils by the increase of the production of total biomass per unit of area since the defining response is associated to multiple and complex interactions (Peporine *et al.*, 1997; Sangwan *et al.*, 2001). The purpose of this study was to evaluate the effect of nitrogen fertilization in the biomass yield and composition of the essential oils in *L. alba* (Miller) N.E.Brown ex Britton & Wilson and *L. origanoides* H.B.K. accessions.

Materials and methods

The field work was done on a clay loam soil, pH 7.4 and high or adequate content of nutrients in the Experimental Center of the Universidad Nacional de Colombia - Palmira (CEUNP), localized in the town of Candelaria (2° 26´ N and 76° 05´ W), El Carmelo, department of del Valle del Cauca, at 927 MASL. Average temperature is 24 °C, relative humidity is 65% and average annual rainfall is 1100 mm. Healthy mother plants from the working collection of the research group on Medicinal, Aromatic and Spicy plants were used. Mother plants of *L. origanoides* Típica were inicially collected in the Chicamocha Canyon, Citrica ones in the Chicamocha Canyon and Jordán Sube, and the ones of the Patía accession in El Bordo, Cauca. The accession Típica from *L. alba* was collected in Puerto López (Colombian Eastern Plains) and the Cítrica accession in Palmira, Valle del Cauca. The cuttings used were of about 12 and 15 cm with 3 – 5 nodes obtained from the middle third part and rooted under the shade in 7 ounces plastic containers with soil-sand (1:1) substrate, which were previously impregnated with *Áloe vera* L. cristals as rooting stimulant. Twenty-eight days after sowing 20 cuttings with roots were transplanted at a distance of 0.50 m between plants and 0.75 m between lines in 6 m^2 plots on a completely randomized block design with three replicates. Fourteen days after transplanting in the field four fertilization treatments were applied, which were equivalent to 50 and 100 kg/ha of N, in urea form (46% N) and chicken manure (2% N), in addition to a control treatment without fertilizers. The manual harvest of leaves was done in three plants, 8 weeks after transplanting. They were dried out on trays at 33 °C till an average of 12% water content.

Extraction of leaf oils was done by stem distillation using 100 g of dry material in the Clevenger type extraction equipment. In the process were obtained essential oil and water which were separated using ethyl ether and anhydrous sodium sulfate. Once the oil concentration was determined, essential oil yield per hectare in each treatment was determined. These oils were stored at an approximately temperature of 5 ºC – 10 ºC, avoiding the direct light beam. The essential oil fraction of each treatment was determined in the laboratories of the Universidad Industrial de Santander (UIS) by gas chromatography coupled to mass spectrometry after the samples were prepared by dilution and direct injection of the essential oils into the chromatography equipment. It was used Agilent Technologies 6890 Plus gas chromatographer coupled to a mass selective detector (MSD, Agilent Technologies 5973) operated in full scan. The column used for the analysis was DB-5MS (J & W Scientific, Folsom, CA, USA) (5%-phenyl polydimethylsiloxane), 60 m X 0,25 mm X 0,25 µm). Injection was performed in the split (5:1) mode with 2 µl injection volume. Data were analyzed with the Statistical Analysis System (SAS version 9.2, 2007) software using the Duncan´s multiple range test to determine comparisons between treatments.

Results and discussion

Dry matter yield

The *L. origanoides* accessions showed the highest average yield for dry biomass (3.32 t/ha) ($P < 0.05$), among them the Cítrica accession has the highest yield (3.83 t/ha). This result is due to the fact that this specie is characterized by taller plants with higher number of ramifications and larger leaf area that *L. alba*, which is a shrub of prostrate initial growth and that can grow up to 1.20 m (Leigh and Walton, 2004).

Application of 100 kg/ha of N as urea increased the dry biomass yield ($P < 0.05$) in the *L. origanoides* accessions. To the opposite, *L. alba* accessions, did not respond significantly to the N application, result that agrees with the findings of Hernández *et al.* (2004) (Table 1).

Leaf essential oils concentrations and yields

The average concentration of the leaf essential oils did not change between the evalua ted species, nitrogen levels and sources ($P > 0.05$); although the *L. origanoides* accessions had higher concentration than the ones of *L. alba*, standing out the Patía accession (Table 2). These results agree with the ones found by Baranauskienė *et al.*

(2003), Prakasa *et al.* (1985) and Azizi *et al.* (2009) in *Thymus* spp., *Cymbopogon* spp. and *Origanun vulgare* in which they found an effect of the nitrogen fertilizers in the dry biomass yield but not in the yield and composition of the essential oils.

The essential oils yield showed highly significant differences ($P < 0.01$) between species. The *L. origanoides* Patía and Cítrica accessions yield 83% more oil than the *L. alba* accessions. In this case, as for the dry matter, the highest yields were obtained with urea application which agrees with the results obtained for *L. alba* by Hernández *et al.* (2004) and Mejía *et al.* (2007) and, by Bommegowda *et al.* (1981) with *Cymbopogon* cultivars. The nitrogen levels applied did not affect the yield of essential oils, due, possibly, to the natural conditions of high fertility in the soil (Table 3).

Chemical composition

There were no differences in the chemical composition of the essential oils due to the nitrogen sources and levels used ($P > 0.05$). The observed differences in the chemical composition of these oils were associated with the species and accessions among them. In the Cítrica accession of *L. alba* the predominant components were geranial

Table 1. Efect of the application of nitrogen sources and doses in the dry biomass yield (t/ha) of *Lippia alba* and *L. origanoides* accessions.

Species/accession	**Control**	**N sources**						**Mean**	
		Urea (kg/ha)			**Organic[a] (kg/ha)**				
	0	**50**	**100**	**Mean**	**50**	**100**	**Mean**	**accession**	**specie**
					Yield (DM, t/ha)				
L. alba:									
Cítrica	1.84	2.22	2.51	2.36	2.10	2.13	2.11	2.16 d	
Típica	1.38	2.03	1.83		1.93	1.41	1.62	1.69 e	
Prom.	1.61	2.12	2.17		2.15	1.75	1.86		1.93 b
L. origanoides:									
Patía	4.07	2.31	3.75	3.03	2.98	3.70	3.34	3.36 b	
Típica	2.75	3.32	2.49	2.90	2.76	2.48	2.62	2.76 c	
Cítrica	2.72	4.50	4.98	4.74.5	3.09	3.82	3.45	3.82 a	
Mean (accesiones)	3.18	3.37	3.74	3.56	2.94	3.33	3.14		3.31 a
Mean (species).	2.39 b*	2.55 c	2.81 a	2.85 a	–	–	2.50 b		

* Means in the same row for N source and on the same column for species followed by the same letter do not significantly differ ($P > 0.05$) according to Duncan´s test. a. Source: chicken manure.

Table 2. Efect of the application of nitrogen sources and doses in the concentration of essential oils (%) of *Lippia alba* and *L. origanoides* accessions.

Species/accession	**Control**	**N sources**						**Mean**	
		Urea (kg/ha)			**Organic[a] (kg/ha)**				
	0	**50**	**100**	**Mean**	**50**	**100**	**Mean**	**accession**	**species**
					Concentration (%)				
L. alba									
Cítrica	2.667	2.889	2.667	2.778	2.667	2.333	2.500	2.644 ab	
Típica	1.923	1.833	2.167	2.000	2.000	1.833	1.916	1.951 c	
Prom.	2.295	2.361	2.417	2.389	2.333	2.083	2.208		2.297 a*
L. origanoides									
Patía	2.333	2.500	4.000	3.250	3.167	2.667	2.917	2.933 a	
Típica	3.500	1.500	1.500	1.500	1.958	1.333	1.645	1.958 c	
Cítrica	2.000	3.333	2.000	2.666	2.500	2.458	2.479	2.458 b	
Mean (accessions)	2.611	2.444	2.500	2.472	2.542	2.153	2.347		2.449 a
Mean (species)	2.453	2.420	2.288	2.430			2.277 a		

* Means in the same row for N source and on the same column for species followed by the same letter do not significantly differ ($P > 0.05$) according to Duncan´s test. a. Source: chicken manure.

Table 3. Efect of the application of nitrogen sources and doses in the yield of essential oils (%) of *Lippia alba* and *L. origanoides* accessions.

Species/accession	Control	N sources						Mean	
		Urea (kg/ha)			Organic[a] (kg/ha)				
	0	50	100	Mean	50	100	Mean	accession	species
					Yield (lt/ha)				
L. alba									
Cítrica	49.2	64.2	67.0	65.6	56.1	49.9	53.0	57.3 b	–
Típica	26.6	37.3	39.7	38.5	28.2	33.5	30.9	33.1 c	–
Prom.	37.9	50.8	53.4	52.1	42.2	41.7	41.9	–	45.2 b*
L. origanoides									
Patía	95.1	57.8	150.1	104.0	94.7	98.9	96.8	99.3 a	–
Típica	96.6	49.8	37.4	43.6	54.1	33.1	43.6	54.2 b	–
Cítrica	54.5	150.1	99.7	124.9	77.3	94.0	85.7	95.1 a	–
Mean (accessions)	82.1	85.9	95.7	90.8	75.4	75.3	75.4	–	82.9 a
Mean (species)	60.0	63.6	66.5		71.4 a			58.6 b	

* Means in the same row for N source and on the same column for species followed by the same letter do not significantly differ (P > 0.05) according to Duncan´s test. a. Source: chicken manure.

(50.1%) and neral (32.1%) (Table 4), while the carvone (47.4%) and limonene (36%) were abundant in the Típica accession (Table 5); results that agree with the ones found by Mejía *et al.* (2007). Thymol was the predominant compound in the Patía (87.5%), Típica (80.7%) and Cítrica (85.7) accessions of de *L. origanoides* (Table 6). These results are in agreement with the findings of previous studies about the essential oils composition of *L. origanoides* grown in the Pará and Minas Gerais states (Brazil) (Gallino, 1987) and with other studies done in Colombia by Ruiz *et al.* (2007)

Table 4. Relative concentration (%) of the compound in the Cítrica accession of *Lippia alba*.

Compound	Concentration (%)
Geranial	50.1
Neral	32.1
Geraniol	7.6
Acetato de geranilo	1.9
trans-β-Cariofileno	1.5
Germacreno D	1.1
(E)-Isocitral	1.1
Nerol	0.8
α-(Z)-Bisaboleno	0.8
Monoterpeno oxigenado, C10H16O	0.7
Oxido de cariofileno	0.6
Linalol	0.6
Citronelal	0.6
β-Elemeno	0.5

* Analysis performed by the Universidad Industrial de Santander, 2009.

who found variable concentrations of thymol, between 33.9 and 77.7%, depending on the extraction method.

Conclusions

- In conditions of theValle del Cauca, the *L. origanoides* accessions presented a higher dry matter yield than the ones of *L. alba*.
- The application of 100 kg/ha of N as urea, increased the dry matter yield in accessions of both species, however the best response was observed in the ones of *L. origanoides*.
- The highest concentrations of essential oils in leaves were shown in the *L. origanoides* accessions and were not affected by the nitrogen doses or the sources used.
- The highest yields of essential oils were obtained in the *L. origanoides* accessions and with the application of 100 kg/ ha of nitrogen as urea, which agrees with a higher dry biomass yield in the accessions of this species
- The chemical composition of the essential oils is probably associated with the species and accessions.

- In the composition of the essential oils of the Cítrica accession from *L. alba* predominate the geranial and the neral; in the Típica accession are carvone and limonene and, in *L. origanoides* accessions the thymol.

Table 5. Relative concentration (%) of the compounds in the Típica accession of *Lippia alba*.

Compound	Concentration (%)	Compound	Concentration (%)
Carvone	47.4	9-*epi*-(E)- Caryophyllene	0.3
Limonen	36.0	Borneol	0.3
Germacrene-D	8.3	Linalool	0.3
Piperitenone	1.2	β-Myrcene	0.3
Piperitone	1.1	*trans*-Carveol	0.2
Oxygenated sesquiterpene C10H16O	0.6	*cis*- Carveol	0.2
Biciclogermacreno	0.5	β-Copaeno	0.2
trans-β-Farnesene	0.5	Germacra-4(15),5,10(14)-trien-1-α-ol	0.1
β-Elemeno	0.5	β-Ylangeno*	0.1
β-Bourboneno	0.5	*trans*- Dihydrocarvone	0.1
trans-β-Ocimene	0.4	*cis*-Dyhidrocarvone	0.1
trans-β-Caryophyllene	0.4	Camphene	0.1
Germacreno D-4-ol + Espatulenol	0.3	α-Pinene	<0.1

* Analysis performed by the Universidad Industrial de Santander, 2009.
Tentative identification.

Table 6. Relative concentration (%) of the compounds in the *Lippia origanoides* accessions.

Compound	Accessions		
	Patía	Típica	Cítrica
Thymol	87.5	80.7	85.7
p-Cimene	4.4	5.2	4
y-Terpinene	2.8	5.3	3.3
trans-13-Caryophyllene	1.0	1.2	1.0
13-Myrcene	1.0	1.4	1.0
α -Terpinene	0.6	1.1	0.7
α -Humuleno	0.6	0.6	0.5
Terpinene-4-ol	0.3	0.4	0.4
Thymol methyl ether	0.3	1	1
N.I. Oxygenated compound (m/z 180)[a]	0.2	0.2	0.3
β-Bisabolene	0.2	0.2	0.1
Ethyl timilo	0.2	0.5	0.6
Umbellulona	0.2	0.1	0.2
Cis-sabinene hydrate	0.2	0.2	0.1
Limonene	0.2	0.2	0.2
α-Tuyeno	0.1	0.3	0.1
β-phellandrene	0.1	0.1	0.1
Eucalyptol	0.1	0.4	0.1
trans-β-Ocimeno	0.1	0.2	0.1
Caryophyllene oxide	0.1	0.1	0.1
α -Pinene	<0.1	0.1	<0.1
α -phelandrene	<0.1	0.2	<0.1
cis- α -Bergamoteno	<0.1	0.4	0.3
11- α -H- Himalacha-1-4- diene-*			0.1

Analysis performed by the Universidad Industrial de Santander, 2009.
*Tentative identification.

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