Effect of fertilization on dry matter production and extraction of nutrients in three accessions of *Lippia origanoides* H.B.K.

Efecto de la fertilización en la producción de materia seca y extracción de nutrientes en tres accesiones de *Lippia origanoides* H.B.K.

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Abstract

An experiment under greenhouse conditions at the Universidad Nacional de Colombia- Palmira to evaluate the relationship among plant nutrition, levels of nutrient uptake and dry matter production of three accessions of *Lippia origanoides* (Patía, Cítrica and Típica), was carried out. Concentrations of foliar nutrients, absorption and extraction rates (of 5 elements N, P, K, Mg and Ca) and their relationship with the production of each accession were evaluated. A split plot design with 11 treatments and three replications was used. The experimental units consisted of a plant sowed in substrate (peat). Fertilization was done by fertigation using the modified Hoagland and Arnon nutrient solution. The Patía accession showed the maximum dry matter yield (292.0 ± 0.1 g plant^{-1}) with the treatment 8 (Mg decreased), the Cítrica accession yielded 287.1 ± 0.1 g plant^{-1} with the treatment 9 (Mg increase) and finally Típica accession (255.2 ± 0.1 g plant^{-1}) with the treatment 8. In terms of absorption of nutrients, potassium is the most required element (13.9 kg t^{-1}), followed by calcium (7.9 kg t^{-1}).

Key words: Aromatic plants, essential oils, foliar nutrients, medicinal plants, nutrient solutions, nutrient uptake, production.

Resumen

En las casas de vegetación de la Universidad Nacional de Colombia sede Palmira (930 m.s.n.m. y 26 °C), se estudió la relación entre la nutrición de plantas de las accesiones Patía, Cítrica y Típica de *Lippia origanoides* H.B.K., los niveles de extracción de nutrientes y la producción y distribución de materia seca (MS). Para el efecto se determinaron las concentraciones de elementos foliares, los niveles de absorción y extracción de N, P, K, Mg y Ca y su relación con la producción de cada accesión. Se utilizó un diseño en parcelas divididas con once tratamientos y tres repeticiones, las unidades experimentales consistieron en una planta sembrada en sustrato (turba). Los elementos se aplicaron con ferti-riego utilizando solución nutritiva de Hoagland y Arnon modificada. Los resultados mostraron que la accesión Patía presentó los mayores rendimientos de MS (292.0 ± 0.1 g/planta) cuando la concentración original de Mg (49 mg/lt) en la solución cambió para 30 mg/lt; en la accesión Cítrica fueron más altos (287.1 ± 0.1 g/planta) con 72 mg/lt de Mg y en la accesión Típica el rendimiento fue mayor (255.2 ± 0.1 g/planta) con la aplicación de 30 mg/lt de Mg. En términos de absorción de nutrientes, el K fue el elemento más requerido (13.9 kg/t), seguido de Ca (7.9 kg/t).

Palabras clave: Absorción de sustancias nutritivas, aceites aromáticos, nutrientes foliares, plantas aromáticas, plantas medicinales, producción, soluciones nutritivas.

Introduction

The biodiversity richness of Colombia has been poorly used, despite of the great possibilities derived from the new tendencies on the demand of organic and natural products. Due to the wide diversity and content of secondary metabolites, several tropical plants have potential uses in fields like medicine, cosmetics, agrobiology, food and drink industries, among others.

In some developing countries, 80% of the population uses natural medicine based on plant active principles extracts to attend their primary health needs (WHO, 2002). In Colombia, the demand for those resources and the opportunities of use are higher every time, as well as the supply based on soil productivity, the variety of thermal floors and the knowledge on these plants. However, the productivity is low and in occasions is based on local knowledge.

Lippia origanoides H.B.K. (native oregano) is an aromatic and medicinal plant, native from some countries in Central America and the Caribbean (Mexico, Guatemala, Cuba) and north of South America (Venezuela, Brazil and Colombia) (Pascual *et al.*, 2001). In Mexico it is known as oregano and it is considered as a substitute of the common oregano (*L. graveolens* Kunth) of that country.

Lippia origanoides is used as seasoning because is similar to oregano. The essential oil and leaves of this plant used in cooking are carminative and improve gastrointestinal function, they have antispasmodic properties like sedatives and tonics for the nervous system, among other uses. The essential oil has bacteriostatic activity against *Nocardia* sp. (Méndez *et al.*, 2007) and antimicrobial activity against some candida species, *Staphylococcus aureus* among others, and inhibitory activity against *Trichophyton rubrum* T544 (Oliveira *et al.,* 2007).

Morais *et al.* (1972) and Gallino (1987) observed that thymol is the most abundant essential oil in *L. origanoides* plants, while carvacrol is only found in trace amounts or is totally absent. Oliveira *et al.* (2007) found in plants of this species 38.6% of carvacrol and 185% of thymol, indicating the existence of both chemotypes. Stashenko *et al.* (2007) in Colombia found two chemotypes of *L. origanoides*: ρ-cimene and 1,8-Cineol for the chemotype I and thymol for the chemotype II. Chemotype I is, apparently, a third chemotype for *L. origanoides*, which does not contain thymol nor carvacrol.

Nutrient requirements differ with the plant species, the level of production, soil type and fertility, and weather, among other factors, thus, it is important to specify the extraction levels to establish fertilization plans and reach the maximum levels of vegetative and reproductive growth. Levels of nutrient extraction are widely known in commercial crops like maize, wheat, rice, soybean, sunflower, sugarcane, among others, however for medicinal or aromatic plants that information is scarce (IPNI, 2009).

In Colombia there have been studies about the total and active components of the essential oils in two chemotypes of *L. origanoides* (Stashenko *et al.*, 2007), yet, there are not known studies on nutrition that indicate the crop requirements, nor the relation between fertilization levels and dry weight yield. Therefore, the present study has as objective to determine the levels of extraction and absorption of nutrients in three accessions of *L. origanoides* and the relation between the applied levels and the dry matter (DM) yield.

Materials and methods

The experiment was performed on the greenhouses and laboratories of the Universidad Nacional de Colombia-Palmira, located in the town of Palmira, Valle del Cauca, at 930 MASL, with average temperature of 26 °C and 65% of relative humidity. Three accessions of *L. origanoides* were used (Patía, Cítrica and Típica) that exist in the in vivo collection of Native Medicinal, Aromatic and Seasoning Plants of the Universidad Nacional de Colombia-Palmira.

Plants were multiplied by vegetative propagation (stem cuttings) in nurseries before they were transplanted to containers with 2 kg of substrate (peat) previously sterilized with boiling water. A split plot design was used, with accessions as principal plots and treatments as subplots. In total there were 11 treatments, three accessions and three replications for a total of 99 plants. For evaluations of MS yield, nutrient leaf concentration and nutrient extraction it was used the Hoagland and Arnon modified nutrient solution (1950) (Table 1).

Irrigation was done when the water content on the peat was 20% less than the field capacity, level that was kept constant to avoid nutrient loss by lixiviation. Nutrient solutions were prepared according to the treatments with analytical grade reactives of Panreac Química Sau (ACS-ISO Y PRSCodex) in distilled water, adjusting the pH to 6.5 with NaOH.

Plants were pruned two months after transplanting to the containers to a height of 35 cm, to proceed with the separation of their components in leaves, flowers and stems. After this homogenizing cut, doses of nutrient solution were adjusted to correct deficiency symptoms observed in plants. After three months of this cut plants were harvested and samples were taken for analysis of tissue of leaves, flowers, stems and roots of each treatment.

Samples obtained were placed on a stove at 65 °C for 48 h before dry biomass for each part of the plant and the total biomass was determined. Then they were grinded on a cutter mill Ika Labortechnik M20 of 2 mm and before all the corresponding analyses. Obtained data were analyzed by analysis of variance (Anova) and mean test, regression and correlation, using the software SAS (2007) version 9.1.3.

Nitrogen (N) was determined by the Kjeldahl method on the laboratory of analysis of the International Center for Tropical Agriculture CIAT; phosphorus (P) was determined by colorimetry with the method of moliybdate-ammonium vanadate according to NTC 234; and potassium (P), magnesium (Mg) and calcium (Ca) by spectrophotometry of atomic absorption according the NTC 5151, on a Varian AA 140 equipment.

Results and discussion

Dry matter yield

The highest yield for the Patía and Típica accessions (292.0 and 255.2 g/plant, respectively) were obtained with the treatment 8 (Mg 30 mg/l), while for the Cítrica accession (287.1 g/plant) was obtained with treatment 9 (Mg 72 mg/l) (Table 2).

The most influential nutrients on DM production were N and P, thus, when their levels were modified in comparison with the control, there was a significant reduction of production ($P \leq 0.05$), in contrast when doses of Mg and K were reduced in the solution, there were no differences ($P > 0.05$) on the DM production compared to the control treatment.

In the Patía accession, the highest plant total DM yield were obtained in the modified treatments for K and Mg (treatments 6, 7 and 8), although the differences with the control treatment were not significant ($P > 0.05$). The highest yield for this accession was shown with the treatment 8 (Mg 30 mg/l) (292 g/plant). On the other hand, it was found that

Table 1. Treatments and nutrient concentrations in the modified nutrient solution of Hogland and Arnon.

Treatment (no)	Modified element	Original concentration (mg/l)	Modified concentration (mg/l)
1	Control (Hoagland and Arnon)		
2	N	210	158
3	N	210	262
4	P	31	23
5	P	31	39
6	K	235	176
7	K	235	294
8	Mg	49	30
9	Mg	49	72
10	N/P	210/235	252/188
11	N/P	210/235	168/282

the modified doses of K did not affect the total DM production of the plant, whereas the reduction of Mg caused an increase in yield in comparison with the control treatment. The lowest yields were displayed with treatments 2, 10 and 11, showing that when N is deficient or when its ratio with respect to K is not suitable, the plant yield is reduced. P modifications in treatments 4 and 5 and the increase in Mg in treatment 9 also affected negatively plant yield when comparing to the control treatment (Table 3).

In the same Patía accession, the highest leaf yield was obtained with treatment 8 (Mg 30 mg/lt) (93.1 g/plant) representing 32% of the total D; production, a value that is lower than the one found by Dordas (2009) in oregano plants, where leaves reached 50% of the total production of DM. Stems (142.9 g/plant) in the same treatment 8 represented 49% of the total DM. In this accession, flowers which also contain essential oils, presented the highest yield in the treatment 6 (K 176 mg/l) (7.1 g/plant), followed by treatment 8 (Mg 30 mg/l) (6.8 g/plant) that represented only 3% of the total DM production per plant. For roots the highest DM yields were observed on the treatment 1 (control) and in treatments 6 to 8 (Table 3).

In the Cítrica accession the highest DM yields were displayed with treatment 1 (control) and treatments 5 to 10 (P > 0.05) (Table 4). Although the highest yield of total DM (278.1 g/plant) was obtained with application

Table 2. Production of total DM by *L. origanoides* accessions with different levels of fertilization using the modified Hoagland and Arnon solution.

Treatment (no)	Modified element	Concentration (mg/l)	Total dry matter (g/plant – accession)			
			Patia	Citrica	Tipica	Media
1	Control	Hoagland & Arnon	259.6 a*	245.0 a	248.6 a	251.1 ± 18.9 a
2	N	I58	162.8 c	118.5 c	183.4 b	154.9 ± 82.4 c
3	N	262	219.2 b	180.(b	237.9 a	212.5 ± 73.2 b
4	P	23	205.9 b	157.9 c	208.0 a	190.6 ± 70.4 b
5	P	39	181.8 b	231.0 a	220.5 a	211.1 ± 64.3 b
6	K	176	251.5 a	264.2 a	246.8 a	254.2 ± 22.5 a
7	K	294	262.2 a	241.3 a	221.9 a	241.8 ± 50.1 a
8	Mg	30	292.0 a	274.4 a	255.2 a	273.9 ± 45.7 a
9	Mg	72	175.8 b	287.I a	194.0 a	219.0 ± 148.4 a
10	N/K	252/18	124.5 c	265.0 a	211.3 a	200.3 ± 176.1 b
11	N/K	198/28	132.5 c	205.0 b	203.6 a	180.4 ± 103.0 c
Average			206.2±16.8 α	224.5±16.1 α	221.0±7.1 α	

*Averages with same letters on the same column are not significantly different ($P \leq 0.05$).

Table 3. Production of DM by *L. origanoides* Patia accession with different levels of fertilization using the modified Hoagland and Arnon solution.

Treatment (no)	Modified element	Concentration (mg/l)	Production dry matter (g/plant)				
			leaves	stem	roots	flowers	Total
1	Control	Hoagland & Arnon	74.5 b	121.9 a	58.25 a	3.7 a	259.6 a
2	N	I58	52.9 c*	74.1 c	31.9 b	3.9 a	162.8 c
3	N	262	73.2 b	107.4 b	34.9 b	3.7 a	219.2 b
4	P	23	71.6 b	98.0 b	30.6 b	5.8 a	205.9 b
5	P	39	61.4 b	85.0 b	31.0 b	4.4 a	181.8 b
6	K	176	80.3 a	119.5 a	44.7 a	7.1 a	251.5 a
7	K	294	81.7 a	132.1 a	43.4 a	5.0 a	262.2 a
8	Mg	30	93.1 a	142.9 a	49.2 a	6.8 a	292.0 a
9	Mg	72	68.3 b	82.7 b	24.2 c	0.6 b	175.8 b
10	N/K	252/18	47.2 c	57.3 c	19.1 c	0.9 b	124.5 c
11	N/K	198/28	40.5 d	63.3 c	25.6 c	3.0 a	132.5 c
Average			67.8±I0.7	98.6±19.2	35.P±8.0	4.1±1.4	206.2±37.4

*Averages with same letters on the same column are not significantly different ($P \leq 0.05$).

of Mg in treatment 9 (72 mg/l) this was not different to the yields on treatments 1, 5 to 8 and 10, indicating that changes in K and Mg concentrations, increases in P, or increases in N with K reduction in the treatment 10 do not affect plant production. The lowest yields were shown in treatments 2 and 4 demonstrating that when N and P are not balanced the yield is reduced. Additionally, increases in N concentration (treatment 3) and reduction of this nutrient with respect to K (treatment 11) affected in a negative way plant yield in comparison to the control. The highest leaf yield of this accession (95.4 g/plant) was obtained in treatment 9 (Mg 72 mg/l) representing 33% of the total dry matter; in the same treatment stems were 47%, flowers were 5% and roots were 15% of the total dry matter.

In the Típica accession the highest total DM was shown in treatment 8 (Mg 30 mg/l) (255.2 g/plant) and the lowest in treatment 2 (N 158 mg/l) showing that N reduction in the solution negatively affects ($P \leq 0.05$) plant yield (Table 5). The highest leaf DM (92.1 g/plant) of this accession are shown in treatment 8 (Mg 30 mg/l) equivalent to 36% of the total DM; in the stems the highest DM yield (120.6 g/plant) was obtained in treatment 1 (control) and represented 46% of the total DM; the highest yield in flowers (20.5 g/plant) were displayed with treatment 9 (Mg 72 mg/l), that together with treatment 11,

Table 4. Production of DM by *L. origanoides* Citrica accession with different levels of fertilization using the modified Hoagland and Arnon solution.

Treatment (no)	Modified element	Concentration (mg/l)	DM production (g/plant)				
			leaves	stem	roots	flowers	Total
1	Control	Hoagland & Arnon	74.5 b*	110.8 a	45.0 a	14.7 a	245.0 a
2	N	I58	43.0 d	53.0 c	16.5 c	6.0 b	118.5 c
3	N	262	58.6 c	89.8 b	20.2 b	11.5 a	180.2 b
4	P	23	59.7 c	69.2 c	20.7 b	8.2 b	157.9 c
5	P	39	76.3 b	109.4 a	30.9 b	14.4 a	231.0 a
6	K	176	83.5 a	116.5 a	49.4 a	14.8 a	264.2 a
7	K	294	79.5 a	112.6 a	37.7 a	11.5 a	241.3 a
8	Mg	30	89.6 a	125.6 a	48.9 a	10.4 a	274.4 a
9	Mg	72	95.4 a	136.0 a	44.5 a	11.2 a	287.1 a
10	N/K	252/18	82.2 a	120.3 a	49.2 a	13.4 a	265.0 a
11	N/K	198/28	60.7 c	98.2 b	34.3 b	11.9 a	205.0 b
Average			73.0±10.5	103.8±16.6.	36.1±8.4	11.6±1.8	224.5±35.8

*Averages with same letters on the same column are not significantly different ($P \leq 0.05$).

Table 5. Production of DM by *L. origanoides* Típica accession with different levels of fertilization using the modified Hoagland and Arnon solution.

Treatment (no)	Modified element	Concentration (mg/l)	DM production (g/plant)				
			leaves	stem	roots	flowers	Total
1	Control	Hoagland & Arnon	80.3 a*	120.6 a	33.9 a	13.7 b	248.6 a
2	N	I58	63.4 b	80.9 b	28.5 a	10.5 b	183.4 b
3	N	262	77.4 a	113.0 a	35.1 a	12.5 b	237.9 a
4	P	23	68.9 b	99.7 a	29.2 a	10.2 b	208.0 a
5	P	39	76.4 a	100.6 a	29.8 a	13.6 b	220.5 a
6	K	176	84.5 a	111.3 a	36.2 a	14.7 b	246.8 a
7	K	294	78.6 a	99.4 a	38.0 a	5.8 c	221.9 a
8	Mg	30	92.1 a	117.8 a	32.8 a	12.5 b	255.2 a
9	Mg	72	65.9 b	80.9 b	26.7 a	20.5 a	194.0 a
10	N/K	252/18	72.2 b	102.2 a	24.9 a	11.9 b	211.3 a
11	N/K	198/28	56.2 c	96.5 a	32.4 a	18.5 a	203.6 a
Average			74.2±6.9	102.1±8.9	3I.6±2.8	13.1±2.7	221.0±15.

*Averages with same letters on the same column are not significantly different ($P \leq 0.05$).

represented 5% of the total DM.

Element concentrations in leaf tissue

K concentrations were lower in leaves of Cítrica accession and the ones of Ca in the Típica accession (Table 6) ($P \leq 0.05$). Studies related with the leaf element concentration in plants of *L. origanoides* are scarce, thus, it is necessary to compare with studies of similar plants. Leaf concentrations in *L. origanoides* plants were higher than those found for similar species (Bergmann, 1992, cited by Dordas, 2009). When they are compared with the leaf concentration of Ca (15.3 g/kg) of *Origanum vulgare* sp. *hirtum* plants is observed that the range of the *L. origanoides* accessions (22.1 – 25.9 g/kg) in this study is over that value. The same happens with Mg concentration (3.3 – 3.7 g/kg) when compared to the value of 2.4 g/kg found on the same plants (Dordas, 2009). This suggests that Ca and Mg requirements are higher for production of *L. origanoides* than for oregano.

Leaf concentrations of *L. origanoides* accessions (4.17 – 4.50 g/kg) in this study were lower than the ones found by Schroeder *et al.*, (2005) in *L. turbinate* plants (6.5 g/kg), however K concentrations (16.6 – 19.6 g/kg) were higher. Average concentration of N in the leaves ranged between 9 and 11.1 g/kg, value that is lower than the one reported for *L. alba* Miller plants (10.6 – 23.3 g/kg) using higher nitrogen fertilizer doses than the ones used in this study (Hernández *et al.*, 2004).

Although *L. origanoides* has a habitat restricted to semiarid zones with dry and nutrient poor soils (Albesiano *et al.*, 2003), it shows high concentrations of Ca, Mg and K compared to similar plants, and its adaptation to this type of environment allows them to develop well with low levels of these nutrients.

Nutrient extraction

Absorption refers to the total amount of nutrients taken by the plant during the first developmental stages and, that for the case of *L. origanoides*, are concentrated mainly in the leaves. Nutrient absorption and extraction presented different values for the same element; for example, K was the most required nutrient for absorption in the crop, followed by Ca; whereas the highest requirements in leaf extraction belong to Ca followed by K. N is the third most demanded element by the crop, both in absorption and extraction (Table 7). P and Mg were element with lower demands, nonetheless, as it is known, this are needed for DM production and to ensure an acceptable concentration of essential oils in the plant.

Analysis of variance showed differences ($P \leq 0.05$) on the nutrient element concentrations in each one of the accessions (Table 7).

Table 6. Nutrient concentration in leaves of *L. origanoides* accessions subjected to fertilization with Hoagland and Arnon nutrient solution (control treatment)

Accession	Concentration (g/kg de MS)				
	N	P	K	Mg	Ca
Patía	10.31 ± 0.01 a*	4.17 ± 0.03 a	19.62 ± 0.01 a	3.297 ± 0.002 a	25.22 ± 0.02 a
Cítrica	11.14 ± 0.01 a	4.50 ± 0.03 a	16.56 ± 0.01 b	4.714 ± 0.003 a	25.89 ± 0.02 a
Típica	9.02 ± 0.01 a	4.37 ± 0.03 a	17.83 ± 0.0I ab	3.553 ± 0.003 a	22.15 ± 0.02 b

*Averages with same letters on the same column are not significantly different ($P \leq 0.05$).

Table 7. Amount of nutrients absorbed by the crop and extracted by the harvesting parts of plant of *L. origanoides* subjected to fertilization with a modified Hoagland and Arnon nutrient solution.

Accession	Absortion (kg/t DM) (aver. ± -x10^{-2})					Extraction (kg/t DM) (aver. ± -x10^{-2})				
	N	P	K	Mg	Ca	N	P	K	Mg	Ca
Patía	5.59±1b*	1.75±2a	13.9±1a	1.49±8b	10.585±03b	3.008±04a	1.18±1a	5.726±04a	0.96±4a	7.358±04 ab
Cítrica	6.74±1a	2.01±2a	13.8±1a	2.44±4a	11.526±04a	3.386±04a	1.14±1a	5.033±03b	1.43±4a	7.869±04 a
Típica	5.55±1b	2.27±2a	13.9±1a	1.77±4b	11.542±04a	2.915±03a	1.40±1a	5.763±04a	1.15±4a	7.158±04 b

*Averages with same letters on the same column are not significantly different ($P \leq 0.05$).

Patía accession displayed the lowest absorptions for N, Mg and Ca, while the Cítrica revealed the highest levels of absorption and extraction of N and Mg, elements that affect both DM production and concentration of essential oils. Típica accession showed the lowest levels of N and Mg absorption and the lowest extraction of Ca, similarly, it was the accession with the highest levels of absorption and extraction of P, which suggests that it is necessary to apply it when cultivated on soils with low contents of this nutrient.

In this study, nutrient extraction of the crop corresponds, in average, to 55% of the total absorption, which happens in the first four months of the plant. These nutrients are accumulated on leaves, the usable part of the plant, while the other 45% stays on structural parts like stems and roots. This information is valuable when programming fertilization plans in these type of crops, because they give an appropriate nutritional balance and avoid fertilizers loses.

Conclusions

- The accessions of *L. origanoides* Patía, Cítrica and Típica have low levels of extraction when compared to reference species.
- Nitrogen and phosphorus were the most influencial elements in aerial biomass production of *L. origanoides*.
- The results showed that the *L. origanoides* accessions showed the highest DM yields in treatments where the original Mg concentration was modified. In terms of nutrient absorption and extraction, K is the most required elements, followed by Ca.

References

Albesiano, S.; Rangel, Ch. J.; and Cadena, A. 2003. La vegetación del cañón del río Chicamocha (Santander, Colombia). Caldasia 25(1):73 - 99.

Dordas, C. 2009. Foliar application of calcium and magnesium improves growth, yield, and essential oil yield of oregano (Origanum vulgare spp. hirtum). Industrial Crops and Products 29:599 - 608.

Gallino, M. A. 1987. Una verbenacea essenziera ricca in Timolo: *Lippia origanoides* H.B.K. Essenze Deriv. Agrum. 57(4):628 - 629.

Hernández V., H.; Bonilla C., C.; and Sánchez O., M. 2004. Efecto de la fertilización nitrogenada en la producción de biomasa y calidad de aceite esencial en *Lippia alba* (Miller), Pronto Alivio. Acta Agronómica 53(1):45 - 52.

Hoagland, D. R.; and Arnon, D. I. 1950. The waterculture method for growing plants without soil. California Agricultural Experiment Station. Circular 347. The College of Agriculture, University of California. Berkeley, California. 32 p.

IPNI (International Plant Nutrition Institute). 2009. Latin America Southern Cone. Archivo Agronómico N° 3: Requerimientos nutricionales de los cultivos. Available online: http://www.ipni.net/ppiweb /ltams.nsf/ [Cited 20 agosto 2009; 13:00]

Méndez, R.; Serrano, J.; Chataing, B.; Jiménez, D.; Mora, D.; Rojas, L.; Usubillaga, A.; and OCallaghan, J. 2007. Estudio comparativo de la actividad biológica del aceite esencial *Protium heptaphyllum* (Aubl.) March y el aceite esencial *Lippia origanoides* H.B.K. sobre tres especies de *Nocardia* sp. Salud & Desarrollo Social 2:49 - 52.

Morais, A. A.; Mourão, J. C.; Gottlieb, O. R.; Silva, M. L.; Marx, M. C.; Maia, J. G.; and Magalhães, E. M. 1972. Óleos essenciais da Amazônia contendo Timol. Acta Amaz. 2(1):45 - 46.

Oliveira, D. R.; Leitão, G. G.; Bizzo, H. R.; Lopes, D.; Alviano, D. S.; Alviano, C. S.; and Leitão. S. G. 2007. Chemical and antimicrobial analyses of essential oil of *Lippia origanoides* H.B.K. Food chem. 101:236 - 240.

Pascual, M. E.; Slowing, K.; Carretero, E.; Mata, D. S.; and Villar, A. 2001. Lippia: traditional uses, chemistry and pharmacology. A review. J. Ethnophar. 76:201 - 214.

SAS: Users guide statistics, SAS Institute Inc., Cary, NC, USA, 2007.

Schroeder, M. A.; López, A. E.; and Sauer, M. V. 2005. Efecto de la fertilización en *Lippia turbinata* Gris. Comunicaciones Científicas y Tecnológicas. Universidad Nacional Del Nordeste. Facultad de Ciencias Agrarias. Resumen A-032. Corrientes, Argentina.

Stashenko, E. E.; Martinez, J. R.; Tunarosa, F.; and Ruiz, C. 2007. Estudio comparativo por GC-MS de metabolitos secundarios volátiles de dos quimiotipos de *Lippia origanoides* H.B.K., obtenidos por diferentes técnicas de extracción. Sci. Tech. 33(05):325 - 328.

WHO (World Health Organization). 2002. Traditional Medicine Strategy: 2002-2005. Geneva, 2002. 70 p. (document WHO/EDM/TRM/2002.1).