Veterinary Parasitology 210 (2015) 118–122

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Short Communication

Acaricidal activity of essential oils from *Lippia alba* genotypes and its major components carvone, limonene, and citral against *Rhipicephalus microplus*

Magna Galvão Peixoto[a], Livio Martins Costa-Júnior[b], Arie Fitzgerald Blank[a], Aldilene da Silva Lima[b], Thays Saynara Alves Menezes[a], Darlisson de Alexandria Santos[c], Péricles Barreto Alves[c], Sócrates Cabral de Holanda Cavalcanti[d], Leandro Bacci[a], Maria de Fátima Arrigoni-Blank[a,*]

[a] *Agronomy Department, Federal University of Sergipe, Av. Marechal Rondon S/N, CEP 49100-000 São Cristóvão, SE, Brazil*
[b] *Animal Science Department, Federal University of Maranhão, Rua São José de Ribamar, n° 15, Boa Vista, CEP 65500-000 Chapadinha, MA, Brazil*
[c] *Chemistry Department, Federal University of Sergipe, Av. Marechal Rondon S/N, CEP 49100-000 São Cristóvão, SE, Brazil*
[d] *Pharmacy Department, Federal University of Sergipe, Av. Marechal Rondon S/N, CEP 49100-000 São Cristóvão, SE, Brazil*

ARTICLE INFO

Article history:
Received 21 November 2014
Received in revised form 18 February 2015
Accepted 12 March 2015

Keywords:
Lippia alba
Volatile oil
Carvone
Citral
Cattle tick

ABSTRACT

The goal of the present study was to evaluate the acaricidal potential of *Lippia alba* essential oil, citral chemotypes (LA-10 and LA-44 genotypes) and carvone chemotypes (LA-13 and LA-57 genotypes), as well as purified citral and enantiomers of carvone and limonene. Efficacy against *Rhipicephalus microplus* was assessed by the larval packet and the engorged female immersion tests. Citral chemotypes had greater larvicidal activity than carvone chemotypes, and this was further supported by larvicidal and adulticidal activity of purified citral with LC_{50} values of 7.0 and 29.8 mg/mL, respectively. While purified enantiomers of carvone exhibited greater larvicidal activity than those of limonene, enantioselectivity of limonene was observed with R-(+) displaying significantly higher efficacy (LC_{50} of 31.2 mg/mL) than S-(−) (LC_{50} of 54.5 mg/mL). The essential oils and purified compounds were much less toxic toward engorged adult females, with the exception of citral, and this may be due to limited cuticular penetration.

Published by Elsevier B.V.

1. Introduction

The cattle tick *Rhipicephalus microplus* is responsible for significant losses in livestock farming in tropical and subtropical regions. In addition to blood loss, pathogen transmission, damage to the hides and irritation of the animals, the rapid and widespread tick resistant to control agents is among the major concerns in this area (Fraga et al., 2003; Castro-Janer et al., 2010; Mendes et al., 2011). Cattle tick control has been achieved basically by means of synthetic chemical agents that are efficient in the short-term. However, chemical control has negative immediate and long-term effects on the whole production system (Alves et al., 2012). The need to develop novel cattle tick control technologies that are more selective and less harmful to humans and the environment has led to the investigation

* Corresponding author. Tel.: +55 79 21056981; fax: +55 79 21056494.
E-mail address: fatima.blank@gmail.com (M.d.F. Arrigoni-Blank).

http://dx.doi.org/10.1016/j.vetpar.2015.03.010
0304-4017/Published by Elsevier B.V.

of many plant species as potential sources of biopesticides to substitute or complement synthetic pesticides (Correa and Salgado, 2011).

L. alba (Mill.) N.E. Brown (Verbenaceae), commonly known as Brazilian lemon balm, is widely used by the population due to its tranquilizing, mild antispasmodic, analgesic, sedative, anxiolytic and slightly expectorant properties (Sette-de-Souza et al., 2014). According to the predominance of specific chemical constituents in its essential oil composition, *L. alba* can be differentiated into chemotypes, such as carvone and citral (Jannuzzi et al., 2010; Hennebelle et al., 2006).

The monoterpenes carvone, limonene and citral are among the most prominent chemical compounds in *L. alba* essential oils. Carvone is an unsaturated monoterpene ketone, and due to the presence of a chiral center, it can be biosynthesized in either its *R*-(−)-carvone or *S*-(+)-carvone enantiomer by some plants. Carvone has antimicrobial and anti-fungal properties and is used in the food and pharmaceutical industries (Carvalho and Fonseca, 2006). Similarly, limonene is a monoterpene hydrocarbon that can be biosynthesized as its *R*-(+)- or *S*-(−)-limonene enantiomer by many plant species. Finally, citral is a mixture of two isomeric acyclic monoterpene aldehydes, geranial (*trans*-citral, citral A) and neral (*cis*-citral, citral B), that possesses a characteristic lemon scent and is widely used in the perfume, food and cosmetics industries (Tavares et al., 2011).

The essential oils of some *Lippia* species, such as *Lippia triplinervis* (Lage et al., 2013) and *Lippia gracilis* (Cruz et al., 2013), have been reported to possess acaricidal activity against ticks. Aiming to broaden the knowledge in the acaricidal activity of essential oils of *Lippia* plant species and their chemical compounds against ticks and identify the better chemotype with focus in a standardization of acaricidal natural compounds, our goal was to evaluate the acaricidal activity of four *L. alba* genotype essential oils, and their major compounds, *R*-(−)- and *S*-(+)-carvone and *R*-(+)- and *S*-(−)-limonene, and citral against *R. microplus* larvae and engorged females.

2. Methods

2.1. Plant, chemicals, and biological materials

Four *L. alba* genotypes were analyzed representing carvone chemotypes (LA-13 and LA-57) and citral chemotypes (LA-10 and LA-44). To extract the essential oils plant leaves were harvested from the field at the Active Germplasm Bank (ABG) for Medicinal and Animal use Plants of the Federal University of Sergipe (Table 1). The ABG was maintained at the "Campus Rural da UFS" Research Farm, located in the municipality of São Cristóvão, Sergipe State, Brazil. The climate of the region is tropical semi-arid, and the soil is classified as Red–Yellow Argisol. The plants received a daily drip irrigation, applying 6 mm day^{-1}. Defoliation was performed manually and leaves dried in a forced-air oven at 40 °C for 5 days.

R. microplus specimens resistant to amidines and synthetic pyrethroids but sensitive to organophosphates were maintained on artificially infected cattle. The present study was approved by the animal research ethics committee of the Federal University of Maranhão under protocol number 23115018061/2011-11.

Purified *R*-(−)- and *S*-(+)-carvone, R-(+)- and S-(−)-limonene, and citral were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA).

2.2. Essential oil extraction and analysis

Essential oils were extracted *via* hydrodistillation (120 min) of 75 g of dried leaves per sample using a Clevenger-type apparatus.

The compounds present in *L. alba* essential oil samples were identified using a gas phase chromatograph coupled to a mass spectrometer (GC-MS; QP 5050A, Shimadzu) equipped with an AOC 20i auto injector (Shimadzu) and a fused-silica capillary column (5% diphenyl–95% dimethylpolysiloxane; 30 m × 0.25 mm ID × 0.25 μm film thickness; J&W Scientific, Folsom, CA, USA), using helium as a carrier gas at 1.2 mL min^{-1} flow. The temperature was set to 50 °C for 1.5 min then programmed to increase to 200 °C at 4 °C/min followed by an increase of 15 °C/min up to 250 °C then remaining constant for 5 min. The injector and detector (or interface) temperature was set at 250 °C and 280 °C, respectively, and 0.5 μL of sample in ethyl acetate was injected at a split ratio of 1:87 and 64.20 kPa of column pressure. The MS conditions were as follows: quadrupole ion-trap mass analyzer operating in electronic ionization mode achieved by electron impact at 70 eV, scanning intervals of 0.85 fragments/s and a fragment detection range between 40 and 550 Da. Quantitative analysis of the chemical constituents was performed by GC on a Shimadzu GC-17A device (Shimadzu Corporation, Kyoto, Japan) with a flame ionization detector (FID) using a ZB-5MS fused-silica capillary column (5% phenyl-arylene–95% dimethylpolysiloxane; 30 m × 0.25 mm ID × 0.25 μm film thickness; Phenomenex, Torrance, CA, USA) under the same conditions as described for the GC–MS column. Each component was quantified by area normalization (%). Compound concentrations were calculated from GC peak areas and arranged in order of elution from the GC column. The essential oil components were identified by matching their mass spectra to spectra available in the database of the device (NIST05, NIST21, and WILEY8). These libraries

Table 1
Identification and origin of *Lippia alba* genotypes from the Active Germplasm Bank of Medicinal Plants from Federal University of Sergipe.

Genotype code	Chemotype	Municipality/state of origin	Origin	UFS herbarium voucher No.
LA-13	Carvone	Fortaleza-Ceará	Federal University of Ceará	13,488
LA-57	Carvone	Rio Real-Bahia	Federal University of Sergipe	13,469
LA-10	Citral	Brasília-Federal District	University of Brasília	13,495
LA-44	Citral	Brasília-Federal District	University of Brasília	14,788

allowed for the comparison of spectral data with a similarity index above 80%. The measured retention indices were also compared with indices from the literature (Adams, 2007). The equation of Vandendool and Kratz (1963) was used to calculate the relative retention indices (RRIs) against a homologous series of *n*-alkanes (C9–C18) injected under the above mentioned GC conditions.

2.3. Larval packet test

The larval packet test was performed with *R. microplus* larvae according to the method developed by Stone and Haydock (1962) and as adapted by the Food and Agriculture Organization (1971) and Leite (1988). The essential oils and the purified compounds were prepared at concentrations varying from 6.0 to 200.0 mg/mL in 3% Triton X-100, and were used in the bioassays treatments. For each concentration, approximately 100 tick larvae were placed between two 2 cm × 2 cm filter papers impregnated with 0.4 mL of solution containing the essential oils or citral or one of the enantiomers to form a packet.

Subsequently, the packet was placed into a clean filter paper envelope (8.5 cm × 8.5 cm) and sealed with plastic clips. The envelopes were transferred to an incubator at 27 ± 1 °C and ≥80% relative humidity for 24 h. After this period, the living and dead larvae were counted. Each treatment was repeated 4 times, and control packets were made using 3% Triton X-100.

2.4. Engorged female immersion test

The engorged *R. microplus* female immersion test was performed according to the method developed by Drummond et al. (1973). Engorged *R. microplus* females were collected from artificially infested calves, washed with running water, dried with paper towels and weighed in groups of 10 specimens, trying to maintain the weights of the groups as homogenous as possible. Each tick group was submerged in the essential oils of *L. alba* (LA-10, LA-13, LA-44, and LA-57 genotypes), *S*-(+)- or *R*-(−)-carvone, *R*-(+)- or *S*-(−)-limonene, or citral at concentrations varying from 10.0 to 200.0 mg/mL for 5 min. Control immersions were performed using 3% Triton X-100. Each treatment was repeated 4 times. Engorged females were then dried in paper towel, placed into Petri dishes and transferred into a biochemical oxygen demand (BOD) incubator at 27 ± 1 °C and ≥80% relative humidity for 30 days for later evaluation of oviposition and hatching.

2.5. Statistics

The 50% lethal concentration (LC_{50}) and confidence limits (CL) of essential oils and pure monoterpenes on larvae and engorged females were calculated by Probit analysis using GraphPad Prism 6.0 software. Compound or essential oil activity is considered significantly different when the 95% CL fails to overlap (Roditakis et al., 2005).

3. Results and discussion

Several studies have reported the diversity of *L. alba* essential oil chemotypes, supporting that the differences between each essential oil vary depending on their major constituents (Senatore and Rigano, 2001; Atti-Serafini et al., 2002; Hennebelle et al., 2006; Jannuzzi et al., 2010). In this study, the major essential oil monoterpenes, *i.e.*, carvone, limonene, and citral (geranial + neral), present in *L. alba* genotypes have exhibited potent acaricidal activity against *R. microplus*.

The essential oil chemical compositions of the four *L. alba* genotypes are listed in Table 2. The LA-13 and LA-57 genotypes were representative carvone chemotypes and consisted primarily of carvone/limonene at 52.9%/26.9% and 63.5%/25.8%, respectively. The LA-10 and LA-44 genotypes were representative citral chemotypes and consisted primarily of geranial/neral at 46.2%/33.5% and 44.2%/31.1%, respectively.

Essential oils from the citral chemotypes (genotypes LA-10 and LA-44) exhibited greater larvicidal activity than the carvone chemotypes (genotypes LA-13 and LA-57), with LC_{50} values of 8.8 mg/mL and 10.9 mg/mL, respectively. The carvone chemotype genotypes exhibited LC_{50} values of 16.8 mg/mL and 27.0 mg/mL, respectively (Table 3). Components such as citral, α- and β-pinene, terpinene and

Table 2
Chemical composition of the essential oils of four *Lippia alba* genotypes.

Compound	RRI[a]	*L.* alba genotype[b]			
		LA-13	LA-57	LA-10	LA-44
Sabinene	969	2.27 ± 0.01	0.03 ± 0.03	–	0.27 ± 0.00
Myrcene	988	0.61 ± 0.01	0.49 ± 0.02	1.25 ± 0.03	2.63 ± 0.03
Limonene	1024	26.95 ± 0.16	25.86 ± 0.47	–	0.54 ± 0.01
Linalool	1095	1.73 ± 0.02	0.87 ± 0.04	0.95 ± 0.01	0.74 ± 0.03
Neral	1235	–	–	33.50 ± 0.19	31.13 ± 0.19
Carvone	1239	52.94 ± 0.17	63.47 ± 0.43	–	0.35 ± 0.35
Geranial	1264	–	–	46.25 ± 0.53	44.17 ± 0.84
Piperitone	1340	1.21 ± 0.03	1.18 ± 0.04	–	–
β-Caryophyllene	1417	–	0.33 ± 0.02	2.42 ± 0.04	2.36 ± 0.04
γ-Muurolene	1478	2.54 ± 0.07	2.25 ± 0.07	0.60 ± 0.01	–
Elemol	1548	4.33 ± 0.10	–	–	–
Caryophyllene oxide	1582	–	–	7.28 ± 0.13	8.06 ± 0.25
Total (%)	92.58	94.48	92.25	90.25	

[a] Relative retention index to an *n*-alkane homologous series (*n*C9–C18) calculated using the Vandendool and Kratz equation (1963).
[b] Means (±SEM) of the content of compounds obtained from three different GC–ID determinations. Dashes indicate that the compound was not found.

Table 3
Lethal concentration 50% (LC_{50}) of *Lippia alba* genotypes, citral, *S*-(+)- and *R*-(−)-carvone, and *R*-(+)- and *S*-(−)-limonene of *Rhipicephalus microplus* larvae and engorged females with their respective 95% confidence limits.

	Genotype	LC_{50} (mg/mL)	95% CL	R^2
Larvae				
Carvone chemotype	LA-13	16.8[b]	16.3–17.4	0.97
	LA-57	27.0[c]	24.9–29.8	0.89
Citral chemotype	LA-10	8.8[a]	6.0–13.0	0.73
	LA-44	10.9[a]	9.9–12.1	0.97
Citral*		7.0[a]	6.8–7.1	0.96
S-(+)-carvone*		10.9[a]	8.7–13.6	0.87
R-(−)-carvone*		9.9[a]	8.5–11.7	0.96
R-(+)-limonene*		31.2[d]	30.8–31.7	0.91
S-(−)-limonene*		54.5[e]	47.8–62.2	0.92
Engorged females				
Carvone chemotype	LA-13	>200.0	–	–
	LA-57	>200.0	–	–
Citral chemotype	LA-10	>200.0	–	–
	LA-44	237.5[b,c]	155.8–362.1	0.88
Citral*		29.8[a]	24.14–36.89	0.91
S-(+)-carvone*		146.2[b]	134.5–159.0	0.96
R-(−)-carvone*		145.8[b]	123.4–172.2	0.86
R-(+)-limonene*		322.7[c]	245.4–424.4	0.94
S-(−)-limonene*		>200.0	–	–

LC_{50} = lethal concentration 50% (mg/mL) *R. Microplus* larvae or engorged females; CL = 95% confidence limit; R^2 = regression coefficient; (−) calculation was not possible.
* Commercial product.
Values down a column with different letters are significantly different.

terpineol extracted from *Eucalyptus* spp. have already been reported to possess acaricidal activity against ticks (Chagas et al., 2002). Citral exhibited the lowest LC_{50} values against both larvae and engorged females. Citral has already been reported in *Cymbopogon* species (*Cymbopogon citratus* and *Cymbopogon flexuosus*), and its toxic effect has been demonstrated on adult *R. microplus* females (Agnolin et al., 2014). Acaricidal differences among essential oils of *Lippia gracillis* genotypes have been reported, however the efficacy was not correlate with their chemotypes (Cruz et al., 2013).

The negative control shown no effect on larvae (0.0%) and engorged female (eggs production index 80.0% and egg hatching 93.5%). Among the pure compounds tested, citral exhibited the highest efficacy against *R. microplus* larvae, with an LC_{50} of 7.0 mg/mL, followed by the *R*-(−)-carvone (LC_{50} 9.9 mg/mL) and the *S*-(+)-carvone (LC_{50} 10.9 mg/mL) enantiomers, while limonene enantiomers achieved LC_{50} values of 31.2 mg/mL for *R*-(+)-limonene and 54.5 mg/mL for *S*-(−)-limonene (Table 3). Ferrarini et al. (2008) reported larvae mortality for limonene in the range of 2.5 μg/mL, but the data were obtained using a different procedure in which larvae were exposed to limonene for longer periods.

Pure citral exhibited the highest efficacy against engorged females, with an LC_{50} of 29.8 mg/mL. In contrast, carvone and citral chemotypes or carvone enantiomers exhibited no efficacy in inducing engorged female mortality with LC_{50} values above 100 mg/mL. Among the enantiomers, the lowest efficiencies were achieved by *R*-(−)-carvone (LC_{50} 145.8 mg/mL) and *S*-(+)-carvone (LC_{50} 146.2 mg/mL). Due to the low efficacy of LA-10, LA-13, and LA-57 genotypes and *S*-(−)-limonene against engorged females the LC_{50} values could not be calculated.

The significance of chirality is widely recognized in insecticides. Although enantiomers have similar physicochemical properties, they interact in a different way in biological systems (Liu et al., 2004). In this case, in addition to satisfactory efficacy against *R. microplus* larvae, limonene enantiomers have exhibited enantioselectivity. Although enantioselectivity has also been observed for limonene in the engorged female assay, potency for both enantiomers against engorged females were lower than those for the larvae.

LA-13 and LA-57 genotypes (carvone chemotype) exhibited intermediate potencies compared to limonene and carvone monoterpenes. This finding suggests the major constituents of the essential oil (carvone and limonene) are acting in an additive or synergic manner, thus contributing equally to the observed toxic effects. However, the synergistic action of other minor constituents cannot be disregarded. Conversely, other studies have reported that essential oils are more efficient than their isolated compounds (Miresmailli et al., 2006; Singh et al., 2009).

In contrast, R-(−)- and S-(+)-carvone enantiomers were less potent than the essential oils and exhibited no significant enantionselectivity for both, *R. microplus* larvae and engorged females.

The efficacy of carvone against *Aedes aegypti* larvae and females (Simas et al., 2004) has been demonstrated. Such differences are probably explained by the evaluation in different species in addition to different mechanisms of action.

With respect to the engorged females, higher LC_{50} values, with the exception of citral, may be explained by the difficulty of the compounds in penetrating the *R. microplus*

cuticle. Furthermore, pesticides may be absorbed orally. Arthropods have two means of cuticle absorption, the hydrophilic and the lipolytic, which influence the functional capacity of a given bioactive substance. It is also important to notice the possible influence of a physical phenomenon, which the product is initially absorbed and then undergoes passivation, a process that forms a layer, preventing the passage of the oil (Chagas et al., 2002).

The study of isolated organic compounds is essential to increase the knowledge on how essential oils may be used as green pesticides (Kalita et al., 2013). It is further important to accomplish in-depth studies on the actions of enantiomers in biological systems, not only because their commercialization represents a global tendency in pharmaceutical production, but also because information about their pharmacokinetic, pharmacodynamic and toxicological interactions could give some insight in the selectivity of a given enantiomer.

4. Conclusions

The results presented herein report the toxicity of essential oils of *L. alba* genotypes against *R. microplus* ticks. The genotypes exhibited potent activity against *R. microplus* larvae, which was accounted for the presence of limonene and carvone as major components of the essential oil. Enantioselectivity has been observed for limonene against larvae and engorged females. These findings provide a greener alternative in cattle tick control.

Acknowledgments

The authors wish to acknowledge CNPq, FAPITEC/SE, CAPES, and RENORBIO for supporting funds.

References

Adams, R.P., 2007. Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. Allured Publ. Corp., Carol Stream, Chicago.

Agnolin, C.A., Olivo, C.J., Parra, C.L.C., 2014. Efeito do óleo de capim limão (*Cymbopogon flexuosus* Stapf) no controle do carrapato dos bovinos. Rev. Bras. Plantas Med. 16, 77–82.

Alves, W.V., Lorenzetti, E.R., Gonçalves, F.C., 2012. Use of plant acaricides in *Rhipicephalus* (*Boophilus*) *microplus* control: a contribution to production and sustainable development. Rev. Bras. Agropecu. Sustent. 2, 14–25.

Atti-Serafini, L., Pancera, M.R., Atti, S.A.C., Rossato, M., Pauletti, G.F., Rotal, D., Parooul, N., Moyna, P., 2002. Variation in essential oil yield and composition of *Lippia alba* (Mill). N.E. Br grows in southern Brazil. Rev. Bras. Plantas Med. 4, 72–74.

Carvalho, C.C.R., Fonseca, M.M.R., 2006. Carvone: why and how should one bother to produce this terpene. Food Chem. 95, 413–422.

Castro-Janer, E., Martins, J.R.S., Mendes, M.C.D., Namindome, A.B., Klafke, G.M.B., Schumaker, T.T.S., 2010. Diagnoses of fipronil resistance in Brazilian cattle ticks *Rhipicephalus (Boophilus) microplus* using in vitro larval biossays. Vet. Parasitol. 173, 300–306.

Chagas, A.C.S., Passos, W.M., Prates, H.T., Leite, R.C., Furlong, J., Fortes, I.C.P., 2002. Acaricide effect of *Eucalyptus* spp. essential oils and concentrated emulsion on *Boophilus microplus*. Braz. J. Vet. Res. Anim. 39, 247–253.

Correa, J.C.R., Salgado, H.R.N., 2011. Insecticidal activities of plants and applications: a review. Rev. Bras. Plantas Med. 13, 500–506.

Cruz, E.M.O., Costa-Junior, L.M., Pinto, J.A.O., Santos, D.A., Araujo, S.A., Arrigoni-Blank, M.F., Bacci, L., Alves, P.B., Cavalcanti, S.C.H., Blank, A.F., 2013. Acaricidal activity of *Lippia gracilis* essential oil and its major constituents on the tick *Rhipicephalus (Boophilus) microplus*. Vet. Parasitol. 195, 198–202.

Drummond, R.O., Ernst, S.E., Trevino, J.L., Gladney, W.J., Graham, O.H., 1973. *Boophilus annulatus* and *Boophilus microplus*: laboratory tests for insecticides. J. Econ. Entomol. 66, 130–133.

FAO, 1971. Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides. Tentative methods for larvae of cattle tick *Boophilus* spp. FAO method No. 7. FAO Plant Proc. Bull., 15–18.

Ferrarini, S.R., Duarte, M.O., Rosa, R.G., Rolim, V., Eifler-Lima, V.L., Poser, G.V., Ribeiro, V.L.S., 2008. Acaricidal activity of limonene, limonene oxide and β-amino alcohol derivatives on *Rhipicephalus* (*Boophilus*) *microplus*. Vet. Parasitol. 157, 149–153.

Fraga, A.B., Alencar, M.M., Figueiredo, L.A., Razook, A.G., Cyrillo, J.N.S.G., 2003. Genetic analysis of the infestation of *Caracu* female cattle breed by cattle tick (*Boophilus microplus*). Rev. Bras. Zootec. 32, 1578–1586.

Hennebelle, T., Sahpaz, S., Dermont, C., Joseph, H., Bailleul, F., 2006. The essential oil of *Lippia alba*: analysis of samples from French overseas departments and review of previous works. Chem. Biodivers. 3, 1116–1125.

Jannuzzi, H., Mattos, J.K.A., Vieira, R.F., Silva, D.B., Bizzo, H.R., Gracindo, L.A., 2010. Agronomic evaluation and identification of chemotypes of lemongrass in Distrito Federal. Hortic. Bras. 28, 412–417.

Kalita, B., Bora, S., Sharma, A.K., 2013. Plant essential oils as mosquito repellent – a review. Int. J. Res. Dev. Pharm. Life Sci. 3, 741–747.

Lage, T.C.A., Montanari, R.M., Fernandes, S.A., Monteiro, C.M.O., Senra, T.O.S., Zeringota, V., Calmon, F., Matos, R.S., Daemon, E., 2013. Activity of essential oil of *Lippia triplinervis* Gardner (Verbenaceae) on *Rhipicephalus microplus* (Acari: Ixodidae). Parasitol. Res. 112, 863–869.

Leite, R.C., (Thesis – Doctor Degree on Preventive Veterinary Medicine) 1988. *Boophilus microplus* (Canestrini, 1887): Susceptibility, Current and Retrospective Usage of Acaricides in Lands from *baixada do Grande-Rio and Rio de Janeiro* Physiogeographic Regions. An Epidemiological Approach. UFMG, Belo Horizonte, pp. 151.

Liu, W., Gan, J., Schlenk, D., Jury, W.A., 2004. Enantioselectivity in environmental safety of current chiral insecticides. Proc. Natl. Acad. Sci. U. S. A. 102, 701–706.

Mendes, M.C., Lima, C.K.P., Nogueira, A.H.C., Yoshihara, E., Chiebao, D.P., Gabriel, F.H., Ueno, T.E., Namindome, A., Klafke, G.M., 2011. Resistance to cypermethrin, deltamethrin and chlorpyriphos in populations of *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae) from small farms of the state of São Paulo, Brazil. Vet. Parasitol. 178, 383–388.

Miresmailli, S., Bradbury, R., Isman, M.B., 2006. Comparative toxicity of *Rosmarinus officinalis* L. essential oil and blends of its major constituents against *Tetranychus urticae* Koch (Acari: Tetranychidae) on two different host plants. Pest. Manage. Sci. 62, 366–371.

Roditakis, E., Roditakis, N.E., Tsagkarakou, A., 2005. Insecticide resistance in *Bemisia tabaci* (Homoptera: Aleyrodidae) populations from Crete. Pest. Manage. Sci. 61, 577–582.

Senatore, F., Rigano, D., 2001. Essential oil of two *Lippia* spp. (Verbenaceae) growing wild in Guatemala. Flavour Fragr. J. 16, 169–171.

Sette-de-Souza, P.H., Carneiro, S.E.R., Macedo-Costa, M.R., Borges, S.B., Medeiros, A.R., Fernandes, T.J.O., Amaro-da-Silva, L.C., Lopes, H.J., Lima, K.C., 2014. Antibacterial activity and phytochemical screening of extracts of *Lippia alba* (Mill). NE Brown. Afr. J. Microbiol. Res. 8 (29), 2783–2787.

Simas, N.K., Lima, E.C., Conceição, S.R., Kuster, R.M., Filho, A.M.O., Lage, C.L.S., 2004. Natural products for dengue transmission control – larvicidal activity of *Myroxylon balsamum* (red oil) and of terpenoids and phenylpropanoids. Quim. Nova 27, 46–49.

Singh, R., Koul, O., Rup, P.J., Jindal, J., 2009. Toxicity of some essential oil constituents and their binary mixtures against *Chilo partellus* (Lepidoptera: Pyralidae). Int. J. Trop. Insect. Sci. 29, 93–101.

Stone, B.F., Haydock, K.P., 1962. A method for measuring the acaricide-susceptibility of the cattle tick *Boophilus microplus* (Can.). Bull. Entomol. Res. 53, 563–578.

Tavares, I.B., Momenté, V.G., Nascimento, I.R., 2011. *Lippia alba*: chemical, pharmacological and agronomical studies. Appl. Res. Agrotec. 4, 204–220.

Vandendool, H., Kratz, P.D., 1963. A generalization of retention index system including linear temperature programmed gas–liquid partition chromatography. J. Chromatogr. 11, 463–471.