Original Article

Braz. J. Vet. Parasitol., Jaboticabal, v. 25, n. 4, p. 401-406, out.-dez. 2016
ISSN 0103-846X (Print) / ISSN 1984-2961 (Electronic)
Doi: http://dx.doi.org/10.1590/S1984-29612016087

Assessment of different *Lippia sidoides* genotypes regarding their acaricidal activity against *Rhipicephalus* (*Boophilus*) *microplus*

Avaliação da atividade carrapaticida do óleo essencial de diferentes genótipos de *Lippia sidoides* sobre *Rhipicephalus* (*Boophilus*) *microplus*

Alexandra Martins dos Santos Soares[1]; Tatiane Aranha Penha[2]; Sandra Alves de Araújo[1]; Elizangela Mércia Oliveira Cruz[3]; Arie Fitzgerald Blank[3]; Livio Martins Costa-Junior[2]*

[1] Curso de Engenharia Química, Universidade Federal do Maranhão – UFMA, São Luís, MA, Brasil

[2] Departamento de Patologia, Universidade Federal do Maranhão – UFMA, São Luís, MA, Brasil

[3] Departamento de Agronomia, Universidade Federal de Sergipe – UFS, São Cristóvão, SE, Brasil

Received May 25, 2016
Accepted October 17, 2016

Abstract

The aim of this study was to select different genotypes of *Lippia sidoides* with the highest activity against larvae and engorged females of the tick *Rhipicephalus* (*Boophilus*) *microplus*. The germplasms studied were LSID006, LSID102, LSID103 and LSID104. The LSID104 genotype, that presented carvacrol as a major constituent, was the germplasm with the worst larvicide effect (LC_{50} 2.99 mg/mL). The LSID006 genotype was the least effective against engorged females (LC_{50} 12.46 mg/mL), and it was chemically similar to the LSID102 genotype, which presented the highest acaricide activity (LC_{50} 2.81 mg/mL). We conclude that chemical similarity of the germplasms was not correlated with acaricide activity against *R.* (*B.*) *microplus*. The essential oil of *L. sidoides* is a potent natural agent. However, the findings of this work provide a better understanding for product development based on this natural product, which must consider synergic effects or the action of minor compounds.

Keywords: Essential oil, acaricidal, chemical diversity, germplasms, carvacrol, thymol.

Resumo

O objetivo deste trabalho foi selecionar genótipos de *Lippia sidoides* que apresentem maiores atividades em larvas e fêmeas ingurgitadas do carrapato *Rhipicephalus* (*Boophilus*) *microplus*. Os genótipos estudados foram LSID006, LSID102, LSID103 e LSID104. O genótipo LSID104, o único a conter o monoterpeno carvacrol como um dos principais constituintes, foi o que apresentou o menor efeito larvicida (CL_{50} 2,99 mg/mL). O genótipo LSID006 apresentou menor efeito sobre fêmeas ingurgitadas (CL_{50} 12,46 mg/mL), entretanto foi quimicamente similar ao genótipo LSID102, que apresentou a maior atividade carrapaticida (CL_{50} 2,81 mg/mL). Conclui-se que a semelhança química dos genótipos não se correlaciona com a atividade carrapaticida contra *R.* (*B.*) *microplus*. O óleo essencial de *L. sidoides* é um agente natural potente e os resultados deste trabalho proporcionam um melhor entendimento para o desenvolvimento de produtos com base neste produto natural, devendo ser considerado os efeitos sinérgicos ou a ação de compostos presentes em menores concentrações.

Palavras-chave: Óleo essencial, acaricida, diversidade química, genótipos, carvacrol, timol.

Introduction

The *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae) cattle tick is a blood-sucking ectoparasite that occurs in tropical and subtropical regions (PEREIRA et al., 2008). This species has caused economic losses to the world livestock, inducing intense irritation of the animals, leather depreciation, decreased weight gain, decreased production of meat and milk, and transmitting *Babesia* spp. and *Anaplasma* spp. (GOMES 1998; FURLONG, 2005; RECK et al., 2014). In Brazil, these losses exceed 3 billion dollars annually (GRISI et al., 2014).

The most widely used control method is the use of synthetic chemical agents. However, chemical control can lead to selected populations of resistant ticks (GUERRERO et al., 2012) and are

***Corresponding author:** Livio Martins Costa-Junior. Departamento de Patologia, Universidade Federal do Maranhão – UFMA, Av. dos Portugueses, 1966, Bacanga, CEP 65080-805, São Luís, MA, Brasil.
e-mail: livio.martins@ufma.br

costly and contaminate the environment with residues harmful to the hosts and also humans (FREITAS et al., 2005). Therefore, new approaches are needed, and natural products are potential candidates for acaricidal drugs. These compounds generally have low environmental impact and can lead to slower tick resistance (BORGES et al., 2003; HU & COATS, 2008).

Lippia sidoides is a Verbenaceae plant known in Brazil as "alecrim-pimenta". Despite the occurrence in northeastern of Brazil, it is now cultivated in several Brazilian states due to its herbal characteristics (MATOS & OLIVEIRA, 1998). *L. sidoides* leaves are approximately constituted with 4% essential oil with carvacrol and thymol as major constituents, depending on the evaluated germplasm (LORENZI & MATOS, 2008; SANTOS et al., 2015). The essential oils of *L. sidoides* have bactericidal, fungicidal and molluscicidal properties which are generally attributed to the major components (MATOS, 2000, 2002; CARVALHO et al., 2003; BOTELHO et al., 2007).

In addition to the activities mentioned above, the acaricide activity of the essential oil of *L. sidoides* with high concentration of thymol and carvacrol has been used against *Tetranychus urticae* (CAVALCANTI et al., 2010), *R.* (*B.*) *microplus, R. sanguineus, Amblyomma cajennense* and *Dermacentor nitens* (GOMES et al., 2012, 2014). However, different genotypes of plants show differences in their chemical composition and thus different bioactivities (CRUZ et al., 2013; COSTA-JÚNIOR et al., 2016). Therefore, the aim of this study was to evaluate the activity of the essential oil of different germplasms of *L. sidoides* plants against larvae and females of *R.* (*B.*) *microplus*.

Materials and Methods

Essential oil

Leaves of *L. sidoides* were collected from the active Germplasm Bank of Medicinal Plants that was established with *L. sidoides* plants from different geographical locations (Table 1) at the research farm of the Federal University of Sergipe, Brazil (SANTOS et al., 2015). The harvests of all genotype were performed at the same time. After manual defoliation, the leaves were dried in a forced air circulation oven for five days at 40 °C.

The essential oils were extracted by hydrodistillation in a Clevenger apparatus for 140 minutes. Each sample consisted of 75 g of dried leaves from four plants. The essential oils extraction, as well the determination and analysis of their chemical composition, were conducted according Santos et al. (2015).

Table 1. Identification and geographical origin of the *Lippia sidoides* genotypes used in the present study.

Code	Origin (State/Country)	Geographical data	Voucher nº
LSID006	Ceará/Brazil	5°14'05.4"S;38°11'35.0"W	8223
LSID102	Sergipe/Brazil	9°58'07.6"S;37°51'49.2"W	8224
LSID103	Sergipe/Brazil	9°58'08.6"S;37°51'50.3"W	8225
LSID104	Sergipe/Brazil	9°58'09.2"S;37°51'50.3"W	8226

Briefly, the chemical composition of the essential oils was determined using a gas chromatograph coupled to a mass spectrometer equipped with an AOC-20i auto injector and a fused-silica capillary column. Quantitative analyses were performed by flame ionization gas chromatography (FID). The essential oil components were identified by comparing their mass spectra with the available spectra in the equipment database (NIST05 and WILEY8). Finally, the measured retention indices were compared with those in the literature (ADAMS, 2007), and the retention times (RT) were determined using the Van Den Dool & Kratz (1963) equation and a homologous series of *n*-alkanes (C_8-C_{18}).

Obtaining the larvae and the engorged females

The larvae and engorged females of *R.* (*B.*) *microplus* ticks used in this work were obtained from colonies maintained at the Biological and Health Science Center of the Federal University of Maranhão (UFMA), Brazil. This study was approved by the Ethics Committee on Animal Use of UFMA under protocol 23115018061/2011-01. Larvae between 14 and 21 days after hatching were used in the Larval Packet Test. Adult engorged female ticks (≥ 4.5 mm in length) were collected from the bodies of artificially infested cattle.

Larval packet test

The larval packet test was performed according to Stone & Haydock (1962) and modified by FAO (1984) and Leite (1988), as described below. Two sheets of filter paper (4 cm^2) (Whatman 80 g) were treated with 400 µL of solution containing 3% dimethyl sulfoxide (DMSO) and essential oil. Twelve concentrations, ranging from 0.0612 to 15.00 mg/mL of essential oil isolated from each of the four *L. sidoides* genotypes, were used for the test. Ten concentrations ranging from 0.0612 to 25.00 mg/mL of thymol (Merck) and carvacrol (Sigma–Aldrich) were performed, tested as published before (CRUZ et al., 2013).

Approximately 100 tick larvae were placed in filter papers folded to form a packet and sealed with a plastic clothespin. The packet was placed in an incubator (27 °C and relative humidity ≥ 80%) for 24 hours. After this time, alive and dead larvae were counted. Ticks that did not move were considered dead. The experiment was performed with four replicates for each treatment. Furthermore, a solution of 3% DMSO was used as the negative control.

Adult immersion test

The adult immersion test was performed as described by Drummond et al. (1973). The adult immersion test shows the activity on mortality as well as the interference in reproduction, by evaluation of oviposition and eggs hatching. Engorged female cattle ticks were collected from artificially infested calves. Groups of ten engorged female ticks were weighed to obtain groups with weights ranging from 2.24 to 2.32 g.

Each tick group was dipped in one of twelve concentrations ranging from 0.0612 to 25.00 mg/mL of essential oil isolated from one of the four *L. sidoides* genotypes, using 3% DMSO as solvent for five minutes. Ten concentrations ranging from 1.00 to 25.00 mg/mL of thymol (Merck) and carvacrol (Sigma–Aldrich) were performed, tested and published before (CRUZ et al., 2013). DMSO (3%) was used for the negative control group. The engorged females were subsequently dried on a paper towel, placed in Petri dishes and maintained in a biochemical oxygen demand (BOD) incubator at 27 ± 1 °C and relative humidity ≥ 80% for 35 days to allow oviposition and hatching of the larvae. The eggs mass were weighed and the hatching was evaluated. The efficacy was calculated according Drummond et al. (1973).

Statistical analysis

Lethal concentrations were calculated using GraphPad Prism 6.0. Significant differences between the average efficiency of each pair of essential oil and/or monoterpene were considered when there was no overlap between the 95% confidence limits of the LC_{50} values (RODITAKIS et al., 2005). The data of the acaricidal activity (on larvae and engorged female) of the essential oils from each genotype were submitted to cluster analysis using DataLab 3.5 software. The dissimilarity matrix was simplified with dendrograms using Ward's clustering method. The dendrograms were drawn using the PHY FY website (FREDSLUND, 2006).

Results

Twenty six components of the *L. sidoides* essential oil were identified (Table 2). The LSID006, LSID102, LSID103, and the LSID104 genotypes presented, respectively, 21, 14, 19 and 16 compounds. The most abundant chemical compound in LSID006, LSID102, and LSID103 was thymol (54.4%; 38.7%; 64.8%, respectively), and the most abundant compound in LSID104 was the thymol isomer, carvacrol (43.7%).

Lippia sidoides oils showed efficacy against larvae and engorged female ticks (Table 3). The LSID104 genotype, which uniquely presented carvacrol as its major constituent, had one of the worst larvicide effects. LSID006 had the highest larvicide effect. LSID103 had the highest amounts of thymol and presented a lower larvicide activity than LSID006. The LSID103 and LSID006 are in different clusters based on acaricidal activity (Figure 1).

LSID102 presented higher efficacy against engorged females (LC50 = 2.81 mg/mL) (Table 3). Similar to the results with larvae, no direct relationship between clustering analysis was found based on the chemical constituents and the acaricidal effect because LSID006 was the least effective compound against engorged females and is chemically similar to the LSID102 genotype (Figure 1). Thymol acetate is present only in LSID102 and LSID103 (Table 2), the two genotypes with the largest acaricidal

Table 2. Essential oil composition (%) from *Lippia sidoides* genotypes characterized by gas chromatography associated with a mass spectrophotometer.

Compound	RT* (min)	LSIDI 006	LSIDI 102	LSIDI 103	LSIDI 104
α-Thujene	6.567	1.55	1.09	1.01	1.66
α-Pinene	6.783	0.69	0.34	0.47	0.49
β-Pinene	8.183	0.32	-	-	-
Myrcene	8.592	3.16	3.35	5.32	3.52
α-Phelandrene	9.167	-	-	-	0.20
δ-3-Carene	9.233	0.23	0.12	0.18	0.10
α-Terpinene	9.542	2.04	1.91	1.53	3.12
p-Cimene	9.817	19.18	34.11	13.89	17.83
Limonene	9.975	0.94	0.49	0.71	0.44
1,8 Cineole	10.083	0.29	-	-	-
β-(Z)-Ocimene	10.233	-	-	-	0.29
γ-Terpinene	11.017	5.10	6.84	4.41	16.56
Linalol	12.583	0.27	-	0.10	-
Ipsdienol	14.133	1.23	-	-	-
NI	15.142	0.20	-	0.40	0.78
Terpinen-4-ol	15.542	1.18	0.66	0.96	0.91
Methyl thymol	17.233	2.16	9.42	1.87	4.13
Thymol	19.625	54.40	38.68	64.82	6.05
Carvacrol	19.858	-	0.60	-	43.69
Thymol Acetate	21.450	-	1.76	2.06	-
E-Methyl Cinnamate	22.808	-	-	0.96	-
β-Cariofilene	24.000	5.04	0.63	0.67	-
Aromadendrene	24.617	0.26	-	-	-
α-Humulene	25.192	0.24	-	0.23	-
β-Selinene	26.350	0.20	-	-	-
β-Bisabolene	26.900	-	-	0.24	0.23
Oxide of Cariophyllene	29.242	1.32	-	0.18	-
Total	**-**	**99.9**	**100.0**	**100.0**	**100.0**

*Retention Time.

Table 3. Acaricidal activity (LC_{50}) of the essential oil from *Lippia sidoides* genotypes.

Access	LC_{50} (mg/mL)	IC 95%	R^2
Larvae			
LSID006	0.93[b]	0.65-1.31	0.97
LSID102	3.36[c]	3.15-3.58	0.96
LSID103	3.90[d]	3.65-4.17	0.94
LSID104	2.99[cd]	1.62-5.50	0.86
Carvacrol*	0.22[a]	0.08-0.60	0.78
Thymol*	3.86[cd]	3.26-4.58	0.82
Engorged females			
LSID006	12.46[d]	11.28-13.77	0.95
LSID102	2.81[a]	2.62-3.01	0.99
LSID103	4.31[b]	3.92-4.74	0.99
LSID104	11.48[d]	11.08-11.91	0.99
Carvacrol*	4.46[b]	4.33-4.60	0.99
Thymol*	5.50[c]	5.41-5.58	0.99

*Tested by our group and published in Cruz et al. (2013). Different letters represent significant differences among the essential oils or monoterpenes ($p > 0.05$).

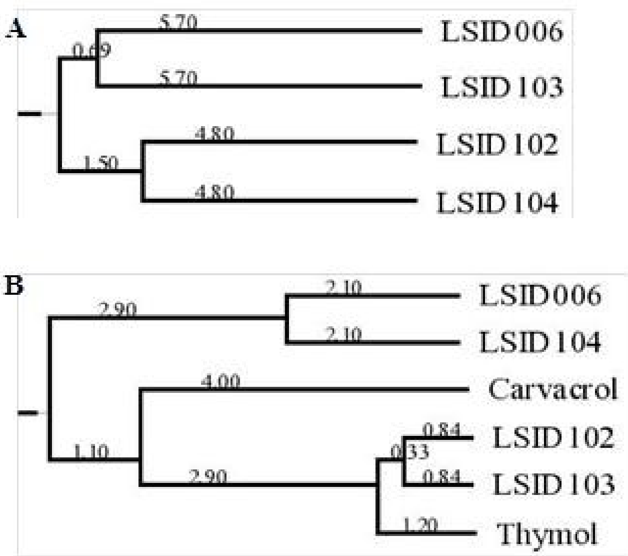

Figure 1. Clustering of *Lippia sidoides* genotypes based on acaricidal activity with the Euclidean distances.

efficacy against engorged females (Table 3), which could indicate the possibility of a synergistic effect of this compound.

Clustering analysis of *L. sidoides* genotypes based on acaricidal activity showed that LSID102 and LSID103 are closely related (Figure 1). These genotypes were the most effective against engorged females (Table 3). Both genotypes are more closely related to thymol than carvacrol, although all of them represent the same cluster (Figure 1).

Discussion

The difficulties in preparing proper formulations, differences in the chemical composition of plants of the same species due to extrinsic and intrinsic factors and differences on the activity of the formulations from the same vegetal species are hindrances that need to be addressed in order to enable progress to transposing the efficacy obtained from the laboratory to the field (BORGES et al., 2011). This study selected *L. sidoides* genotypes with highest efficacies on *R.* (*B.*) *microplus* advancing knowledge for the standardization of a compound.

The susceptibility to acaricidal compounds is related to the life stages of the tick, as well as the physiological process involving blood feeding. In general, the immature stages of ticks seem to be more susceptible to synthetic acaricide effects than others stages (PINHEIRO, 1987). The essential oil of *L. sidoides* was most efficient against larvae than nymphs of *R. sanguineus* and *A. cajennense* (GOMES et al., 2014). However the animals are always infested by ticks at different life stages and the best compound should be effective against all of them. In the present study a cluster analysis was performed to select *L. sidoides* genotypes with the highest efficacies against *R. (B.) microplus* larvae and engorged female.

Several phytochemical studies demonstrated the presence of thymol as the major compound of *L. sidoides* (CAVALCANTI et al., 2010; VERAS et al., 2012; GOMES et al., 2014). The exception observed in LSID104 (CAVALCANTI et al., 2010) could be a result of the chemical distance explained by the clustering analysis of *L. sidoides* genotypes, where LSID104 was grouped alone and the other genotypes showed considerable chemical similarity (SANTOS et al., 2015).

Carvacrol was the most efficient compound against larvae mainly organophosphate resistant strain larvae (CRUZ et al., 2013; COSTA-JUNIOR et al., 2016), the essential oil that presented as its major constituent, had one of the worst larvicide effects. This result suggests that despite carvacrol alone having elevated larvicide action, it is not the main bioactive compound, and the different oil constitutions play an important role in the acaricidal action. We hypothesize that the high levels of thymol (54.4%) in LSID006 are responsible for this action, but LSID103 has higher amounts of thymol and presented a lower larvicide activity because LSID103 and LSID006 are in the same cluster based on chemical constituents (SANTOS et al., 2015) but are in different clusters based on acaricidal activity (Figure 1). β-bisabolene is present only in LSID103 and could have antagonistic activity. Additionally, some compounds exist only in LSID006 (β-pinene; 1,8 cineole; ipsdienol; aromadrendene; β-selinene) and could contribute to the elevated larvicide action of this genotype. The synergistic effects of β-pinene, 1,8 cineole and aromadendrene have recently been reported (MULYANINGSIH et al., 2010; RODENAK et al., 2014; ZHANG et al., 2015), but to the best of our knowledge, the minor compounds of *L. sidoides* essential oil have not been studied to evaluate their synergistic capabilities in *R.* (*B.*) *microplus*.

The synergy studies conducted in *Lippia* spp. corroborate our results. The activity of the essential oil of *L. sidoides* and *L. gracilis* and chemical components against the fungus *Thielaviopsis paradoxa* recently have been reported. The compounds p-cymene, 1.8 cineole, α-terpinene and β-caryophyllene had no efficiency when tested alone, and the authors proposed that they act in synergy with other compounds because the thymol concentration required to control the fungus was higher than the concentration of the *L. sidoides* essential oil (CARVALHO et al., 2013).

Different plant genotypes can present distinct essential oil profiles (GIL et al., 2002; DRAGLAND et al., 2005; PEIXOTO et al., 2015). In this context, it is important to study the relation between these composition variations and the interference in the bioactivity. For example, LSID102 and LSID104 presented similar leishmanicidal activity (Concentration that inhibits 50% - IC_{50} = 74.1 and 54.8 μg/mL, respectively) (FARIAS-JUNIOR et al., 2012), although both genotypes are in different clusters based on their chemical constitution (SANTOS et al., 2015). In addition, no significant differences were observed in the acaricidal activity of the essential oils of different *L. sidoides* genotypes against *T. urticae,* and after acaricidal analysis with selected compounds it was suggested that the evaluated components act synergistically to achieve the acaricidal effect (CAVALCANTI et al., 2010).

The bioactivity of thymol has been reported against ticks and insects (NOVELINO et al., 2007; WALIWITIYA et al., 2010; CRUZ et al., 2013), and the larvicide activity of *L. sidoides* essential oil against *Aedes aegypti* was attributed to this monoterpene (CARVALHO et al., 2003). The LSID102 and LSID103 genotypes were the most effective against engorged females (Table 3). Both genotypes are more closely related to thymol than carvacrol, although all of them represent the same cluster (Figure 1). The presence of thymol in these genotypes is associated with the activity against engorged females.

Although the genotypes with higher efficiency against larvae (LSID006) and engorged females (LSID102) have thymol as their major constituent, which could be an indicative that this monoterpene is involved in the acaricidal effect, the general chemical balance among the essential oil compounds leads to different acaricidal effects. Both genotypes are in different clusters based on acaricidal activity (Figure 1), which suggests that there could be different action modes of these essential oils during different life stages of the *R.* (*B.*) *microplus*. Although the activity of *L. sidoides* essential oil has been described on *R.* (*B.*) *microplus*, to our knowledge, this is the first evaluation of the relationship among the activity of different genotypes of *L. sidoides* and the acaricide effect.

Conclusions

The results indicated that the chemical differences in the *L. sidoides* genotypes influence the acaricidal activity against *R.* (*B.*) *microplus*. In addition, the clustering analysis of *L. sidoides* genotypes based on acaricidal activity suggests that the essential oils have different modes of action in larvae and in engorged females. We conclude that the different constitutions of the essential oils, as well as the relationships among the compounds, play important roles in the acaricidal action. However, further studies are needed to verify the global acaricidal effects of the minor compounds of *L. sidoides* essential oil. The findings of this work facilitate the understanding and the development of innovative strategies aimed to control the cattle tick *R.* (*B.*) *microplus*.

Acknowledgements

The authors wish to thank CNPq (The Brazilian National Council for Scientific and Technological Development) for awarding a fellowship to L.M. Costa-Júnior, and A.F. Blank, CAPES (Brazilian Federal Agency for support and evaluation of graduate education) for the scholarship to T.A. Penha and E.M.O. Cruz and FAPEMA (Maranhão State Research Foundation) for the scholarship to S.A. Araújo. We also thank CNPq and FAPEMA for financial support.

References

Adams RP. *Identification of essential oil components by gas chromatography/mass spectroscopy*. 4th ed. Illinois: Allured Publishing Corporation; 2007.

Borges LMF, Ferri PH, Silva WJ, Silva WC, Silva JG. *In vitro* efficacy of extracts of *Melia azedarach* against the tick *Boophilus microplus. Med Vet Entomol* 2003; 17(2): 228-31. PMid:12823842. http://dx.doi.org/10.1046/j.1365-2915.2003.00426.x.

Borges LMF, Sousa LAD, Barbosa CS. Perspectives for the use of plant extracts to control the cattle tick *Rhipicephalus* (*Boophilus*) *microplus. Rev Bras Parasitol Vet* 2011; 20(2): 89-96. PMid:21722481. http://dx.doi.org/10.1590/S1984-29612011000200001.

Botelho MA, Nogueira NAP, Bastos GM, Fonseca SC, Lemos TL, Matos FJ, et al. Antimicrobial activity of the essential oil from *Lippia sidoides*, carvacrol and thymol against oral pathogens. *Braz J Med Biol Res* 2007; 40(3): 349-356. PMid:17334532. http://dx.doi.org/10.1590/S0100-879X2007000300010.

Carvalho AFU, Melo VMM, Craveiro AA, Machado MIL, Bantim MB, Rabelo EF. Larvicidal activity of the essential oil from *Lippia sidoides* Cham. against *Aedes aegypti* Linn. *Mem Inst Oswaldo Cruz* 2003; 98(4): 569-571. PMid:12937776. http://dx.doi.org/10.1590/S0074-02762003000400027.

Carvalho RRC, Laranjeira D, Carvalho-Filho JLS, Souza PE, Blank AF, Alves PB, et al. *In vitro* Activity of essential oils of *Lippia sidoides* and *Lippia gracilis* and their major chemical components against *Thielaviopsis paradoxa*, causal agent of stem bleeding in coconut palms. *Quim Nova* 2013; 36(2): 241-244. http://dx.doi.org/10.1590/S0100-40422013000200007.

Cavalcanti SCH, Niculau ES, Blank AF, Câmara CAG, Araújo IN, Alves PB. Composition and acaricidal activity of *Lippia sidoides* essential oil against two-spotted spider mite (*Tetranychus urticae* Koch). *Bioresour Technol* 2010; 101(2): 829-832. PMid:19758799. http://dx.doi.org/10.1016/j.biortech.2009.08.053.

Costa-Júnior LM, Miller RJ, Alves PB, Blank AF, Li AY, Pérez de León AA. Acaricidal efficacies of *Lippia gracilis* essential oil and its phytochemicals against organophosphate-resistant and susceptible strains of *Rhipicephalus (Boophilus) microplus. Vet Parasitol* 2016; 228: 60-64. PMid:27692332. http://dx.doi.org/10.1016/j.vetpar.2016.05.028.

Cruz EM, Costa-Júnior LM, Pinto JA, Santos DA, Araujo SA, Arrigoni-Blank MF, et al. Acaricidal activity of *Lippia gracilis* essential oil and its major constituents on the tick *Rhipicephalus (Boophilus) microplus. Vet Parasitol* 2013; 195(1-2): 198-202. PMid:23337330. http://dx.doi.org/10.1016/j.vetpar.2012.12.046.

Dragland S, Rohloff J, Mordal R, Iversen TH. Harvest regimen optimization and essential oil production in five tansy (*Tanacetum vulgare* L.) genotypes under a northern climate. *J Agric Food Chem* 2005; 53(12): 4946-4953. PMid:15941340. http://dx.doi.org/10.1021/jf047817m.

Drummond RO, Ernst SE, Trevino JL, Gladney WJ, Graham OH. *Boophilus annulatus* and *Boophilus microplus*: Laboratory test of insecticides. *J Econ Entomol* 1973; 66(1): 130-133. PMid:4690254. http://dx.doi.org/10.1093/jee/66.1.130.

Farias-Júnior PA, Rios MC, Moura TA, Almeida RP, Alves PB, Blank AF, et al. Leishmanicidal activity of carvacrol-rich essencial oil from *Lippia sidoides* Cham. *Biol Res* 2012; 45(4): 399-402. PMid:23558998. http://dx.doi.org/10.4067/S0716-97602012000400012.

Food and Agriculture Organization – FAO. *Ticks and tick borne disease control: a practical field manual*. Roma: FAO; 1984.

Fredslund J. PHY FI: fast and easy online creation and manipulation of phylogeny color figures. *BMC Bioinformatics* 2006; 7(1): 315. PMid:16792795. http://dx.doi.org/10.1186/1471-2105-7-315.

Freitas DRJ, Pohl PC, Vaz IS. Characterization of acaricide resistance in *Boophilus microplus. Acta Sci Vet* 2005; 33(2): 109-117.

Furlong J. *Carrapato: problemas e soluções*. Juiz de Fora: Embrapa Gado de Leite; 2005.

Gil A, De La Fuente EB, Lenardis AE, López Pereira M, Suárez SA, Bandoni A, et al. Coriander essential oil composition from two genotypes grown in different environmental conditions. *J Agric Food Chem* 2002; 50(10): 2870-2877. PMid:11982413. http://dx.doi.org/10.1021/jf011128i.

Gomes A. *Controle do carrapato do boi: um problema para quem cria raças européias*. Campo Grande: Embrapa; 1998.

Gomes GA, Monteiro CMO, Julião LS, Maturano R, Senra TOS, Zeringóta V, et al. Acaricidal activity of essential oil from *Lippia sidoides* on unengorged larvae and nymphs of *Rhipicephalus sanguineus* (Acari: Ixodidae) and *Amblyomma cajennense* (Acari: Ixodidae). *Exp Parasitol* 2014; 137: 41-45. PMid:24333789. http://dx.doi.org/10.1016/j.exppara.2013.12.003.

Gomes GA, Monteiro CMO, Senra TOS, Zeringota V, Calmon F, Matos RS, et al. Chemical composition and acaricidal activity of essential oil from *Lippia sidoides* on larvae of *Dermacentor nitens* (Acari: Ixodidae) and larvae and engorged females of *Rhipicephalus microplus* (Acari: Ixodidae). *Parasitol Res* 2012; 111(6): 2423-2430. PMid:22983219. http://dx.doi.org/10.1007/s00436-012-3101-9.

Grisi L, Leite RC, Martins JRS, Barros ATM, Andreotti R, Cançado PHD, et al. Reassessment of the potential economic impact of cattle parasites in Brazil. *Rev Bras Parasitol Vet* 2014; 23(2): 150-156. PMid:25054492. http://dx.doi.org/10.1590/S1984-29612014042.

Guerrero FD, Lovis L, Martins JR. Acaricide resistance mechanisms in *Rhipicephalus* (*Boophilus*) *microplus. Rev Bras Parasitol Vet* 2012; 21(1): 1-6. PMid:22534937. http://dx.doi.org/10.1590/S1984-29612012000100002.

Hu D, Coats J. Evaluation of the environmental fate of thymol and phenethyl propionate in the Laboratory. *Pest Manag Sci* 2008; 64(7): 775-779. PMid:18381775. http://dx.doi.org/10.1002/ps.1555.

Leite RC. *Boophilus microplus (Canestrini, 1887): susceptibilidade, uso atual e retrospectivo de carrapaticidas em propriedades das regiões fisiográficas da baixada do Grande-Rio e Rio de Janeiro, uma abordagem epidemiológica* [Thesis]. Rio de Janeiro: Universidade Federal Rural do Rio de Janeiro, 1988.

Lorenzi H, Matos FJA. *Plantas medicinais no Brasil: nativas e exóticas*. Nova Odessa: Plantarum; 2008.

Matos FJA. *Plantas medicinais: guia de seleção e emprego de plantas usadas em fitoterapia no nordeste do Brasil*. Fortaleza: Imprensa Universitária; 2000.

Matos FJA. *Farmácias vivas: sistema de utilização de plantas medicinais projetado para pequenas comunidades*. Fortaleza: UFC; 2002.

Matos FJA, Oliveira F. *Lippia sidoides* Cham.: farmacognosia, química e farmacologia. *Rev Bras Farm* 1998; 79(3-4): 84-87.

Mulyaningsih S, Sporer F, Zimmermann S, Reichling J, Wink M. Synergistic properties of the terpenoids aromadendrene and 1,8-cineole from the essential oil of *Eucalyptus globulus* against antibiotic-susceptible and antibiotic-resistant pathogens. *Phytomedicine* 2010; 17(13): 1061-1066. PMid:20727725. http://dx.doi.org/10.1016/j.phymed.2010.06.018.

Novelino AMS, Daemon E, Soares GLG. Avaliação da atividade repelente do timol, mentol, salicilato de metila e ácido salicilico sobre larvas de *Boophilus microplus* (Canestrini, 1887) (Acari: Ixodidae). *Arq Bras Med Vet Zootec* 2007; 59(3): 700-704. http://dx.doi.org/10.1590/S0102-09352007000300023.

Peixoto MG, Costa-Júnior LM, Blank AF, Lima AS, Menezes TSA, Santos DA, et al. Acaricidal activity of essential oils from *Lippia alba* genotypes and its major components carvone, limonene, and citral against *Rhipicephalus microplus. Vet Parasitol* 2015; 210(1-2): 118-122. PMid:25837783. http://dx.doi.org/10.1016/j.vetpar.2015.03.010.

Pereira MC, Labruna MB, Szabó MPJ, Klafke GM. *Rhipicephalus (Boophilus) microplus: biologia, controle e resistência*. São Paulo: Medvet; 2008. 192 p.

Pinheiro VRE. *Avaliação do efeito carrapaticida de alguns piretróides sintéticos sobre o carrapato Amblyomma cajennense (Fabricius, 1787) (Acarina: Ixodidae)* [Dissertation]. Rio de Janeiro: Universidade Federal Rural do Rio de Janeiro, 1987.

Reck J, Marks FS, Rodrigues RO, Souza UA, Webster A, Leite RC, et al. Does *Rhipicephalus microplus* tick infestation increase the risk for myiasis caused by *Cochliomyia hominivorax* in cattle? *Prev Vet Med* 2014; 113(1): 59-62. PMid:24176137. http://dx.doi.org/10.1016/j.prevetmed.2013.10.006.

Rodenak KB, Polo M, Montero Villegas S, Galle M, Crespo R, García de Bravo M. Synergistic antiproliferative and anticholesterogenic effects of linalool, 1,8-cineole, and simvastatin on human cell lines. *Chem Biol Interact* 2014; 214: 57-68. PMid:24613879. http://dx.doi.org/10.1016/j.cbi.2014.02.013.

Roditakis E, Roditakis NE, Tsagkarakou A. Insecticide resistance in *Bemisia tabaci* (Homoptera: Aleyrodidae) populations from Crete. *Pest Manag Sci* 2005; 61(6): 577-582. PMid:15712366. http://dx.doi.org/10.1002/ps.1029.

Santos PL, Araújo AA, Quintans JS, Oliveira MG, Brito RG, Serafini MR, et al. Preparation, Characterization, and Pharmacological Activity of *Cymbopogon winterianus* Jowitt ex Bor (Poaceae) Leaf Essential Oil of β-Cyclodextrin Inclusion Complexes. *Evid Based Complement Alternat Med* 2015; 2015: 502454. PMid:26246838. http://dx.doi.org/10.1155/2015/502454.

Stone BF, Haydock KP. A method for measuring the acaricide-susceptibility of the cattle tick *Boophilus microplus* (Can.). *Bull Entomol Res* 1962; 53(3): 563-578. http://dx.doi.org/10.1017/S000748530004832X.

Van Den Dool H, Kratz PD. A generalization of retention index system including linear temperature programmed gas–liquid partition chromatography. *J Chromatogr* 1963; 11: 463-471. PMid:14062605. http://dx.doi.org/10.1016/S0021-9673(01)80947-X.

Veras HNH, Rodrigues FFG, Colares AV, Menezes IRA, Coutinho HD, Botelho MA, et al. Synergistic antibiotic activity of volatile compounds from the essential oil of *Lippia sidoides* and thymol. *Fitoterapia* 2012; 83(3): 508-512. PMid:22245085. http://dx.doi.org/10.1016/j.fitote.2011.12.024.

Waliwitiya R, Belton P, Nicholson RA, Lowenberger CA. Effects of the essential oil constituent thymol and other neuroactive chemicals on flight motor activity and wing beat frequency in the blowfly *Phaenicia sericata. Pest Manag Sci* 2010; 66(3): 277-289. PMid:19890946. http://dx.doi.org/10.1002/ps.1871.

Zhang Z, Guo S, Liu X, Gao X. Synergistic antitumor effect of α-pinene and β-pinene with paclitaxel against non-small-cell lung carcinoma (NSCLC). *Drug Res* 2015; 65(4): 214-218. PMid:25188609.