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Baccharis dracunculifolia DC (Asteraceae) leaf and flower essential oils to control *Rhipicephalus microplus* Canestrini (Arachnida: Ixodidae) in the free-living stage

Óleo essencial das folhas e flores de *Baccharis dracunculifolia* DC (Asteraceae) para controle de *Rhipicephalus microplus* Canestrini (Arachnida: Ixodidae) na fase de vida livre

Aceite essencial de hojas y flores de *Baccharis dracunculifolia* DC (Asteraceae) para el control de *Rhipicephalus microplus* Canestrini (Arachnida: Ixodidae) en la etapa de vida libre

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Abstract

Baccharis dracunculifolia, native to Brazil and the main source of "green propolis", has been reported with several biological activities, and may be a source of bovine tick control substituting synthetic acaricides. Objective: to evaluate the *in vitro* and *ex situ* acaricidal activity of *B. dracunculifolia* leaf and flower essential oils against *Rhipicephalus microplus*. Methodology: the essential oils were extracted by hydrodistillation and analyzed by a gas chromatography coupled to mass spectrometry; the acaricidal activity of the essential oil was evaluated *in vitro* against adult females and against the egg hatchability; moreover, the

acaricidal activity against tick larvae was evaluated *in vitro* and *ex situ*. Results: the major class of the essential oils was oxygenated sesquiterpene (55.1% leaves 50.4% flowers) and the main compounds were (21.5% leaves; 20.6% flowers) and spathulenol (21.8% leaves; 20.3% flowers). The essential oil at 500 mg/mL was effective to control egg hatchability with a reduction of egg laying capacity and decrease of number of adult ticks and larvae. The larvicidal activity of the essential oil had $LC_{99.9}$ from 35 to 37 mg/mL by probit analysis, and the essential oil from 11 to 14 mg/mL presented 85 to 95% of treatment efficiency in the *ex situ* test. Conclusion: *B. dracunculifolia* leaf and flower essential oils are stable and have application potential to control bovine ticks.

Keywords: Wild rosemary; Cattle tick; Nerolidol; Spathulenol; *Ex situ* test.

Resumo

Baccharis dracunculifolia nativa do Brasil, principal fonte da "própolis verde", tem sido reportada com diversas atividades biológicas, podendo ser uma fonte de controle do carrapato bovino em substituição aos acaricidas sintéticos. Objetivo: avaliar a atividade acaricida *in vitro* e *ex situ* do óleo essencial das folhas e das flores de *B. dracunculifolia* contra *Rhipicephalus microplus*. Metodologia: os óleos essenciais foram extraídos por hidrodestilação e analisados por cromatografia em fase gasosa acoplada à espectrometria de massas; a atividade acaricida dos óleos essenciais foi avaliada *in vitro* contra fêmeas adultas e na eclodibilidade dos ovos; ainda a ação acaricida sobre larvas do carrapato foram avaliadas *in vitro* e *ex situ*. Resultados: a classe majoritária dos óleos essenciais foi o sesquiterpeno oxigenado (55.1% folha e 50.4% flor) e os compostos principais foram nerolidol (21.5% folha; 20.6% flor) e spathulenol (21.8% folha; 20.3% flor). O óleo essencial a 500 mg/mL foi efetivo para controlar a eclodibilidade de ovos com redução da capacidade de oviposição e redução do número de carrapatos adultos e larvas. A atividade larvicida do óleo essencial teve $CL_{99,9}$ de 35 a 37 mg/mL pela análise de probitos e no teste *ex situ* o óleo essencial de 11 a 14 mg/mL teve 85 e 95% de eficácia de tratamento. Conclusão: o óleo essencial da folha e flor de *B. dracunculifolia* tem estabilidade e potencial de aplicação para o controle de carrapatos bovinos.

Palavras-chave: Alecrim-do-campo; Carrapato bovino; Nerolidol; Espatulenol; *Ex situ* teste.

Resumen

Baccharis dracunculifolia, originaria de Brasil, principal fuente de "propóleo verde", ha sido reportada con varias actividades biológicas y puede ser una fuente de control de garrapatas

bovinas en sustitución de insecticidas sintéticos. Objetivo: evaluar la actividad acaricida *in vitro* y *ex situ* del aceite esencial de hojas y flores de *B. dracunculifolia* contra *Rhipicephalus microplus*. Metodología: los aceites esenciales fueron extraídos por hidrodestilación y analizados por cromatografía de gases acoplada a espectrometría de masas; la actividad acaricida de los aceites essenciales se evaluó *in vitro* contra hembras adultas y en la incubabilidad de huevos; también se evaluó *in vitro* y *ex situ* la acción acaricida sobre larvas de garrapatas. Resultados: la clase principal de aceites esenciales fue el sesquiterpeno oxigenado (55,1% hoja y 50,4% flor) y los compuestos principales fueron nerolidol (21,5% hoja; 20,6% flor) y espatulenol (21,8% hoja; 20,3% flor). El aceite esencial hasta 500 mg/mL fue eficaz en el control de la incubabilidad de los huevos con capacidad de oviposición reducida y reducción en el número de garrapatas adultas y larvas. La actividad larvicida del aceite esencial tuvo $LC_{99.9}$ de 35 a 37 mg/mL por análisis probit y en la prueba *ex situ*, el aceite esencial de 11 a 14 mg/mL tuvo una eficacia de tratamiento de 85 a 95%. Conclusión: el aceite esencial de hoja y flor de *B. dracunculifolia* tiene estabilidad y potencial aplicación para el control de garrapatas bovinas.

Palabras clave: Alecrim-do-campo; Garrapata del ganado; Nerolidol; Espatulenol; Ensayo *ex situ*.

1. Introduction

Brazil is one the biggest producers and exporters of bovine meat. In 2018, Brazil had 31.9 million cattle slaughtered and produced 24.45 billion liters of industrially processed milk (IBGE, 2019). However, livestock farming profitability is significantly decreased by *Rhipicephalus microplus* Canestrini (Arachnida: Ixodidae) in cattle, requiring high costs with equipment and synthetic acaricides for infestation control (Grisi et al., 2014). Bovine tick is considered one of the most harmful parasites to cattle productivity in Brazil with losses around USD 3.24 billion per year (Grisi et al., 2014; Chagas et al., 2016). This hematophagous ectoparasite can affect animal welfare by transmitting pathogens such as *Anaplasma marginale* (Teruel et al., 2009; Silva, Fonseca, & Barbosa, 2015), *Babesia bovis*, and *Babesia bigemina* (Romero-Salas et al., 2016). Moreover, it is responsible for cattle mass reduction and milk, meat, and leather production decreases (Chagas et al., 2012). Synthetic acaricides have been used indiscriminately to control bovine ticks (Ramírez et al., 2016), resulting in development of tick resistance and chemical contamination of meat and milk products (Shaker & Elsharkawy, 2015; Robbertse et al., 2016).

Several natural compounds from plants have been reported and widely used as insecticide and/or acaricide such as pyrethrum from *Chrysanthemum cinerariifolium* Trev. [current scientific name *Tanacetum cinerariifolium* (Trev.) Sch. Bip.; Asteraceae], nicotine from *Nicotiana tabacum* L. (Solanaceae), azadirachtin from *Azadirachta indica* A. Juss. (Meliaceae), among others (Callejon et al., 2016). Other secondary metabolites from plants such as extracts, essential oils and isolated compounds have shown promising biological activities (Ribeiro et al., 2010; Bispo, Almeida, & Nunes, 2020) such as the sesquiterpene nerolidol, a major compound found in *B. dracunculifolia* essential oil to control bovine tick (Lage et al., 2015).

Baccharis dracunculifolia DC. (Asteraceae) is a perennial ligneous bush plant native to Brazil with distribution in Cerrado, Atlantic Rainforest, and Pampa biomes (Heiden & Schneider, 2015). It is popularly used in traditional medicine (leaf infusion) for liver problems, stomach dysfunction, and as an anti-inflammatory activity, and is popularly known as “wild rosemary” and “vassourinha” in Brazil (Trindade, Facioni, & Borba, 2007). In addition, it is considered the main botanical source for honeybees (*Apis mellifera* L.) to produce the “Brazilian green propolis”, a propolis with a characteristic green color and biological activities (Park, Paredes-Guzmán, Aguiar, Alencar, & Fujiwara, 2004; Alencar, Aguiar, Paredes-Guzmán & Park, 2005).

Baccharis dracunculifolia essential oil has several reported biological activities such as antiviral (Búfalo et al., 2009; Pereira et al., 2011), antioxidant (Luchesi, Paulus, Busso, Frata & Oliveira, 2020), antifungal (Bonett & Cerri, 2011; Fonseca et al., 2015; Luchesi, Paulus, Busso, Frata & Oliveira, 2020), antibacterial (Pereira, Costa, Liporoni, Rego, & Jorge, 2016; Salazar et al., 2018; Cazellla et al., 2019), antiulcerogenic (Massignani et al., 2009), antiprotozoal (Parreira et al., 2010), and acaricidal activities (leaf essential oil) against *R. microplus* (Lage et al., 2012).

Considering that natural compounds from plants are still of interest to control bovine tick and to reduce tick resistance to synthetic acaricides, and that there have been no studies on the acaricidal activity of *B. dracunculifolia* essential oil in *ex situ* conditions, this study aimed to evaluate the chemical composition and *in vitro* and *ex situ* acaricidal activity of *B. dracunculifolia* leaf and flower essential oil against *R. microplus*.

2. Methodology

2.1. Plant material and botanical identification

Baccharis dracunculifolia leaves and flowers were harvested in Guaraniaçu, Brazil, at latitude 25º06'03''S, longitude 52º52'41''W, and 923 m de altitude, from 7-8 h in the morning, in April 2016, during the flowering phenophase. The sample was identified by Dr. Gustavo Heiden and deposited in the collection of the Herbarium of the State University of West Paraná, campus of Cascavel, Paraná, Brazil, under the registration number UNOP-8655. This species is registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen, acronym in Portuguese) under the number A72A04D.

2.2. Essential oil extraction and chemical composition

Baccharis dracunculifolia leaf or flower essential oil was extracted separately by hydrodistillation in a Clevenger apparatus for 2 h (Miranda, Cardoso, Batista, Rodrigues, & Figueiredo, 2016). At the end of distillation, the essential oil was removed from the apparatus, transferred to amber vials, and stored at –20 °C (Pereira, Costa, Liporoni, Rego, & Jorge, 2016).

The essential oil chemical identification was carried out by a gas chromatographer (Agilent 7890B) coupled to a mass spectrometer (Agilent 5977A MSD) and a HP5-MS UI column (Agilent fused silica capillary of 30 m × 250 µm × 0.25 µm; Agilent Technologies), with initial oven temperature from 40 °C (2 min) to 230 ºC (3 ºC/min), and kept at this temperature for 20 min. Helium was utilized as the carrier gas at the linear speed of 1 mL/min up to 300 °C, and pressure release of 56 kPa. The injector temperature was 250 ºC; the injection volume was 1 µL; the injection occurred in split mode (20:1). Temperatures of the transfer line, ion source, and quadrupole were 280, 230, and 150 ºC, respectively. The mass spectrometry detection system was utilized in "scan" mode at the mass/charge rate/load (*m/z*) of 40-600 with "solvent delay" of 3 min. The compounds were identified by comparing them to mass spectra found in Wiley 275 libraries and by comparing the retention indices (RI) obtained by a homologous series of *n*-alkane standard (C_7-C_{28}) (Adams, 2017).

2.3. Acaricidal activity

2.3.1. Adult immersion test

The adult immersion test (AIT) was performed according to Drummond, Ernst, Trevino, Gladney and Graham (1973). Engorged female adult ticks (900) from dairy cattle of Northeastern region of Paraná state, Brazil, which had not been exposed to acaricides for 60 days, were utilized. The ticks were washed with ultrapure water and selected according to their healthy appearance, body integrity, and full engorgement (Leite, Labruna, Oliveira, Monteiro & Caetano Junior, 1995).

Baccharis dracunculifolia leaf or flower essential oil was diluted in 2% polysorbate-80 (mass: volume) emulsion at final concentrations of 500.00, 400.00, 300.00, 200.00, 100.00, 50.00, 25.00, 12.50, 6.25, 3.12, 1.56, and 0.78 mg/mL. A 2% polysorbate-80 (mass: volume) emulsion was utilized as negative control and a 1.25 mL/L broad-spectrum commercial ectoparasiticide solution (Colosso®), containing 150.00 mg/mL cypermethrin, 250.00 mg/mL chlorpyrifos, and 10.00 mg/mL citronellal, was utilized as positive control. Groups of 30 engorged female ticks had body mass measured and immersed for 5 min, at 28 ºC, in 10 mL essential oil suspension, synthetic solution, or 2% polysorbate-80 emulsion, and then transferred to Petri dishes (10 ticks per plate) in a chamber at 28 °C with 80% relative humidity for 14 days until oviposition. After 14 days, the egg mass of each female tick was recorded, placed in assay tubes, and kept at 28 °C in a chamber with 80% relative humidity for 21 days until hatching. After 21 days, the larvae were killed by immersion in sulfuric ether and counted in order to obtain the hatching rate. All the tests were performed in triplicate. The estimated reproduction (ER) and the product efficacy (PE) were calculated by the tick mass of engorged adult females, eggs, and egg hatching rate, according to Equations 1 and 2 (Drummond, Ernst, Trevino, Gladney, & Graham, 1973).

ER (%) = egg mass / engorged adult female mass × egg hatching rate × 20000 (Equation 1)

PE (%) = ER negative control / ER treatment group / ER negative control × 100 (Equation 2)

2.3.2. Larval packet test

Engorged adult female ticks without previous treatment with acaricides were kept in a controlled environment to produce larvae. The obtained larvae were placed in a closed paper

filter envelope (2 × 2 cm) impregnated with essential oil, positive or negative control solutions according to the larval packet test (LPT) (Fernandes, Bessa, & Freitas, 2008; Chagas et al., 2012). The essential oil was applied at final concentrations of 50.00, 25.00, 12.50, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19, 0.09, 0.04, and 0.02 mg/mL. The positive and negative controls were the same ones utilized in AIT. The filter paper containing larvae was kept in a Petri dish in a chamber at 28 °C and after 24 h the living larvae were separated from the dead ones (Leite, Labruna, Oliveira, Monteiro & Caetano Junior, 1995). The treatments were carried out in triplicate and the larval mortality was determined according to Equation 3.

Mortality (%) = dead larvae / total larvae × 100 (Equation 3)

All the tests were done in triplicate. The essential oil with lethal concentration to kill 99.9% ($LC_{99.9}$) of larvae was utilized for the *ex situ* test of tick control in vases in a protected environment.

2.3.3. Ex situ test (free-living stage)

Plastic vases (n = 9), 25 cm height and 25 cm diameter, were filled up with 2.2 kg soil, previously autoclaved at 121 ºC for 2 h. Six seeds of *Brachiaria eminii* (Mez) Robyns (synonym *Brachiaria decumbens* Stapf) were sown at 2 cm soil depth in each vase. The plants were kept in a greenhouse for three months with irrigation. The leaves of *B. eminii* were trimmed at 40 cm from the soil surface and an adhesive tape was placed around the edge of each vase as a physical barrier to contain larvae (30 mg) on the soil surface of each vase. After 24 h, the larval migration to the apex of the grass leaves was observed (Araújo et al., 2015). Each treatment consisted of a group of three vases. The obtained $LC_{99.9}$ in LPT (*in vitro* test) for the essential oils and positive control were used for the treated and control group.

A 1.25 mL/L Colosso® commercial solution (150.00 mg/mL cypermethrin, 250.00 mg/mL chlorpyrifos, and 10.00 mg/mL citronellal) was utilized as positive control and 2% polysorbate-80 (mass: volume) emulsion was utilized as negative control in the same LPT and AIT concentrations utilized before. For each treatment, 4 mL of each solution per vase was sprayed starting from the leaf apex until the soil in order to simulate l commercial applications of acaricide in pastures for the herd. After 24 h, the grass leaves were trimmed with the help of an entomological lens and larvae without movement after touching were considered dead. Next, the number of living larvae in the negative group, and living larvae in

the treated group with the essential oils, or with positive control Colosso® were determined. From these data, the efficacy of treatments of essential oils were calculated by the Equation 4 and according to Bittencourt, Bahiense, Fernandes and Souza (2003).

Efficacy of treatment of essential oil (%) = (A - B) / A × 100 (Equation 4)

where A = number of living larvae in the negative control group and B = number of living larvae in the test or control group.

2.4. Statistical analysis

The experiment, a quantitative study (Pereira, Shitsuka, Parreira, & Shitsuka, 2018), had a completely random design. The data were submitted to analysis of variance (ANOVA) and the differences among arithmetic averages with standard deviations were determined by Scott-Knott test at 5% significance. The lethal concentrations that killed 50% (LC_{50}) and 99.9% ($LC_{99.9}$) of adult and larvae ticks with the respective confidence interval (CI; $\alpha = 0.05$) were calculated by probit analysis (ED 50 Plus version 1.0). All the tests were carried out in triplicate.

3. Results

Forty-eight compounds were identified in *B. dracunculifolia* leaf and flower essential oil such as oxygenated sesquiterpenes with 55.1% (leaf) and 50.4% (flower), hydrocarbon sesquiterpenes with 28.6% (leaf) and 29.9% (flower), and hydrocarbon monoterpenes with 10.9% (leaf) and 14.9% (flower) (Table 1). The major compounds of the essential oil were nerolidol with 21.5% (leaf) and 20.6% (flower), and spathulenol with 21.8% (leaf) and 20.3% (flower) with low variation between the amount of each compound for the leaf and the flower essential oil (Table 1 and Figure 1 and 2).

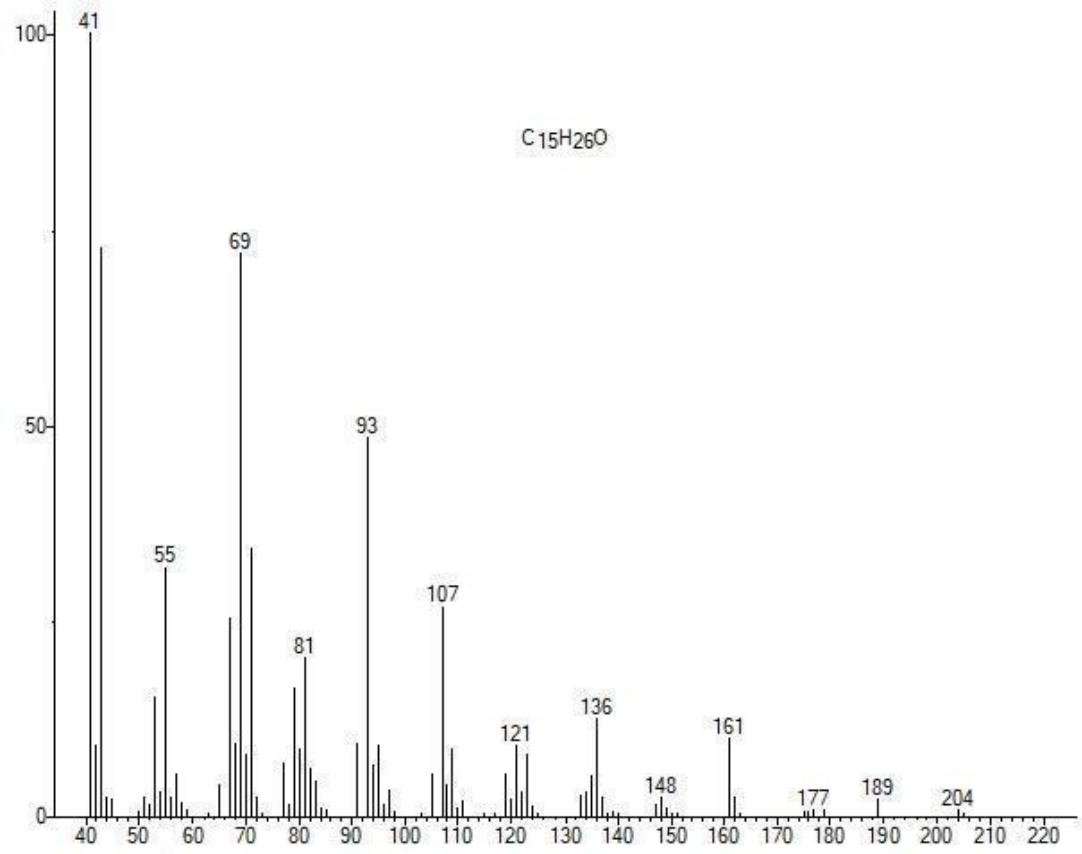
Table 1 - Chemical composition of *Baccharis dracunculifolia* leaf (LEO) and flower (FEO) essential oils.

Peak	[a]Compounds	Relative area %		[b]RI	Identification methods
		LEO	FEO		
	Hydrocarbon monoterpenes	**(10.87)**	**(14.87)**		
1	*α*-thujene	0.07	0.12	952	a, b, c
2	*α*-pinene	1.59	2.04	954	a, b, c
3	Camphene	0.03	0.04	957	a, b, c
4	Thuja-2,4(10)-diene	0.03	0.04	958	a, b, c
5	*β*-pinene	5.20	6.65	964	a, b, c
6	*α*-phellandrene	0.02	0.02	1018	a, b, c
7	*δ*-3-carene	0.02	0.01	1019	a, b, c
8	*α*-terpinene	0.03	0.04	1020	a, b, c
9	*o*-cymene	0.13	0.15	1022	a, b, c
10	Limonene	3.46	5.49	1024	a, b, c
11	*cis*-*β*-ocimene	0.13	0.13	1025	a, b, c
12	Terpinolene	0.16	0.14	1064	a, b, c
	Oxygenated monoterpenes	**(4.59)**	**(3.89)**		
13	Linalool	0.16	0.18	1066	a, b, c
14	Fenchol	0.02	0.02	1118	a, b, c
15	*trans*-*p*-mentha-2,8-dien-1-ol	0.16	0.20	1119	a, b, c
16	*α*-campholenal	0.05	0.03	1121	a, b, c
17	*trans*-pinocarveol	0.87	0.66	1122	a, b, c
18	*cis*-verbenol	0.59	0.49	1126	a, b, c
19	Myrtenol	1.87	1.54	1165	a, b, c
20	Verbenone	0.03	0.06	1216	a, b, c
21	*cis*-carveol	0.40	0.21	1217	a, b, c
22	*cis*-*p*-mentha-1(7),8-dien-2-ol	0.29	0.35	1220	a, b, c
23	Geraniol	0.15	0.15	1260	a, b, c
	Hydrocarbon sesquiterpenes	**(28.70)**	**(30.14)**		
24	*α*-ylangene	0.38	0.46	1354	a, b, c
25	*α*-copaene	0.69	0.64	1358	a, b, c
26	*β*-bourbonene	0.17	0.16	1408	a, b, c
27	*β*-elemene	0.95	0.98	1409	a, b, c
28	*α*-gurjunene	0.49	0.48	1411	a, b, c
29	(Z)-caryophyllene	4.37	5.02	1412	a, b, c
30	*β*-copaene	0.23	0.23	1413	a, b, c
31	*α*-guaiene	0.07	1.25	1414	a, b, c
32	Aromadendrene	3.71	3.54	1452	a, b, c
33	*γ*-muurolene	0.32	0.30	1458	a, b, c
34	Germacrene D	5.68	5.73	1508	a, b, c
35	Viridiflorene	4.48	4.85	1509	a, b, c
36	*α*-muurolene	0.76	0.66	1509	a, b, c
37	*δ*-cadinene	6.40	5.84	1513	a, b, c
	Oxygenated sesquiterpenes	**(55.49)**	**(50.42)**		

38	Nerolidol	21.51	20.59	1557	a, b, c
39	Spathulenol	21.78	20.31	1560	a, b, c
40	*epi*-*α*-cadinol	2.35	1.62	1652	a, b, c
41	*α*-Cadinol	3.23	1.59	1654	a, b, c
42	Cedren-13-ol, 8-	2.39	2.17	1656	a, b, c
43	*cis*-*β*-santalol	1.86	2.50	1706	a, b, c
44	Murolan-3,9(11)-diene-10-peroxy	2.30	1.44	1710	a, b, c
45	Velerdiol	0.07	0.20	1939	a, b, c
	Hydrocarbon diterpene	**(0.10)**	**(0.47)**		
46	Cembrene	0.10	0.47	1940	a, b, c
	Oxygenated diterpene	**(0.10)**	**(0.13)**		
47	Phytol	0.10	0.13	1945	a, b, c
	Linear saturated hydrocarbon	**(0.02)**	**(0.04)**		
48	Heptacosane	0.02	0.04	2701	a, b, c
	Total identified	**99.87**	**99.96**		

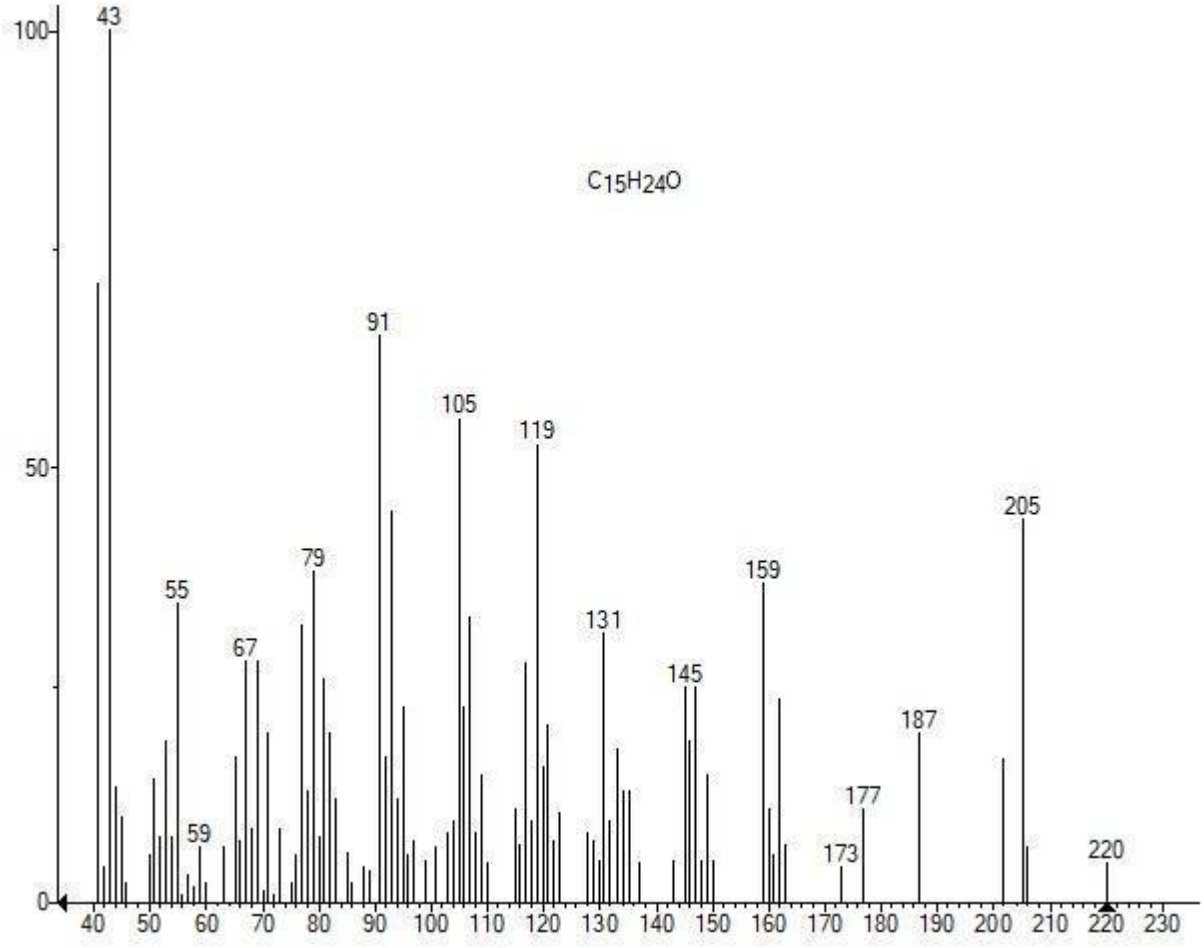
[a]Compounds listed in order of elution in column HP-5MS; [b]**RI** = Identification based on retention index (RI) using a homologous series of *n*-alkane C7-C28 on Agilent HP-5MS column; [c]**MS=** identification based on comparison of mass spectra using Wiley 275 libraries; Relative area (%) = percentage of the area occupied by the compounds in the chromatogram. Source: Authors.

Figure 1 - Mass spectrum of nerolidol (*m/z* = 222.19) from *Baccharis dracunculifolia* leaf (21.5%) and flower (20.6%) essential oils by GC-MS.



Source: Authors.

Figure 2 - Mass spectrum of spathulenol (*m/z* = 220.18) from *Baccharis dracunculifolia* leaf (21.8%) and flower (20.3%) essential oils by GC-MS.



Source: Authors.

Other compounds of *B. dracunculifolia* essential oils with expressive amounts were hydrocarbon monoterpenes such as *β*-pinene with 5.2% (leaf) and 6.6% (flower), and limonene with 3.5% (leaf) and 5.5% (flower); and hydrocarbon sesquiterpenes such as *δ*-cadinene with 6.4% (leaf) and 5.8% (flower), germacrene D with 5.7% (leaf) and 5.7% (flower), (*Z*)-caryophyllene with 4.4% (leaf) and 5.0% (flower), viridiflorene with 4.5% (leaf) and 4.8% (flower), and aromadendrene with 3.7% (leaf) and 3.5% (flower). This suggests that there is little variation in the amount of each compound in the leaf and flower essential oils.

Baccharis dracunculifolia leaf and flower essential oils had ovicidal activity at 500 mg/mL and at 400 mg/mL the leaf essential oil had 65% hatchability reduction (Table 2).

Table 2 - Hatchability and adult female and egg mass of *Rhipicephalus microplus* submitted to different concentrations of *Baccharis dracunculifolia* leaf (LEO) and flower (FEO) essential oils by adult immersion test.

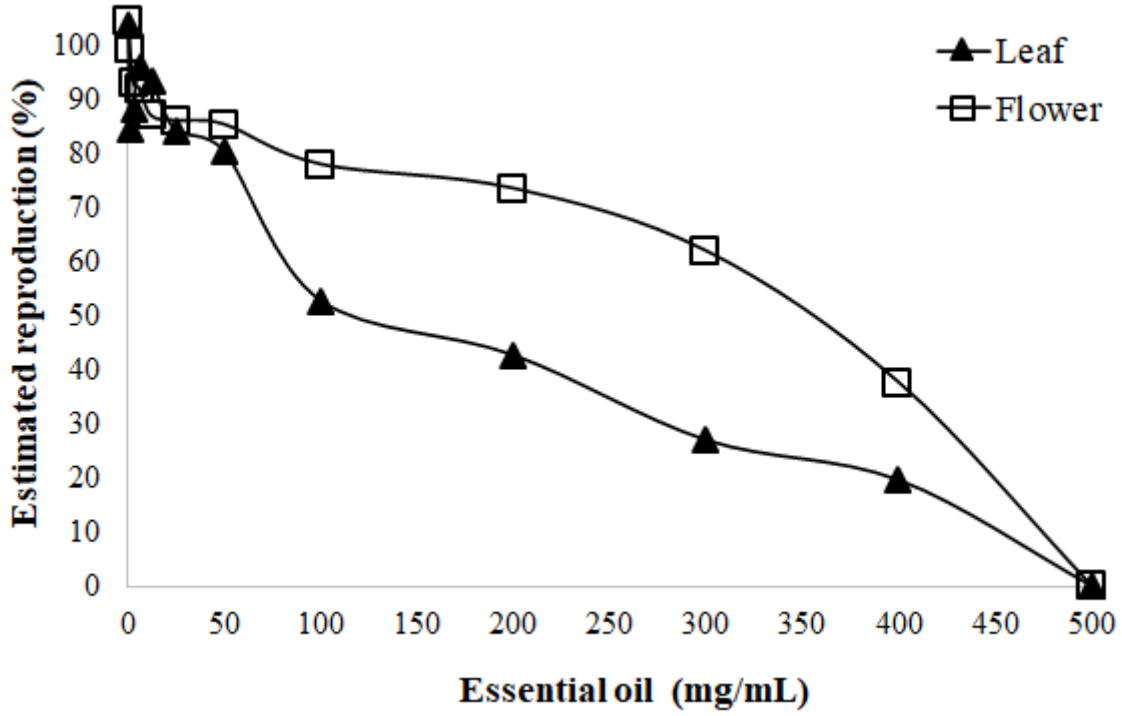
Essential oil (mg/mL)	Adult female (mg)	Egg (mg)	Hatchability (%)
	LEO FEO	LEO FEO	LEO FEO
PC	241.00 ± 7.00D 242.00 ± 7.00b	0.00 ± 0.00A 0.00 ± 0.00a	0.00 ± 0.00A 0.00 ± 0.00a
500	249.00 ± 3.00C 233.00 ± 5.00c	21.00 ± 5.00B 27.00 ± 7.00b	0.00 ± 0.00A 0.00 ± 0.00a
400	234.00 ± 4.00D 236.00 ± 6.00c	66.00 ± 5.00C 78.00 ± 3.00c	35.03 ± 3.55B 57.30 ± 7.23b
300	250.00 ± 3.00C 245.00 ± 8.00b	76.00 ± 5.00D 113.00 ± 4.00d	44.73 ± 4.29C 67.40 ± 2.39c
200	246.00 ± 6.00C 233.00 ± 5.00c	94.00 ± 4.00E 112.00 ± 3.00d	55.96 ± 2.85D 76.86 ± 1.99d
100	249.00 ± 4.00C 242.00 ± 7.00b	96.00 ± 3.00E 110.00 ± 4.00d	68.93 ± 2.63E 85.90 ± 1.41e
50	251.00 ± 4.00C 237.00 ± 4.00c	115.00 ± 3.00F 106.00 ± 3.00d	88.26 ± 1.22F 95.86 ± 0.91f
25	283.00 ± 6.00A 244.00 ± 5.00b	124.00 ± 4.00G 107.00 ± 3.00d	96.83 ± 1.05G 98.36 ± 0.50f
12.5	241.00 ± 5.00D 241.00 ± 5.00b	113.00 ± 5.0F 105.00 ± 3.00d	100.00 ± 0.00G 100.00 ± 0.00f
6.25	262.00 ± 6.00B 230.00 ± 5.00c	126.00 ± 4.00G 106.00 ± 2.00d	100.00 ± 0.00G 100.00 ± 0.00f
3.12	260.00 ± 6.00B 232.00 ± 3.00c	115.00 ± 5.00F 108.00 ± 2.00d	100.00 ± 0.00G 100.00 ± 0.00f
1.56	262.00 ± 6.00B 259.00 ± 5.00a	111.00 ± 5.00F 129.00 ± 8.00e	100.00 ± 0.00G 100.00 ± 0.00f
0.78	264.00 ± 5.00B 262.00 ± 4.00a	137.00 ± 2.00H 137.00 ± 5.00e	100.00 ± 0.00G 100.00 ± 0.00f
NC	234.00 ± 6.00D 234.00 ± 6.00c	108.00 ± 4.00F 107.00 ± 9.00d	100.00 ± 0.00G 100.00 ± 0.00f

Averages ± standard deviation with different uppercase letters (LEO) and lowercase letters (FEO) in the same column differ statistically by Scott-Knott test ($p \leqslant 0.05$). PC = positive control [1.25 mL/L Colosso® commercial solution (cypermethrin 150 mg/mL, chlorpyrifos 250 mg/mL, and citronellal 10 mg/mL)]; NC = negative control (2% polysorbate-80 emulsion). Source: Authors.

In addition, there is a reduction of the tick oviposition from 38 to 80% for leaf essential oil from 400 to 500 mg/mL and reduction from 27 to 74% for flower essential oil from 400 to 500 mg/mL (Table 2). The leaf essential oil from 300 to 400 mg/mL has ER

values of 73 and 81% (Figure 3); for the flower essential oil the ER was of 38 and 63%, respectively (Figure 3). It suggests that the leaf essential oil is more efficient at a lower concentration than the flower essential oil. The leaf essential oil compared to the flower essential oil had slightly higher amounts of each chemical compound such as nerolidol, spathulenol, and *δ*-cadinene that might be responsible for a more efficient activity.

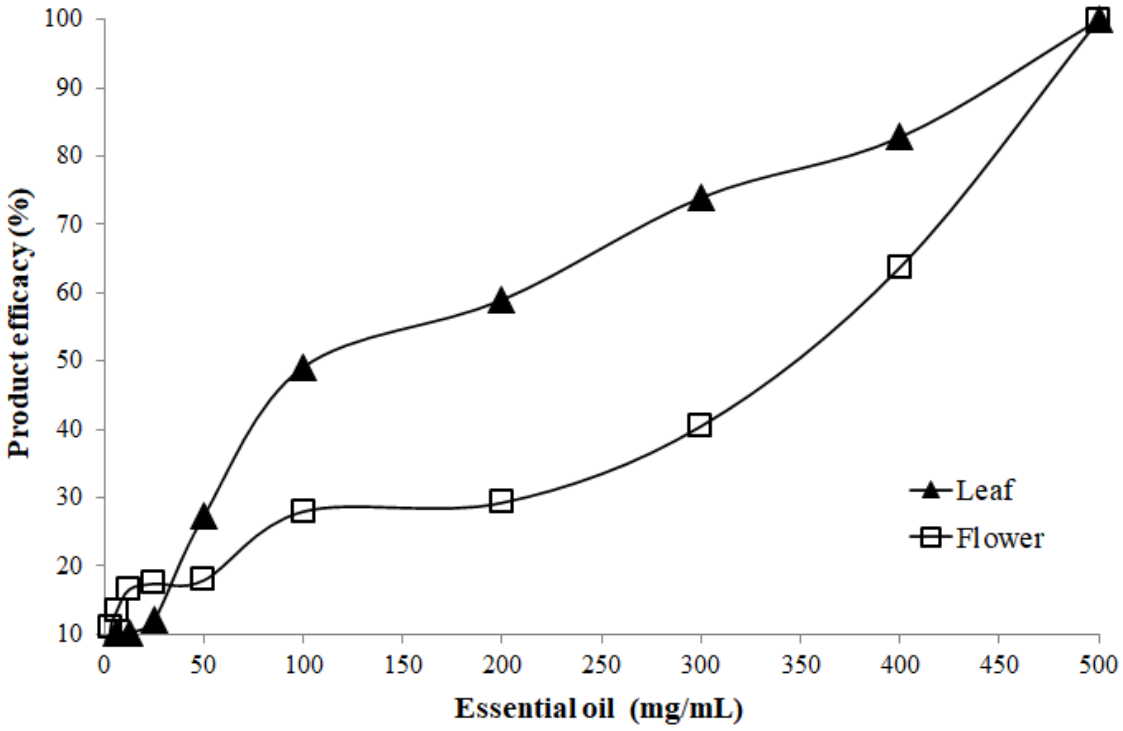
Figure 3 - Estimated reproduction (ER) of *Rhipicephalus microplus* adult females submitted to different concentrations of *Baccharis dracunculifolia* leaf and flower essential oils. Positive control = 1.25 mL/L Colosso® commercial solution (150 mg/mL cypermethrin, 250 mg/mL chlorpyrifos, and 10 mg/mL citronellal); negative control = 2% polysorbate-80 emulsion.



Source: Authors.

The leaf essential oil at 400 mg/mL had PE greater than 80%, but for the flower essential oil the PE was 64% against adult female ticks (Figure 4). The adult female tick mortality was more effective at lower concentrations of leaf essential oil compared to flower essential oil. Thus, *B. dracunculifolia* essential oil should be used at the concentration of 500 mg/mL to be considered an acaricidal product with efficiency over 95% according to the Brazilian Ministry of Agriculture (Brasil, 1997).

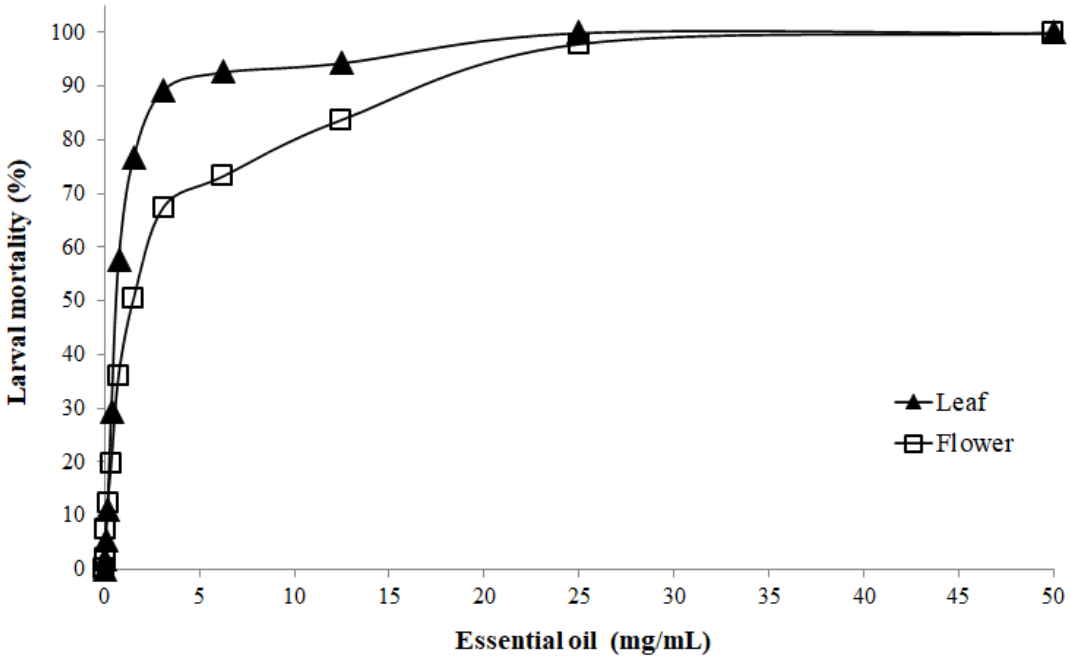
Figure 4 - Product efficacy (%) of *Baccharis dracunculifolia* leaf and flower essential oils at different concentrations against *Rhipicephalus microplus* adult females by adult immersion test (AIT). Positive control = 1.25 mL/L Colosso® commercial solution (150 mg/mL cypermethrin, 250 mg/mL chlorpyrifos, and 10 mg/mL citronellal); negative control = 2% polysorbate-80 emulsion.



Source: Authors.

The larvicidal activity of 95% calculated for *B. dracunculifolia* essential oils (probit analysis) were effective at 13.36 mg/mL of leaf essential oil and 31.06 mg/mL of flower essential oil (Figure 5). The acaricidal activity of essential oils was more effective against larvae and at a lower concentration than against adults.

Figure 5 - Acaricidal activity of *Baccharis dracunculifolia* leaf and flower essential oils at different concentrations against *Rhipicephalus microplus* larval mortality by larval packet test (LPT). Positive control = 1.25 mL/L Colosso® commercial solution (150 mg/mL cypermethrin, 250 mg/mL chlorpyrifos, and 10 mg/mL citronellal); negative control = 2% polysorbate-80 emulsion.



Source: Authors.

For tick larvae, it was determined by probit analysis that the essential oils had LC_{50} ranging from 10.5 to 13.5 mg/mL and $LC_{99.9}$ from 34.6 to 36.6 mg/mL (Table 3). The greatest efficiency at the lowest concentration with larvicidal activity was found for *B. dracunculifolia* leaf essential oil with LC_{50} of 10.5 mg/mL and $LC_{99.9}$ of 34.6 mg/mL (Table 3).

Table 3 - Lethal concentration (LC_{50} and $LC_{99.9}$) of *Baccharis dracunculifolia* leaf (LEO) and flower (FEO) essential oils on *Rhipicephalus microplus* larval mortality by probit analysis.

Essential oil	Larval mortality			
	LC_{50} (mg/mL)	CI	$LC_{99.9}$ (mg/mL)	CI
PC	0.019	0.007–0.030	0.21	0.20–0.22
LEO	10.52	10.43–10.61	34.65	34.52–34.73
FEO	13.48	13.40–13.52	36.60	36.58–36.68

CI = confidence interval. PC = positive control (commercial solution at 1.25 mL/L Colosso® (cypermethrin 150 mg/mL, chlorpyrifos 250 mg/mL, and citronellal 10 mg/mL). Source: Authors.

Baccharis dracunculifolia leaf and flower essential oils had treatment efficacy from 85 to 95% for the *ex situ* test in a protected environment against tick larvae (Table 4). The leaf essential oil was 34.6 mg/mL and the flower essential oil was at 36.6 mg/mL according to probit analysis (Table 3). In the *ex situ* test, the flower essential oil was more effective than the leaf essential oil probably because of a better stability of this essential oil in adverse conditions.

Table 4 - Acaricidal activity of *Baccharis dracunculifolia* leaf (LEO) and flower (FEO) essential oils on *Rhipicephalus microplus* larval mortality by *ex situ* test.

Treatment	Number of living larvae	Efficacy of treatment (%)
PC	0.0 ± 0.0^{A}	100.0^{A}
LEO at 34.65 mg/mL	88.7 ± 6.6^{C}	85.3^{C}
FEO at 36.60 mg/mL	29.3 ± 3.0^{B}	95.1^{B}
NC	602.3 ± 6.6^{D}	0.00^{D}

Average ± standard deviation with different letters in the same column differ statistically by the Scott-Knott test ($p \leqslant 0.05$). PC = positive control (1.25 mL/L Colosso® commercial solution (cypermethrin 150 mg/mL, chlorpyrifos 250 mg/mL, and citronellal 10 mg/mL); NC = negative control (2% polysorbate-80 emulsion). Source: Authors.

4. Discussion

The major compounds of *B. dracunculifolia* leaf and flower essential oils in our study were mainly nerolidol and spathulenol, corroborating the literature on this plant (Massignani et al., 2009; Queiroga et al., 2014). In addition, Lage et al. (2015) reported that the essential oil from *B. dracunculifolia* fresh aerial parts (leaves) from Viçosa, Brazil, had nerolidol

(22.3%), germacrene D (7.2%), limonene (6.9%), *β*-pinene (6.7%), bicyclogermacrene (6.5%), and spathulenol (5.3%) as major compounds; Parreira et al. (2010) reported as major compounds from France (*E*)-nerolidol (33.5%), spathulenol (16.2%), *α*-muurolol (4.7%), *δ*-cadinene (3.7%), bicyclogermacrene (3.4%), *β*-caryophyllene (2.3%), and germacrene D (2.2%); Chaaban et al. (2017) reported major compounds from Canelinha, Brazil, *β*-pinene (9.9%), D-limonene (9.6%), (*E*)-nerolidol (7.9%), caryophyllene (7.7%), spathulenol (6.7), *α*-muurolol (6.7%), and α-pinene (5.3%). These differences in the chemical composition of the essential oil for this species may be related to genetic, biotic and/or abiotic factors (Morais, 2009), because they are from very distinct regions.

The presence of sesquiterpenes nerolidol and spathulenol in *B. dracunculifolia* essential oils indicates that they are partially responsible for the biocidal activity. Spathulenol (sesquiterpene alcohol) has been used for insect control (Enan, 2013; Kennedy & Schmidt, 2017) and nerolidol (sesquiterpene alcohol) has been reported with acaricidal activity against *R. microplus* larvae and engorged females with 100% larval mortality at 15 mg/mL and reduction of egg mass and hatchability at 30 to 50 mg/mL (Lage et al., 2015). Moreover, nerolidol inhibited *in vitro* protozoa such as *B. bovis*, *B. bigemina*, *Babesia ovata*, and *Babesia caballi* (Aboulaila, Sivakumar, Yokoyama, & Igarashi, 2010), insects such as *Pediculus humanus capitis* De Geer (Di Campli et al., 2012), and mites such as *Tetranychus urticae* Koch (Araújo, Câmara, Born, Moraes, & Badji, 2012). Nerolidol can inhibit acetylcholinesterase enzyme (BxACE-1, BxACE-2 and BxACE-3) (Kang, Kim, Lee, & Park, 2013), one of the mechanisms involved in the insecticidal activity. It also presented antimalarial (Lopes et al. 1999), antileishmanial (Camargos et al., 2014), and anti-*Schistosoma mansoni* activities (Silva et al., 2014).

Baccharis dracunculifolia leaf and flower essential oils reduced the reproductive efficiency of *R. microplus* engorged females in our study which is significant due to the possible occurrence of three to four generations of ticks per year. Considering that a female can produce approximately 3,000 new ticks per cycle and that half of them will be females (Furlong, 1998), the reduction of the female reproductive efficiency is relevant to control the tick population. In addition, if there is an association with the egg hatching rate (non-parasitic phase) with reduction of the tick population (parasitic phase), there will be a smaller tick infestation in the subsequent generations in pastures as well as in animals (Martins, Evans, Ceresér, & Corrêa, 2002; Furlong, & Prata, 2005).

Lage et al. (2015) observed that the essential oil from *B. dracunculifolia* aerial parts (leaves) showed intense activity on different development phases of *R. microplus* and that the

tick mortality rate was dosage-dependent. The acaricidal activity by AIT showed 7.8% hatchability and reduction in the amount and quality of produced eggs at 60 mg/mL essential oil. The modified acaricidal activity by LPT showed 99.6% larval mortality of the essential oil at 15 mg/mL. These results are in accordance to the ones found in our study because the highest activity of the leaf and flower essential oils occurred against bovine tick larvae. The susceptibility of adult bovine ticks seems to be substantially lower compared to larval bovine ticks submitted to essential oils (Castro et al., 2018). Bovine larvae are more vulnerable than adult females because their cuticle is thinner, allowing the active compounds to penetrate in the tick (El Amri et al., 2014). Moreover, the cuticle of engorged female ticks can increase from 32 to 43% in the feeding phase (Flynn and Kaufman, 2011); thus, the thinner cuticle in larvae could explain the higher acaricidal activity against ticks in the larval phase than in the adult phase (Chagas, Leite, Furlong, Prates, & Passos, 2003).

Baccharis dracunculifolia leaf essential oil in our study was more active to inhibit egg hatchability of ticks probably due to the difference in the chemical composition of the essential oils. The oxygenated monoterpenes and sesquiterpenes were found at greater concentrations in the leaf essential oil (4.6% and 55.1%, respectively) than in the flower essential oil (3.9% and 50.4%, respectively). Oxygenated sesquiterpenes have shown greater acaricidal potential than hydrocarbon sesquiterpenes because the presence of oxygenated functional groups in the molecule and the capacity to form hydrogen bindings can potentialize the biological activity of these compounds (Eldoksch, Ayad, & El-Sebae, 2009; Amaral et al., 2017). Gross, Temeyer, Day and Léon (2017) reported that oxygenated terpenes acted as agonists (pulegone) or modulators (piperonyl alcohol), (1,4-cineole, carvacrol and isoeugenol) of *R. microplus* tyramine receptors, causing anatomical alterations in the digestive tract and in tick mortality.

The emergence of resistant mite populations to acaricides has been increasing in all regions where the parasite finds favorable conditions to its development (Klafke et al., 2017; Reginato, Cadore, Menezes, Sangioni, & Vogel, 2017). In the field, after a maturation period on pasture, larvae move to leaf extremities to increase the chances to reach the livestock and complete its development cycle (Gonzales, 1974). This phase of the tick reproductive cycle corresponds to the free-living stage of larvae when 95% of the larvae are distribute in pasture and only 5% are parasites of the animal (Powell & Reid, 1982). In our study, the spraying of *B. dracunculifolia* leaf and flower essential oils killed 85.3% and 95.1% of *R. microplus* larvae on *B. eminii* leaves, simulating the conditions of free-living stage of the parasite in the larval phase. Moreover, when exposed to open environment conditions, the essential oils can

volatize or oxidize, reducing the efficiency reported in *in vitro* tests to control ticks (Borges, Souza, & Barbosa, 2011). Therefore, the results in our study suggest that the flower essential oil instead of the leaf one is more stable under adverse conditions of the *ex situ* test and, thus, has greater utilization potential.

5. Final Considerations

Baccharis dracunculifolia leaf and flower essential oils have potential to control *R. microplus* in two important life cycles of ticks, reducing their egg hatchability and killing larvae. The stability of the essential oils from this plant makes them an alternative to synthetic chemical products against bovine ticks, and also for further studies on the effect on non-target organisms and residual effect on the environment.

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Disclosure statement

There is no conflict of interest for this research work.

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