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Control of bovine tick (*Rhipicephalus microplus*) with essential oil from *Psidium rufum* DC leaves

Controle de carrapato bovino (*Rhipicephalus microplus*) com óleo essencial de folhas de *Psidium rufum* DC

Control de garrapata del ganado bovino (*Rhipicephalus microplus*) con aceite esencial de hojas de *Psidium rufum* DC

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Luiz Antônio Branco
ORCID: https://orcid.org/0000-0003-4757-7758
Paranaense University, Brazil
E-mail: luiz.branco@edu.unipar.br
Herika Line Marko de Oliveira
ORCID: https://orcid.org/0000-0003-3446-9408
Paranaense University, Brazil
E-mail: herika.marko@edu.unipar.br
Wanessa de Campos Bortolucci
ORCID: https://orcid.org/0000-0002-7233-8313
Paranaense University, Brazil
E-mail: wanessa.bortolucci@edu.unipar.br
Carla Maria Mariano Fernandez
ORCID: https://orcid.org/0000-0001-7324-5533
Paranaense University, Brazil
E-mail: carlamfernandez@prof.unipar.br
José Eduardo Gonçalves
ORCID: https://orcid.org/0000-0003-2035-4167
Cesumar Institute of Science, Technology and Innovation, Brazil
E-mail: jose.goncalves@unicesumar.edu.br
Zilda Cristiani Gazim
ORCID: https://orcid.org/0000-0003-0392-5976
Paranaense University, Brazil
E-mail: cristianigazim@prof.unipar.br

Daniela Dib Gonçalves
ORCID: https://orcid.org/0000-0001-8322-8905
Paranaense University, Brazil
E-mail: danieladib@prof.unipar.br
Emerson Luiz Botelho Lourenço
ORCID: https://orcid.org/0000-0002-1798-7871
Paranaense University, Brazil
E-mail: emerson@prof.unipar.br
Jessé Lahos Borges
ORCID: https://orcid.org/0000-0003-2886-0996
Paranaense University, Brazil
E-mail: lahos@outlook.com
Vanderson dos Santos Silva
ORCID: https://orcid.org/0000-0002-5925-3585
Paranaense University, Brazil
E-mail: vanderson.silva.80@edu.unipar.br
Ranulfo Piau Junior
ORCID: https://orcid.org/0000-0003-4765-6544
Paranaense University, Brazil
E-mail: piau@prof.unipar.br

Abstract

Objetive: The present study was to evaluate the acaricidal and larvicidal activity of the essential oil from *Psidium rufum* leaves against engorged *Rhipicephalus microplus* females and larvae. Methodology: Essential oil from fresh *P. rufum* leaves was extracted by hydrodistillation (2h) and analyzed by gas chromatography coupled to mass spectrometry (GC-MS). The acaricidal and larvicidal activities were performed using the adult immersion and larval immersion (Larval Paket Test) techniques using the essential oil at concentrations ranging from 300 to 0.001 mg/mL . As positive control was utilized as a commercial solution (15% cypermethrin; 25% chlorpyrifos; 1% citronellal) at 0.005%, and as negative control, an aqueous solution of 2.0% polysorbate (80) was used. Lethal concentrations (LC_{50} and LC_{99}) were determined by Probit analysis. Results: In the essential oil, 55 compounds were identified, with hydrocarbon sesquiterpenes being the majority class (70.07%), with majority being 1.8-cineole (19.00%); followed by α-longipinene (18.62%). The results showed that the

essential oil of P. rufum at 6.20 mg / mL was effective in controlling 73% of the hatchability of eggs, reducing oviposition and the number of adult ticks and larvae. The CL_{99} for larvae was 2.50 mg / mL, and for engorged females CL_{99} was 513.57 mg / mL. Conclusion: essential oil from *P. rufum* leaves demonstrated potential to control *R. microplus* in the life cycle that corresponds to the larval stage, indicating that it is a promising plant for the development and application of products against bovine ticks.

Keywords: 1,8-cineole; α-longipinene; *Araçá-roxo*; Larvicide; *Rhipicephalus microplus*.

Resumo

Objetivo: avaliar a atividade acaricida e larvicida do óleo essencial das folhas de *Psidium rufum* frente a fêmeas ingurgitadas e larvas de *Rhipicephalus microplus.* Metodologia: o óleo essencial das folhas frescas de *P. rufum* foi extraído por hidrodestilação (2h) e analisado por cromatografia em fase gasosa acoplada à espectrometria de massas (CG-EM). As atividades acaricida e larvicida foram realizadas pela técnica de imersão de adultos e imersão larval (Larval Paket Test) utilizando o óleo essencial nas concentrações de 300 a 0,001 mg/mL. Como controle positivo foi utilizado uma solução comercial (cipermetrina 15%; clorpirifós 25%; citronelal 1%) a 0,005%, e como controle negativo uma solução aquosa de polissorbato (80) a 2,0%. As concentrações letais (CL_{50} e CL_{99}) foram determinadas pela análise de Probitos. Resultados: no óleo essencial, foram identificados 55 compostos, sendo a classe majoritária os sesquiterpenos hidrocarbonetos (70,07%), e como majoritários o 1,8-cineole (19,00%); seguido do *α*-longipinene (18,62%). Os resultados evidenciaram que o óleo essencial de *P. rufum* a 6.20 mg/mL foi efetivo para controlar 73% da eclodibilidade de ovos reduzindo a oviposição e o número de carrapatos adultos e larvas. A CL_{99} para as larvas foi 2,50 mg/mL, e para as fêmeas ingurgitadas CL_{99} de 513,57 mg/mL. Conclusão: o óleo essenciaL das folhas de *P. rufum* demonstrou potencial para controlar *R. microplus* no ciclo de vida que corresponde a fase larval, indicando tratar-se de uma planta promissora para o desenvolvimento e aplicação de produtos contra carrapatos bovinos.

Palavras-chave: 1,8-cineole; *α*-longipinene; Araçá-roxo; Larvicida; *Rhipicephalus microplus*.

Resumen

Objetivo: evaluar la actividad acaricida y larvicida del aceite esencial de las hojas de *Psidium rufum* ante hembras ingurgitadas y larvas de *Rhipicephalus microplus.* Metodología: el aceite esencial de las hojas frescas de *P. rufum* fue extraído por hidrodestilación (2h) y analizado por

cromatografía gaseosa acoplada a la espectrometría de masa (CG-EM). Las actividades acaricida y larvicida fueron realizadas por la técnica de inmersión de adultos y inmersión larval (Larval packet Test) utilizando el aceite esencial en las concentraciones de 300 a 0,001 mg/mL. Como control positivo fue utilizado una solución comercial (cipermetrina 15%; clorpirifós 25%; citronelal 1%) a 0,005%, y el control negativo una solución acuosa de polisorbato (80) a 2,0%. Las concentraciones letales (CL_{50} y CL_{99}) fueron determinadas por el análisis de Probitos. Resultados: en el aceite esencial, fueron identificados 55 compuestos, siendo el tipo mayoritario los sesquiterpenos hidrocarbonetos (70,07%), teniendo como mayoritario el 1,8-cineole (19,00%); seguido del *α*-longipinene (18,62%). Los resultados encontrados mostraron que el aceite esencial de *P. rufum* a 6.20 mg/mL fue efectivo para controlar 73% de la incubabilidad de huevos con reducción de la capacidad de oviposición y del número de garrapatas adultas y larvas. La actividad larvicida del aceite esencial tuvo CL_{99} de 2,50 mg/mL, comparado con las hembras ingurgitadas CL_{99} de 513,57 mg/mL.Conclusión: el aceite esencial de las hojas de *P. rufum* demostró un potencial para controlar *R. microplus* en el ciclo de vida que corresponde a la etapa larval, mostrando que se trata de una planta promisora para el desarrollo y aplicación de productos contra garrapatas bovinas.

Palabras clave: 1,8-cineole; *α*-longipinene; Guayava cimarrón; Larvicida; *Rhipicephalus microplus*.

1. Introduction

Livestock is of fundamental importance to the Brazilian economy, which stands out as the main world's beef producer, reaching more than 70 million tons produced in 2018. With around 209 million head of cattle, the activity today represents a considerable part of the country's Gross domestic product (Gomes et al., 2017).

However, the tropical climate where cattle herds are found has provided favorable conditions for the occurrence of bovine tick infestations, causing the emergence of parasitic diseases, which have become endemic, requiring special attention and health management (Andreotti et al., 2019; Bispo et al., 2020). *Rhipicephalus microplus* Canestrini (Acari: Ixodidae) is an ectoparasite with 21-day parasitic life cycle in live host and its free-living stage is up to 300 days in pastures without live host (Medeiros et al., 2019). This tick is responsible for the transmission of hemotozoas such as *Babesia bovis*, *Babesia bigemina*, and *Anaplasma marginata*, which cause diseases and may lead to animal death (Bortolucci et al., 2018; Cazella et al., 2020). The tick can cause anemia, mass reduction, milk production

reduction, birth rate reduction, increase in mortality rates, labor and material costs, mainly for the treatment of bovine parasite sadness, in addition to reducing bovine leather due to the animal's inflammatory response to the ectoparasite (Sugauara et al., 2019; Pires Filho et al., 2020).

According to Andreotti et al. (2019), losses caused by bovine ticks are estimated at R$ 9 billion per year. This value tends to increase significantly due to the increasing resistance of ticks against the active ingredients used and also due to global warming, which is causing the appearance of the tick in previously unaffected regions, such as part of the southern region of Brazil and increase in infestations in other regions.

Another factor that causes damage is the high expenses in an attempt to control this ectoparasite, for example, costs with the use of acaricides and expenses related to their application. There is also the appearance of tick populations resistant to tick medications, which is mainly triggered by the incorrect or disordered use of acaricides. The emergence of resistant tick populations is one of the major obstacles in cattle production, as resistance reports cover a wide range of commercially used acaricides (Andreotti et al., 2019).

The integration of control methods and the conscious use of available products can improve the quality of food produced, contributing to public health safety. The technological intensification of the livestock production system requires, among other aspects, innovations with regard to animal nutrition and health control, respecting the soil-plant-animal integration (Andreotti et al. (2019). The adoption of technologies less harmful to the animal and human environment in the Brazilian livestock activity has been the great challenge of the sector. In this context, bioinsecticides from secondary plant metabolites are an alternative to control bovine tick, mostly due to the rising demand for environmentally friendly and economically feasible products. Essential oils from plants have been a promising alternative for pest control (Fernandez et al., 2018).

Brazil has a great diversity of plants and *Psidium rufum* is a fruit tree belonging to the Myrtaceae family. It is a species native to Brazil found in the Cerrado and Atlantic forest biomes, popularly known as *araçá-cagão*, *araçá-perinha* and *araçá-roxo* (Sobral et al., 2014). The species is a small tree, with four to five meters in height, globose and dense canopy and tortuous trunk, reaching up to 30 cm in diameter at chest height. Its flowers are white, axillary, solitary, and bloom exuberantly from August to September and fruits ripen from May to June. It has edible but laxative fruits, which are consumed by birds (Lorenzi, 2009).

There are no studies highlighting the biological potential of *P. rufum* leaves, but based on species belonging to the Myrtaceae family, they are rich in essential oils found in their leaves and flowers (Cerqueira et al., 2009; Tedesco et al., 2020). However, in preliminary tests in our laboratory, essential oil from *P. rufum* leaves showed acaricidal and larvicidal activity against *Rhipicephalus microplus* (unpublished data).

Thus, this study aimed to identify the chemical compounds present in *P. rufum* leaves by gas chromatography coupled to mass spectrometry (GC-MS) and to evaluate the acaricidal and larvicidal activity against *Rhipicephalus microplus*.

2. Material and methods

2.1 Plant material

Araçá-roxo culture is located in northwestern state of Paraná at coordinates (Latitude 23º 43 ’37’ ’S; Longitude 53º 29’ 58’’ W, and 418 m a.s.l.). Leaves were collected in the month of April in the morning between 07:00 and 09:00 am, the time when *araçá-roxo* had only leaves. Identified as *Psidium rufum* DC, the material was deposited on the Herbarium of the Medicinal Plant Collection at Campus 2 of UNIPAR, under registration number 325. This species is registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen, acronym in Portuguese) under registration number A3440B2.

2.2 Essential oil extraction and GC-MS analysis

For essential oil extraction, leaves were dried at room temperature and submitted to the hydrodistillation process for 2 h in modified Clevenger apparatus. Essential oil was extracted from the apparatus with n-hexane, filtered in anhydrous sodium sulfate (Na_2SO_4), stored in amber flask and kept at -4 ºC until complete solvent evaporation. Essential oil yield was determined through the ratio between mass of dry leaves and fresh flower bud (g) and essential oil mass (g) (%).

Essential oil chemical identification was carried out by gas chromatographer (Agilent 7890B) coupled to mass spectrometer (Agilent 5977A MSD) (GC-MS). The capillary column was HP5-MS UI 5% (30 m × 250µm × 0.25µm; Agilent Technologies) with initial oven temperature from 60 °C to 280 °C (3 °C/min and maintained for 1 min). Helium was used as

carrier gas at linear speed of 1 mL/min up to 300 °C, and pressure release of 8.23 psi. The injector temperature was 220 ºC; the injection volume was 1 µL; injection occurred in split mode (20: 1) with injector temperature kept at 220 °C. Temperatures of the transfer line, ion source, and quadrupole were 260, 230 and 150 ºC, respectively. Mass spectrometry detection system was used in "scan" mode, in the mass/charge range from 40 to 500 *m/z* with 3-min solvent delay. Compounds were identified by comparing mass spectra found in NIST 11.0 libraries with retention indices (RI) obtained by a homologous series of n-alkane standard (C_7-C_{28}) (Adams, 2017).

2.3 Acaricidal activity of essential oil by adult immersion test

For our study, 340 fully engorged *R. microplus* females were collected from dairy cattle of Paranaense University (UNIPAR), Umuarama, northeastern region of the state of Paraná, Brazil, which has not been exposed to acaricide application for 60 days. Ticks were washed with ultrapure water and selected according to their healthy appearance, whole body, and full engorgement (Bortolucci et al., 2020).

For the adult immersion test (AIT) (Stone & Haydock, 1962, Drummond et al., 1971, Drummond et al., 1973), 30 fully engorged females were used, divided into 10 ticks per plate, for each essential oil concentration (400; 300; 200; 100; 50; 25; 12.50; 6.25; 3.12; 1.56; 0.70; 0.39 mg/mL). A 2% polysorbate-80 (w/v) emulsion was used as negative control and a 1.25 mL/L broad-spectrum commercial ectoparasiticide solution (Colosso®), containing 15% cypermethrin, 25% chlorpyrifos, and 1% citronellal was used as positive control (Camilotti et al., 2015; Raimundo et al., 2017).

Initially, ticks were weighed and immersed for 5 min in essential oil, positive and negative control solutions. After the immersion time had elapsed, fully engorged females were transferred to Petri dishes (10 ticks per plate), kept at room temperature and controlled humidity. After 14 days, the mass of laid eggs was weighed and stored in assay tubes to observe the beginning of hatching. After 21 days, hatching occurred and larvae were killed by immersion in sulfuric ether; then, the egg-hatching rate was evaluated by measuring the number of eggs and larvae present in each sample, (eggs + larvae = 100%), thus finding the hatchability percentage at each concentration. All tests were performed in triplicate. Estimated reproduction (ER) (Equation 1) and efficacy of the product (EP) (Equation 2) were calculated according to Drummond et al. (1971), using the fully engorged female mass (g), egg mass (g),

egg hatching rate (%), assuming that each 1 g of egg contains about 20,000 larvae (Drummond et al., 1971, Drummond et al., 1973).

(ER) = (egg mass weight / female weight) * hatching rate * 20000 (Equation 1)

(EP) = [(ER negative control / ER treatment group) / ER negative control] * 100 (Equation 2)

Lethal concentrations that killed 50% (LC_{50}) and 99.9% ($LC_{99.9}$) of the population of ingurgitated adult female ticks with respective confidence intervals (CI) were calculated by Probit analysis (ED 50 Plus version 1.0). All tests were performed in triplicate. According to the Brazilian legislation, acaricides must have efficiency above 95% in order to be marketed in the country (Mapa, 1997).

2.4 Larvicidal activity of essential oil by larval packet test

For the larval packet test (LPT), fully engorged *R. microplus* females without previous treatment with acaricides were kept in controlled environment to produce larvae. Approximately 100 larvae were placed in closed paper filter envelope (2 x 2 cm) impregnated with different essential oil concentrations (300; 200; 100; 50; 25; 12.50; 6.25; 3.12; 1.56; 0.78; 0.39; 0.19; 0.09; 0.04; 0.02; 0.01; 0.006; 0.003; 0.001 mg/mL). Positive and negative controls were the same as those used in the AIT (Fernandes et al., 2008; Chagas et al. 2012). The filter paper containing larvae was kept in Petri dish at room temperature (28 °C) and after 24 h, live larvae were separated from dead ones with the help of entomologic lens (Leite et al., 1995). Treatments were carried out in triplicate and larval mortality was determined according to Equation 3.

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

2.5 Statistical analysis

The experimente, a quantitative study (Pereira, Shitsuka, Parreira, & Shitsuka, 2018), had a completely random design. Data were submitted to analysis of variance (ANOVA) and differences among arithmetical averages were determined by Tukey's test at 5% significance (IBM SPSS Statistics 20). Lethal concentration (LC) that killed 50% (LC_{50}) and 99.9%

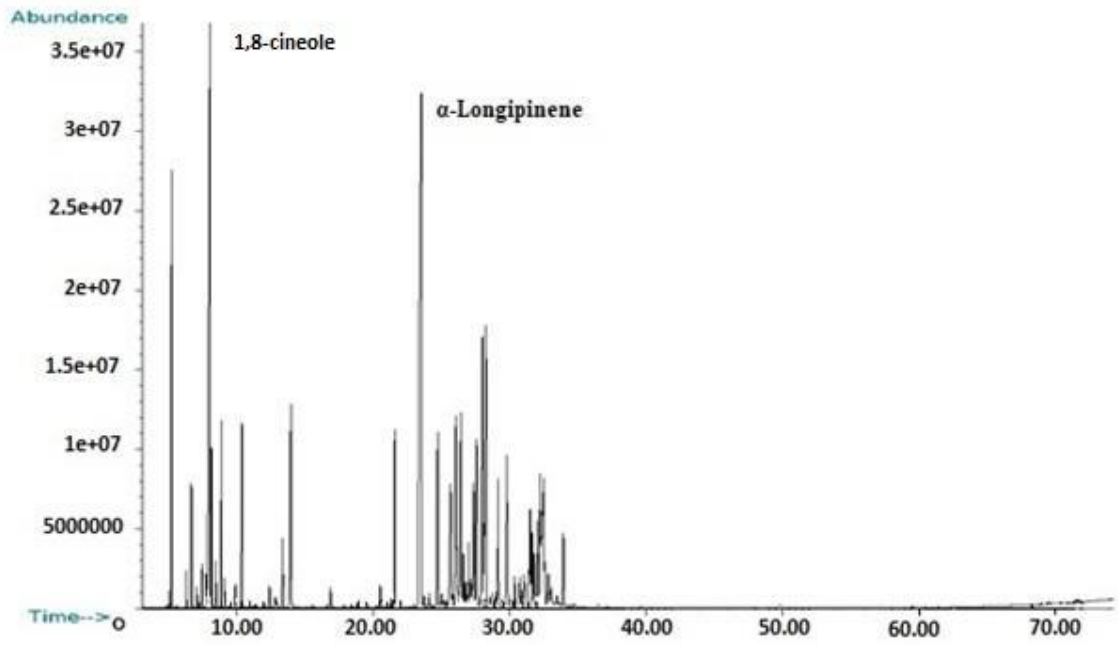
($LC_{99.9}$) of adult ticks and larvae and the respective confidence interval (CI) ($\alpha = 0.05$) were calculated by Probit analysis (ED 50 Plus version 1.0). All the tests were carried out in triplicate.

3. Results and Discussion

By the hydrodistillation technique, essential oil was obtained from fresh *P. rufum* leaves, with yield of 0.70%. This result can be considered high when compared to essential oil from *Psidium myrsinitis* leaves (0.13%) obtained in studies by Castelo et al. (2010), and yields of 0.14% and 0.62% of essential oils from *Psidium guajava* and *Psidium widgrenianum* leaves, respectively, reported in studies by Ramos et al. (2006).

Psidium rufum essential oil was analyzed by GC-MS, and the chromatogram is shown in Figure 1. From these results, it was possible to identify compounds and define the chemical composition of essential oil described in Table 1.

Figure 1 - Chromatogram of the essential oil from *Psidium rufum* leaves obtained by gas chromatography coupled to mass spectrometer.



Source: Authors.

Table 1 - Chemical composition of the essential oil from *Psidium rufum* leaves.

Peak	[a]Compounds	[b]IR_{calc}	Relative area (%)	MI
	Hydrocarbon monoterpenes			
1	Sabinene	967	0.17	a, b, c
2	*β*-pinene	980	0.61	a, b, c
3	*δ*-3-carene	998	t	a, b, c
4	Limonene	1004	t	a, b, c
5	*β*-phellandrene	1008	1.35	a, b, c
	Oxygenated monoterpenes			
6	1,8-cineole	1018	19.00	a, b, c
7	*Cis*-sabinene hydrate	1029	0.70	a, b, c
8	Linalol	1032	0.16	a, b, c
9	*Trans*-sabinene hydrate	1044	0.90	a, b, c
10	Camphene hydrate	1050	0.33	a, b, c
11	Terpinen-4-ol	1074	0.10	a, b, c
12	*α*-terpineol	1086	1.87	a, b, c
13	Fragranol	1146	0.03	a, b, c
14	*Trans*-geraniol	1160	0.94	a, b, c
15	Geraniol	1176	3.83	a, b, c
	Hydrocarbon sesquiterpenes			
16	*γ*-elemene	1247	0.34	a, b, c
17	*α*-cubebene	1436	1.75	a, b, c
18	*α*-longipinene	1500	18.62	a, b, c
19	*α*-ylangene	1505	0.18	a, b, c
20	*α*-copaene	1515	0.14	a, b, c
21	Isoledene	1531	1.38	a, b, c
22	*β*-patchouli	1539	0.29	a, b, c
23	*β*-elemene	1557	2.52	a, b, c
24	Longifolene	1575	4.12	a, b, c
25	*Trans*-caryophyllene	1579	3.29	a, b, c
26	*α*-gurjunene	1583	0.56	a, b, c
27	*β*-cedrene	1589	0.25	a, b, c
28	*α*-guaiene	1592	0.51	a, b, c
29	Aromadendrene	1597	0.34	a, b, c
30	*α*-humulene	1664	1.47	a, b, c
31	*α*-patchouli	1710	2.49	a, b, c
32	*γ*-curcumene	1713	3.71	a, b, c
33	*β*-selinene	1717	0.46	a, b, c
34	*α*-amorphene	1725	3.39	a, b, c
35	*γ*-selinene	1730	0.23	a, b, c
36	Zingiberene	1743	0.32	a, b, c
37	Valencene	1743	1.42	a, b, c
38	*α*-selinene	1773	5.00	a, b, c
39	Bicyclogermacrene	1783	0.13	a, b, c
40	*α*-murolene	1787	0.23	a, b, c

41	Epizonarene	1791	0.27	a, b, c
42	*β*-bisabolene	1800	0.59	a, b, c
43	*Trans*-*α*-bisabolene	1885	0.75	a, b, c
44	*γ*-cadinene	1903	1.67	a, b, c
45	*δ*-cadinene	1908	0.86	a, b, c
46	*Cis*-calamenene	1915	1.05	a, b, c
47	*Cis*-*α*-bisabolene	1921	1.97	a, b, c
48	*α*-cadinene	1926	2.62	a, b, c
49	Germacrene B	1928	2.55	a, b, c
50	Longipinene	1931	1.57	a, b, c
51	*Allo*-aromadendrene	1938	0.77	a, b, c
52	Calarene	1944	0.53	a, b, c
53	Eremophilene	1944	0.46	a, b, c
54	Aristolene	1947	1.29	a, b, c
55	Ledene	1952	t	a, b, c
	Total identified		99.99	
	Hydrocarbon monoterpenes		2.04	
	Oxygenated monoterpenes		27.86	
	Hydrocarbon sesquiterpenes		70.09	

MI = identification method (a, b, c); [a]Compound listed in order of elution from HP-5 column; [b]IR: Retention index calculated using n-alkane C_7 – C_{30} in HP-5 column; [c]IR: Identification based on comparison of mass spectra using NIST 11.0 library; Relative area (%): percentage of area occupied by compounds in the chromatogram; t= trace. Source: Authors.

In *P. rufum* essential oil, 55 compounds were identified (Table 1), with hydrocarbon sesquiterpenes being the predominant class (70.07%) followed by oxygenated monoterpene (29.15%), with 1.8-cineole (19.00%) and α-longipinene (18.62%) (Figure 1) as major compounds.

This is the first report in literature on the chemical composition of the essential oil from *P. rufum* leaves. Essential oil presented hydrocarbon sesquiterpenes as a major class, as well as other *Psidium* species. In studies by Ramos et al. (2006), essential oils from *Psidium guajava* and *Psidium widgrenianum* leaves (Myrtaceae) presented hydrocarbon sesquiterpenes (62%; 33%, respectively) as the predominant class, and the major compounds were α-humulene (15%), caryophyllene (12%) and α-selinene (10%) for *P. guajava* essential oil, and caryophyllene (21%), α-pinene (14%), 1,8-cineole (8.4%) and eudesmol isomers (16.7%) for *P. widgrenianum* essential oil.

In relation to major compounds, *P. rufum* essential oil showed different chemical composition with 1,8-cineole (19.00%) and α-longipinene (18.62%). However, the chemical composition of essential oils varies considerably depending on the species, climatic parameters, agronomic factors, development phase, as well as extraction process, physiology

and plant genetics (Gobbo-Neto & Lopes, 2007), which explains differences in the chemical composition of essential oils from *Psidium* species.

Table 2 presents the results of the acaricidal activity found for *P. rufum* essential oil on the mortality of engorged females (%), egg hatchability (%) and product efficiency (%).

Table 2 - Average ± standard error of the mortality of engorged females (%), female mass (g), egg mass (g), hatchability (%) and product efficiency (%) on engorged *Rhipicephalus microplus* females after immersion at different *Psidium rufum* essential oil concentrations.

Oil concentration (mg/mL)	Female mortality (%)	Female mass (g)	Egg mass (g)	Hatchability (%)	Product efficiency (EP - %)
PC	100.00±0.00^{a}	0.26±0.00^{a}	0.00±0.000^{a}	0.00±0.00^{d}	100.00^{a}
400	100.00±0.00^{a}	0.24±0.04^{a}	0.01±0.030^{a}	0.00±0.00^{d}	72.31^{b}
300	66.66±2.88^{b}	0.24±0.05^{a}	0.03±0.035^{a}	0.00±0.00^{d}	72.19^{c}
200	40.00±5.56^{c}	0.24±0.04^{a}	0.03±0.020^{a}	0.00±0.00^{d}	72.07^{d}
100	46.66±2.88^{c}	0.26±0.03^{a}	0.08±0.032^{a}	7.65±1.55^{d}	70.23^{e}
50	20.00±1.73^{d}	0.25±0.05^{a}	0.09±0.037^{a}	8.32±1.22^{d}	66.83^{f}
25	13.33±5.77^{d,e}	0.25±0.03^{a}	0.10±0.029^{a}	9.30±3.60^{d}	65.70^{g}
12.5	6.66±2.88^{e,f}	0.24±0.04^{a}	0.10±0.025^{a}	24.51±6.49^{c}	51.17^{h}
6.20	6.66±2.08^{e,f}	0.23±0.00^{a}	0.07±0.024^{a}	27.19±3.14^{c}	48.59^{i}
3.10	0.00±0.00^{f}	0.24±0.03^{a}	0.07±0.007^{a}	58.06±7.59^{b}	35.98^{j}
1.50	0.00±0.00^{f}	0.25±0.03^{a}	0.10±0.028^{a}	85.40±10.66^{a}	11.12^{k}
0.70	0.00±0.00^{f}	0.25±0.03^{a}	0.08±0.007^{a}	89.12±1.39^{a}	6.74^{l}
0.39	0.00±0.00^{f}	0.25±0.04^{a}	0.10±0.007^{a}	89.14±2.19^{a}	6.72^{l}
NC	0.00±0.00^{f}	0.26±0.03^{a}	0.11±0.006^{a}	90.35±2.44^{a}	0.00^{m}

PC: Positive control (15% cypermethrin; 25% chlorpyrifos; 1% citronellal - 0.005% solution); NC = negative control (2.0% aqueous polysorbate solution) Means followed by the same lowercase letter in columns do not differ from each other by the Tukey test ($p \leq 0.05$). Source: Authors.

As shown in Table 2, *P. rufum* essential oil caused 100% and 66.66% of female mortality at concentrations of 400 mg/mL and 300 mg/mL. It was also observed that essential oil inhibited egg hatchability at concentrations ranging from 400 to 200 mg/mL, and considerably reduced egg hatchability at concentration of 3.10 mg/mL. This reduction in egg hatchability caused by *araçá-roxo* essential oil affected the reproductive efficiency of engorged *R. microplus* females. Considering that three to four tick generations occur during the year, and that one female produces about 3,000 new ticks (Furlong, 2005), a reduction in reproductive efficiency exponentially decreases the tick population in a given location, contributing to less infestation in subsequent tick generations in pastures and animals (Martins et al., 2002; Furlong 2005). It is noteworthy that *P. rufum* essential oil showed efficiency

above 70% at concentrations ranging from 400 to 100 mg/mL, with potential control over this ectoparasite.

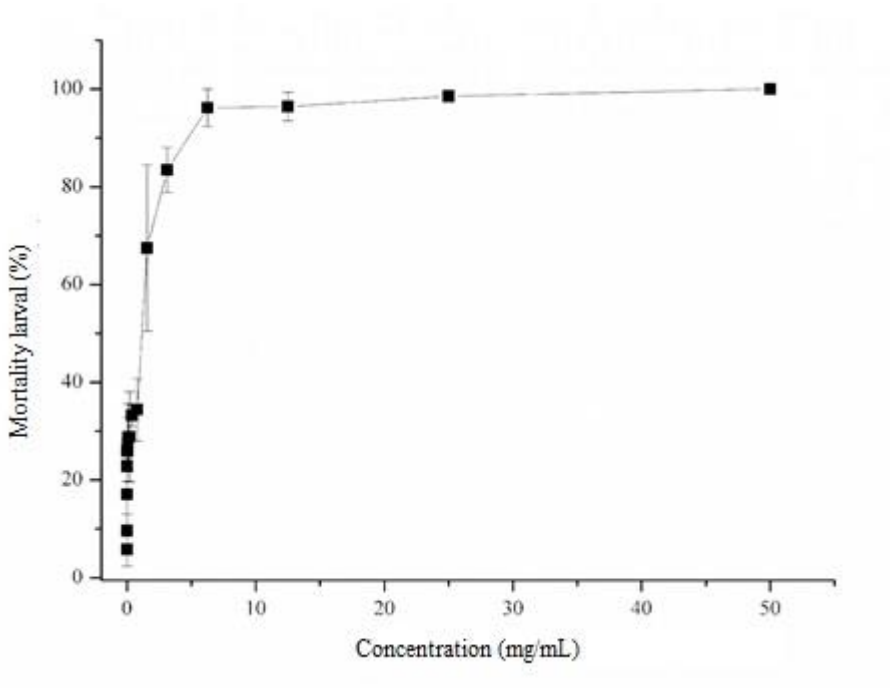
In the present experiment, the effect of essential oil on bovine tick larvae was also investigated, and the results are described in Table 3 and Figure 2. It was observed that *araçá-roxo* essential oil up to the concentration of 50.00 mg/mL killed 100% of larvae of this ectoparasite.

Table 3 - Mean ± standard error of mortality rate (%) of *Rhipicephalus microplus* larvae by the larval packet test at different *Psidium rufum* essential oil concentrations.

Essential oil concentration (mg/mL)	Mortality rate (%)
CP	98.91 ± 0.55[a]
300.00	100.00 ± 0.00[a]
200.00	100.00 ± 0.00[a]
100.00	100.00 ± 0.00[a]
50.00	100.00 ± 0.00[a]
25.00	98.51 ± 0.80[a]
12.50	96.37 ± 2.91[a]
6.25	96.15 ± 3.84[a]
3.12	83.44 ± 4.62[a,b]
1.56	67.45 ± 17.01[b]
0.78	34.35 ± 6.35[c]
0.39	33.20 ± 2.25[c]
0.19	28.84 ± 9.23[c]
0.095	28.75 ± 6.84[c]
0.047	27.51 ± 5.28[c,d]
0.023	25.92 ± 2.64[c,d]
0.011	22.79 ± 3.06[c,d,e]
0.006	17.02 ± 7.34[c,d,e]
0.003	9.63 ± 3.37[d,e]
0.001	5.77 ± 3.39[e]
CN	0.00± 0.00[f]

PC: Positive control (15% cypermethrin; 25% chlorpyrifos; 1% citronellal - 0.005% solution); NC: 2.0% aqueous polysorbate solution (80). Means followed by the same lowercase letter in column do not differ by the Tukey test ($p \leq 0.05$). Source: Authors.

Figure 2 - Mortality rate (%) of *Rhipicephalus microplus* larvae by the larval packet test at *Psidium rufum* essential oil concentrations from 50.00 to 0.001 mg/mL.



Source: Authors.

From data found on the mortality rate of larvae (%) (Table 4) at different *araçá-roxo* essential oil concentrations, the lethal concentration (LC) capable of killing 50% of larvae (LC_{50}) and 99% (LC_{99}) was determined, and results are shown in Table 4.

Table 4 - Lethal concentrations (LC) and confidence intervals (CI) of *Psidium rufum* essential oil on engorged *Rhipicephalus microplus* females and larvae through Probitos analysis.

	LC_{50} (CI)	**LC_{99}** (CI)
Mortality rate of engorged females	195.22[b] (180.17 - 211.95)	513.57[b] (472.42 – 564.70)
Mortality rate of larvae (mg/mL)	2.50[a] (2.15 – 2.88)	12.57[a] (11.23– 14.30)
PC	0.019[a] (0.018 – 0.020)	0.21[a] (0.20 – 0.22)

PC: Positive control (15% cypermethrin; 25% chlorpyrifos; 1% citronellal - 0.005% solution). Means followed by the same lowercase letter in column do not differ by Tukey test ($p \leq 0.05$). Source: Authors.

P. rufum essential oil showed LC_{50} of 2.50 mg/mL and LC_{99} of 12.57 mg/mL. According to literature, it was observed that the results obtained in the present study for the larvicidal activity of *araçá-roxo* essential oil were superior to results found by Chagas et al. (2002), who evaluated the acaricidal activity of essential oils from *Eucalyptus* sp. species on bovine tick larvae and found $LC_{99.9}$ of 100.00 mg/mL for *Eucalyptus staigeriana* and *Eucalyptus citriodora* essential oil and $CL_{99.9}$ of 200 mg/mL for *Eucalyptus globulus* essential oil.

The results found in Tables 3 and 4 indicated that *araçá-roxo* essential oil showed greater efficiency on larvae (LC_{99} = 12.57 mg/mL) when compared to engorged females (LC_{99} = 513.57 mg/mL). This result can be explained by the increase in the cuticle thickness of engorged females due to the hormonal stimulus, so that proteins acquired in the blood meal do not leak (Furlong et al., 2007). This impairs the penetration of essential oil into the cuticle of teleogens, causing this contact to occur through joints and natural orifices, making the tick less vulnerable to intoxication, requiring greater essential oil concentrations for better performance against teleogens, different from larvae (Santos et al., 2015).

The presence of monoterpene 1,8-cineole in *P. rufum* essential oil indicates that it is partially responsible for the acaricidal activity. Monoterpene 1,8-cineole shows larvicidal activity against *Rhipicephalus microplus* (Canestrini), as reported by Prates et al. (1998). In addition to its larvicidal activity, it has analgesic and anti-inflammatory properties (Menezes et al., 1990), being also used in the treatment of cough, rheumatism, neurosis, muscle pain, asthma, kidney stones and in cosmetics (Miyzawa et al., 2001). However, there are no reports in literature about the larvicidal potential of α-longipinene, but there are studies showing its therapeutic potential against anxiety (Li et al., 2012).

Therefore, establishing at which stage of the bovine tick reproductive cycle the *araçá-roxo* essential oil acted was fundamental for this research. Thus, it could be concluded that *araçá-roxo* essential oil showed greater activity in the larval phase of the tick when compared with results found in the other reproductive cycle stages of this ectoparasite. Therefore, these results open new research perspectives, searching for biomolecules responsible for the larvicidal potential, aiming at the development of products for bovine tick control.

4. Concluding Remarks

Essential oil from *Psidium rufum* leaves demonstrated potential to control *R. microplus* in two stages of the tick's reproductive cycle, reducing egg hatchability and killing

larvae. This study shows the potential of this species as well as its leaves, indicating that it is a promising plant for the development and application of products against bovine ticks.

However, further studies are needed in different stages of development from the cattle tick, as well as studies about the effect on non-target organisms and the 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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Percentage of contribution of each author in the manuscript

Luiz Antônio Branco – 20%

Herika Line Marko de Oliveira – 10%

Wanessa de Campos Bortolucci – 10%

Carla Maria Mariano Fernandez – 10%

José Eduardo Gonçalves – 5%

Zilda Cristiani Gazim – 10%

Daniela Dib Gonçalves – 5%

Emerson Luiz Botelho Lourenço – 5%

Jessé Lahos Borges – 5%

Vanderson dos Santos Silva – 5%

Ranulfo Piau Junior – 15%