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Acaricidal and larvicidal activity of leaves and fractions of rose pepper (*Schinus terebinthifolius* Raddi. (Anacardiaceae) essential oil against *Rhipicephalus* (*Boophilus*) *microplus*

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Abstract

This experiment aimed to investigate the chemical composition and the acaricidal and larvicidal activities of essential oil (EO) and fractions from rose pepper (*Schinus terebinthifolius* (Raddi) leaves against *Rhipicephalus* (*Boophilus) microplus.* The EO was obtained from fresh leaves of adult rose pepper specimens by hydrodistillation (2h), then fractioned by column chromatography and analyzed by gas chromatography coupled to mass spectrometry (GC/MS). The acaricidal activity was determined by Adult Immersion Test (AIT) and Larval Packet Test (LPT). The concentrations of EO for AIT were from 500.00 to 0.19 mg/mL, and for LPT were from 12.50 to 0.00018 mg/mL. The mortality percentage (%) of female and larvae ticks were obtained by calculating the average ± standard error utilizing the Microsoft Excel® program. The data were submitted to analysis of variance (ANOVA) and compared utilizing Sisvar 5.6 program by Scott-Knott's test ($p<0.05$). The values of lethal concentration (LC_{50} and $LC_{99.9}$) and their respective confidence intervals (CI) were calculated by Probit analysis. Bioassays showed that EO from leaves killed 40.00% of the females and inhibited 97.06% of egg hatchability at the dose of 500.00 mg/mL, and high activity on larvae, presenting LC_{50} 0.0026 ± 0.0004 mg/mL and $LC_{99.9}$ of 8.58 ± 0.03 mg/mL. The probable action mechanism of EO and fractions was established by the bio-autographic method, measuring the inhibition potential on the acetylcholinesterase enzyme, indicating an inhibition until the concentration of 0.0003 mg/mL. These results allow new perspectives to propose new biomolecules as a way to minimize tick resistance against conventional acaricides.

Keywords: bicyclogermacrene; germacrene D; Adult Immersion Test; Larval Immersion Test; Acetylcholinesterase.
Abbreviations: GC/MS_gas chromatographer coupled to mass spectrometer; AChE_ Acetylcholinesterase, LC_lethal concentration; $LC_{99.9}$_lethal concentration to eliminate 99.9% of larvae and ticks, LC_{50}_lethal concentration to eliminate 50% of larvae and ticks, EO_essential oil, FR_fraction, CI_confidence interval.

Introduction

In year of 2016, 29.70 million cattle heads were slaughtered in the livestock sector of Brazil, estimating a production of 9.5 thousand tons of meat in 2017, and projecting the growth of 2.10% a year (BRASIL, 2017). In this context, cattle tick, *Rhipicephalus* (*Boophilus*) *microplus* Canestrini (Acari: Ixodidae), stands out as a problem that demands attention as it causes world economic losses estimated in more than 3 billion dollars a year (Robbertse et al., 2016). This ectoparasite causes cutaneous lesions, resulting in economic losses for the leather trade. It also reduces milk and beef production as well as it affects natality directly (Alvaréz et al., 2008). Moreover, it is responsible for the transmission of hemotozoas such as *Babesia bovis, Babesia bigemina* and *Anaplasma marginata*, which cause diseases and may cause the animal's death (Grisi et al., 2014). The control against cattle tick is done by vaccination, integrated management practices, and the continuous utilization of chemical acaricides and larvicides such as pyrethroids, organophosphates and avermectins (Benelli et al., 2016). These acaricides must be effective throughout the tick's life cycle, which occurs for 21 days and consists of a free life phase when the teleoginae detach themselves from the animal to lay eggs in the soil. The free larval life is the longest phase of animal infestation because the ticks remain in the soil for until 6 months, making their resistance capacity against adverse conditions evident (Nunes et al.,

2011). As they find the host, these larvae migrate and start the parasitic life cycle when they will be attached to the host and feed themselves with lymph and blood (Furlong et al., 2007; Nunes et al., 2011).

The utilization of chemical acaricides and larvicides is the most used strategy to fight this ectoparasite; however, this practice has been causing tick resistance besides its small environmental safety with the death of non-target organisms and with traces of these chemical compounds in milk and beef (Mendes et al., 2007, Singh et al., 2017). Therefore, it is necessary to implement research studies and technologies to propose new biomolecules. One of the alternatives is the utilization of natural products with pesticide action. In our study, we propose the utilization of *Schinus terebinthifolius* Raddi (Anacardiaceae) popularly known as rose pepper, Brazilian pepper, red aroeira, poivre rosé (Oliveira et al., 2014). This species is native to Brazil and found throughout the territorial extension of the Atlantic Forest, and has been broadly utilized in reforestation processes on river banks (Cesario and Ganclione, 2008). It is economically important because its fruits are utilized as food spices, and the essential oil (EO). These fruits have been used in the production of perfumes, body creams and oils (Cavalcante et al., 2015).

Thus, this study aimed to evaluate the acaricidal and larvicidal potential of EO from *Schinus terebinthifolius* leaves and fractions against *R. (B.) microplus* to propose new biomolecules as a way to minimize the resistance to conventional acaricides.

Results and Discussion

Chemical characterization of essential oil and fractions from Schinus terebinthifolius leaves

Through chemical analysis (Table 2), it was possible to identify 44 chemical compounds in EO from leaves, and the predominant class was sesquiterpenes hyrodcarbons (72.10%). The major compounds were: bicyclogermacrene (27.57%), *β*-phelandrene (7.30%), germacrene D (7.16%), isolongipholene (7.11%), *β-cis*-farnesene (6.38%) and *α*-pinene (6.30%). Jeribi et al*.* (2012) also reported bicyclogermacrene (23.56%), and *α*-pinene (9.63%) in EO of Tunisia's pepper rose leaves. Santana et al*.* (2012) also obtained germacrene D (23.70%) and bicyclogermacrene (15.00%) from the leaves of the pepper rose of São Paulo, Brazil, corroborating with the major compounds found in the pepper rose EO in this study.

In plants, volatile monoterpenes and sesquiterpenes are responsible for the pungent smell and taste of specific vegetal tissues, protecting them from insect attacks, acting to attract pollinators as well as responding to possible damages (Khan et al*.,* 2015). The difference between the amount of monoterpene and sesquiterpene compounds in the same plant is related to the vegetative phase of plant (Lange and Ahkami, 2013).

The fractioning of rose pepper EO leaves and hexane fraction (FR 1) from leaves revealed that the major class was hydrocarbon sesquiterpenes, mainly bicyclogermacrene (30.94%) and germacrene D (10.94%). The dichloromethane fraction (FR 2) presented spathulenol (28.93%) and *epi-α*-cadinol (11.43%) as major compounds. The ethyl acetate fraction (FR 3) had viridiflorol (10.25%), and the methanol fraction (FR 4) and the compound 1,2-benzenedicorboxilic acid (26.13%).

The antifungal activity of the bicyclogermacrene and germacrene D compounds were reported in the literature (Silva et al*.,* 2007); however, there are no reports on their acaricidal and larvicidal activity against *R.* (*B.*) *microplus* as found in this study, according to the results presented in Tables 3 and 4.

Adult Immersion Test (AIT)

The chemical characteristics of this EO makes possible to verify the acaricidal activity against engorged *R. microplus* females (Table 3).

It is possible to verify that EO from leaves promoted different mortality rates (%) of engorged females. EO from leaves presented a mortality rate of 40.00 and 16.60% at concentrations of 500.00 and 200.00 mg/mL, respectively. Another important aspect to be evaluated during a tick's reproductive cycle is the action of active ingredients and principles that inhibit egg hatchability. Therefore, it was possible to verify that EO from leaves presented greater inhibition of hatchability (Table 3), from 97.10% at 500.00 mg/mL to 35.91% at 3.12 mg/mL. These results made evident that EO from leaves inhibited egg hatchability.

This is the first study in which the effect of EO and isolated fractions from *S. terebinthifolius* leaves against cattle tick is evaluated. However, within the *Schinus* genus, the species *Schinus molle* is the one that stands out in research studies in different phases of the reproductive cycle of this ectoparasite. Within this context, Avelar et al. (2016) investigated the effect of EO from *S. molle* leaves on the mortality of engorged *R.* (*B.*) *microplus* female ticks at the concentration of 40.00 mg/mL, finding mortality of 12.40% and 20.00 mg/mL (1.10%). The comparing of those results to the EO from *S. terebinthifolius* leaves in this study shows that there is 6.6 and 3.3% of mortality at 50.00 mg/mL 25.00 mg/mL of teleoginae, respectively (indicating a greater action of rose pepper EO on the mortality of female ticks.

Larval Packet Test (LPT)

Evaluation of active EO from leaves on reproductive cycle of tick showed that larvae stage is the most sensitive stage; therefore, the fractions (FR) of EO from rose pepper leaves were tested in the larval cycle. However, the best results were obtained from EO with (LC_{50}= 0.0026 and $LC_{99.9}$= 8.5850 mg/mL) followed by FR 1 (LC_{50}= 0.0355 and $LC_{99.9}$= 8.8317 mg/mL) (Table 4). This high activity against larvae can be explained due to the presence of bicyclogermacrene and germacrene D compounds in FR1, which are the major compounds of OE. Research on the substitution of conventional larvicides for biologically active molecules has been carried out with several plant species. *Schinus molle* is a species that has been tested. In this context, Avelar et al. (2016) utilized *S. molle* EO leaves against cattle tick larvae, and found 10.80% of mortality at the concentration of 10.00 mg/mL, and 1.70% at 5.00 mg/mL. In another study, Torres et al. (2012) evaluated the *S. molle* OE of the aerial parts, in the concentrations of 0.025 to 0.000625 mg/mL, finding no mortality on the larvae.

Table 1. Analysis of pH, base saturations, micro and macro nutrients of the soil for the cultivation of *Schinus terebinthifolius*.

pH and Macronutrients								
			cmol/dm^{-3}				mg/dm^{-3}	mg/dm^{-3}
pH ($CaCl_2$)	Al^{3+}	$H^+ + Al^{3+}$	Ca^{2+} Mg^{2+}	K^+	BA	CEC	P	C
5.7	0	4.61	14.75	0.23	14.98	19.59	27.8	16.75
Micronutrients								
(%)			cmol/dm-3					
V	Ca^{2+}	Mg^{2+}	K^+	Ca/Mg	Ca/K	Mg/K		
52.43	10.25	4.50	0.23	2.28	44.42	19.5		

pH in $CaCl_2$ (pH), phosphorus (P), carbon (C), organic matter (OM), total nitrogen (N), aluminum (Al^{3+}), potential acidity (H^++Al^{3+}), calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^+), base addition (BA), cationic exchange capacity (CEC) and base saturation (V).

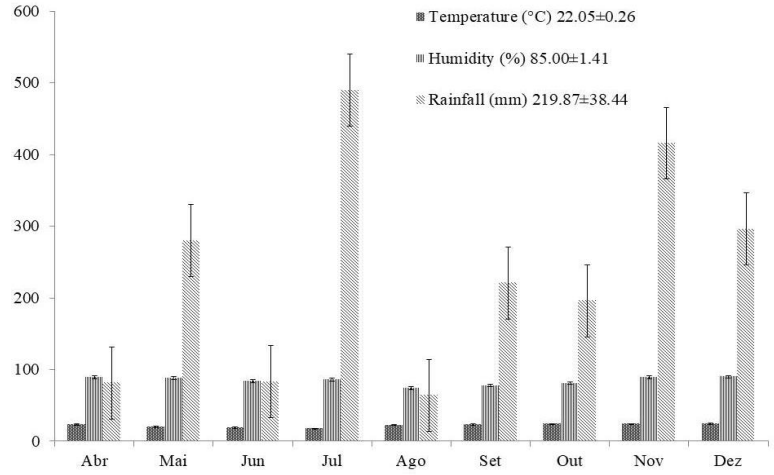

Fig 1. Average ± standard deviation of rainfall index (mm), relative humidity (%) and temperature (ºC) determined from 04/01/2015 to 12/31/2015 in the location of *Schinus terebinthifolius* vegetal material (leaves) collection.

Table 2. Chemical composition of essential oil from leaves of *Schinus terebinthifolius.*

Peak	[a]Compounds	[b]Calculated RI	Relative Area (%)	Peak	[a]Compounds	[b]Calculated RI	Relative Area (%)	Identification Method
1	*trans*-2-hexenol	838	0.31	26	*β*-selinene	1466	0.16	b,c
2	*α*-pinene	926	6.30	27	Biciclogermacrene	1482	27.57	b,c
3	Sabinene	962	0.22	28	*α*-muurolene	1483	0.37	b,c
4	*β*-pinene	970	1.91	29	germacrene A	1486	1.66	b,c
5	Myrcene	980	0.12	30	*δ*-cadinene	1535	0.51	b,c
6	*α*-phellandrene	993	0.07	31	n.i.	1537	0.83	b,c
7	Limonene	1015	0.16	32	*α*-cadinene	1539	1.98	b,c
8	*β*- phellandrene	1019	7.30	33	selina-3,7(11)-diene	1540	1.57	b,c
9	*β*-*trans*-ocimene	1038	0.36	34	n.i.	1547	0.53	b,c
10	n.i.	1309	0.12	35	germacrene B	1560	2.01	b,c
11	*δ*-elemene	1325	1.68	36	n.i.	1563	0.11	b,c
12	*α*-cubebene	1341	0.32	37	caryophyllene alcohol	1566	2.41	b,c
13	*α*-ylangene	1372	0.35	38	Spathulenol	1573	1.02	b,c
14	*α*-copaene	1376	5.08	39	*ar*-turmerol	1576	0.39	b,c
15	Isolongifolene	1389	7.11	40	caryophyllene oxyde	1584	0.75	b,c
16	Ciperene	1393	1.65	41	Viridiflorol	1592	0.09	b,c
17	*β*-longipinene	1399	4.35	42	Ledol	1604	0.30	b,c
18	*α*-gurjunene	1402	0.11	43	n.i.	1610	0.11	b,c
19	*α*-cedrene	1410	0.17	44	1,10-di-*epi*-cubenol	1620	0.30	b,c
20	*trans*-caryophyllene	1417	0.94	45	γ-eudesmol	1624	1.49	b,c
21	*γ*-elemene	1430	0.11	46	epi-*α*-cadinol	1629	0.21	b,c
22	Aromadendrene	1439	0.46	47	epi-*α*-muurolol	1633	0.26	b,c
23	*β*-*cis*-farnesene	1442	6.38	48	*α*-cadinol	1648	1.58	b,c
24	*allo*-aromadendrene	1453	0.40	49	*α*-bisabolol	1700	0.43	b,c
25	germacrene D	1463	7.16			Total identified: 98.3		
Hydrocarbon Monoterpenes:			16.44		Oxygenated Monoterpenes:		0.12	
Hydrogenated Sesquiterpenes:			72.1		Oxygenated Sesquiterpenes:		9.23	

[a]Compounds listed according to elution order from DB-5; [b] calculated retention index (RI) utilizing n-alkanes c_7 to c_{26} in capillary column (HP-5MS); [c] Identification based on the comparison of mass spectra from Wiley 275 libraries; n.i: non-identified; (-): absent.

Table 3. Average ± standard deviation of female mortality rate (%), female mass (mg), egg mass (mg), hatchability (%) and product efficiency (%) in engorged *Rhipicephalus* (*Boophilus*) *microplus* females by adult immersion test in essential oil from *Schinus terebinthifolius* leaves.

concentration (mg/mL)	Mortality of Female (%)	Mass of Female (mg)	Egg mass (mg)	Hatchability (%)	Product efficiency (%)
PC	$100.00^{a} \pm 0.00$	$213.50^{a} \pm 0.00$	0.00^{a}	0.00^{a}	$100.00^{a} \pm 0.00$
500.00	$40.00^{b} \pm 0.09$	$211.60^{a} \pm 0.00$	$35.91^{b} \pm 0.00$	$2.94^{a} \pm 0.53$	$99.37^{a} \pm 0.00$
400.00	$27.00^{c} \pm 0.08$	$212.30^{a} \pm 0.00$	$50.19^{b} \pm 0.00$	$9.80^{b} \pm 1.78$	$97.10^{a} \pm 0.00$
300.00	$27.00^{c} \pm 0.08$	$212.40^{a} \pm 0.00$	$62.73^{c} \pm 0.00$	$15.41^{b} \pm 2.8$	$95.15^{a} \pm 0.00$
200.00	$16.60^{d} \pm 0.06$	$212.80^{a} \pm 0.00$	$76.13^{c} \pm 0.00$	$20.08^{c} \pm 3.66$	$90.92^{a} \pm 0.00$
100.00	$10.00^{e} \pm 0.05$	$213.80^{a} \pm 0.00$	$91.55^{d} \pm 0.00$	$27.90^{d} \pm 5.09$	$84.90^{a} \pm 0.00$
50.00	$6.60^{e} \pm 0.04$	$212.00^{a} \pm 0.00$	$95.08^{d} \pm 0.00$	$34.76^{e} \pm 6.34$	$80.29^{b} \pm 0.01$
25.00	$3.30^{f} \pm 0.03$	$212.90^{a} \pm 0.00$	$101.74^{d} \pm 0.00$	$43.14^{f} \pm 7.87$	$73.94^{b} \pm 0.00$
12.50	0.00^{f}	$215.00^{a} \pm 0.00$	$119.27^{e} \pm 0.00$	$55.12^{g} \pm 10.06$	$61.36^{c} \pm 0.01$
6.25	0.00^{f}	$212.60^{a} \pm 0.00$	$132.14^{e} \pm 0.02$	$61.77^{h} \pm 11.27$	$51.70^{d} \pm 0.03$
3.12	0.00^{f}	$211.50^{a} \pm 0.00$	$140.25^{e} \pm 0.00$	$75.26^{i} \pm 13.74$	$36.91^{b} \pm 0.02$
1.56	0.00^{f}	$211.70^{a} \pm 0.00$	$151.66^{f} \pm 0.00$	$87.62^{j} \pm 15.99$	$20.82^{f} \pm 0.01$
0.78	0.00^{f}	$215.70^{a} \pm 0.00$	$162.18^{f} \pm 0.00$	$94.57^{l} \pm 17.26$	$12.53^{g} \pm 0.00$
0.39	0.00^{f}	$212.00^{a} \pm 0.00$	$163.19^{f} \pm 0.00$	$99.96^{m} \pm 18.24$	$2.74^{h} \pm 0.00$
0.19	0.00^{f}	$212.10^{a} \pm 0.00$	$168.69^{f} \pm 0.00$	$100.00^{m} \pm 18.25$	0.00^{h}
NC	0.00^{f}	$214.20^{a} \pm 0.00$	$169.43^{f} \pm 0.00$	$100.00^{m} \pm 18.25$	0.00^{h}

PC: positive control [commercial solution containing 150.00 mg/mL cipermetrine, 250.00 mg/mL of chlorpyrifos and 10.00 mg/mL de citronellal]; NC: negative control [aqueus solution of polysorbate 80 at 2.00%]; (PE) product efficacy.

Table 4. Average ± standard deviation and confidence interval (CI) of lethal concentration (LC_{50} and $LC_{99.9}$) of *Schinus terebinthifolius* EO and fractions from leaves on *Rhipicephalus (Boophilus) microplus* larvae by Probitos Analysis.

Mortality of *R. (B.) microplus* larvae		LC_{50} (mg/mL) (CI)	$LC_{99.9}$ (mg/mL) (CI)
Essential oil from rose pepper fresh leaves	EO rose pepper leaves	$0.0026^{a} \pm 0.0004$ (0.0018 – 0.0030)	$8.5850^{a} \pm 0.0308$ (8.5281 – 8.6417)
	FR 1	$0.0355^{b} \pm 0.0006$ (0.0354 – 0.0357)	$8.8317^{b} \pm 0.0249$ (8.8038 – 8.8875)
	FR 2	$0.1628^{c} \pm 0.0047$ (0.1575 – 0.1735)	$8.9449^{c} \pm 0.0502$ (8.8887 – 9.0574)
	FR 3	$1.9500^{d} \pm 0.0450$ (1.8997 – 2.0507)	$9.8545^{d} \pm 0.0155$ (9.8371 – 9.8893)
	FR 4	$4.6723^{e} \pm 0.0691$ (4.5950 – 4.8269)	$12.7716^{e} \pm 0.2662$ (12.4739 – 13.3670)

FR1: hexane fraction; FR2: dichloromethane fraction; FR3: ethyl acetate fraction; FR4: methanol fraction; LC_{50}: lethal concentration 50%; $LD_{99.9}$: lethal concentration 99.9%; CI: confidence interval; Small letters: comparison between treatments of essential oil from rose pepper leaves.

Table 5. Inhibiting activity of Acetylcholinesterase enzyme at different concentrations of *Schinus terebinthifolius* essential oil from leaves and fractions by autobiography method.

	Inhibition of Acetylcholinesterase enzyme					
Concentration (mg/mL)	Leaves	FR 1	FR 2	FR 3	FR 4	PC
1.5625	+	+	+	+	+	+
0.7812	+	-	+	+	+	+
0.3906	+	-	+	+	+	+
0.1953	+	-	+	+	+	+
0.0976	+	-	+	-	-	+
0.0488	+	-	+	-	-	+
0.0244	+	-	-	-	-	+
0.0122	+	-	-	-	-	+
0.0061	+	-	-	-	-	+
0.0030	+	-	-	-	-	+
0.0015	+	-	-	-	-	+
0.0007	+	-	-	-	-	+
0.0003	+	-	-	-	-	+
0.0001	-	-	-	-	-	+

FR1: hexane fraction; FR2: dichloromethane fraction; FR3: ethyl acetate fraction; FR4: methanol fraction; PC: positive control [commercial solution containing 150.00 mg/mL cipermetrine, 250.00 mg/mL of chlorpyrifos and 10.00 mg/mL of citronellol].

When comparing the results found for *S. terebinthifolius* (Table 4), with those observed for *S. molle* it was observed that the rose pepper had a higher mortality rate, killing 99.90% of the larvae in the concentration of 8.58 mg/mL. In this phase of the experiment, it was possible to conclude that *S. terebinthifolius* is more active in the mortality of engorged female cattle ticks and larvae when compared to *S. molle.*

Another point to be discussed is regarding the greater activity of EO and FR on larvae, when compared to female adult ticks. The difference is probably because of the increase in the engorged female cuticle thickness resulting from hormonal stimulus, which prevents proteins acquired during chemical repast not to overflow (Furlong et al*.,* 2007). This makes the EO penetration more difficult on engorged female cuticle and forces this contact to happen through natural articulations and orifices, making the tick less vulnerable to intoxication, and making the use of higher concentrations of EO necessary to better act against

engorged females, differently from larvae (Santos et al., 2015).

Anticholinesterase activity of Schinus terebinthifolius essential oil

The activity of AChE enzyme was carried out by bioautographic method to verify the possible action mechanism of EO from leaves on *R. (B) microplus* engorged females and larvae (Table 5).
The results point out that EO from leaves presented high anticholinesterase potential, inhibiting enzyme until the concentration of 0.00037 mg/mL, and was superior to fractions from leaves: FR 1 (1.56 mg/mL), FR 2 (0.048 mg/mL), FR 3 (0.19 mg/mL) and FR 4 (0.19 mg/mL). These values present greater effectiveness on the enzyme, when compared to $LC_{99.9}$ utilized in *in vitro* larvicidal and acaricidal activity, according to results shown in Tables 3 and 4.
The difference between the action of EO applied on the enzyme by the bioautographic method and on larvae can be explained by the absence of physiological conditions that interfere in biochemical reactions since it is done in controlled environment with all pre-established conditions, without interferences of cellular wall permeability, characteristics of molecular absorptions as well as the solubility in hydrophilic and lipophilic means inherent to a living being (Chagas et al., 2012).
Thus, this study allows new studies such as the isolation of major compounds of EO from leaves to verify toxicity of this oil, a possible development of products or their association, which is a possible alternative for *R.* (*B.*) *microplus* control.

Materials and Methods

Vegetal matter and soil analysis

S. terebinthifolius fresh leaves were collected from an adult plant, located in Juranda city, northwestern region of Paraná state, Brazil, at coordinates S 24º 21' 28.2456" and WO 52º 36'6276" and altitude of 419 m. An accession was deposited in the Herbarium of the Universidade Estadual do Oeste do Paraná – UNIOESTE, under the registration number 1717. The cultivation soil was collected at 0-20 cm depth in different points to obtain a composed sample. For the soil analysis, Ca, Mg, Al were extracted with 1 M KCl; P and K were extracted according to Mehlich method; H+Al was determined by Shoemaker, McLean and Pratt method; carbon by Walkley and Black method; and the sum of bases and cation exchange capacity according to Silva (2009).
According to the data obtained from the soil analysis (Table 1), *S terebinthifolius* tree grew naturally in the clayey soil, classified as red-yellow Latosol. The soil had clayey texture and its granulometric composition was 54.00% of clay, 27.10% of silt and 18.20% of sand, and was classified as clayey soil according to the Normative Ruling Number 2, from October 9, 2008 of the Ministério da Agricultura, Pecuária and Abastecimento (BRASIL, 2008). The soil pH was moderately acid (pH 5.70). It had reddish color and high fertility due to the organic matter content (OM 28.88 g/dm^3) and base saturation (V 52.43%), according to the criteria adopted by Embrapa Solos (2006).

Meteorological data

Temperature (°C), humidity (%) and rainfall index (mm) values were provided by Secretaria de Agricultura e Abastecimento (SEAB) of the city of Juranda during the period of the vegetal material harvest (April to December, 2015).
The results from the meteorological data indicated an average temperature of 22.05ºC ± 0.26, where humidity was kept at 85.00% ± 1.41 and the rainfall was 219.87 ± 38.44 mm (Figure 1). The *S. terebinthifolius* can be developed in a wide geographical distribution and adapt to different climates (Cesario and Gaglianone, 2008). The climate in which the tree was located corresponds to humid subtropical according to the Köppen-Geiger classification (Seidel et al., 2012), and does not interfere in the development of this plant species.
According to Morais (2009), the chemical composition as well as the biological activities is directly influenced by biotic and abiotic factors. Therefore, the soil characteristics as well as the meteorological data where the rose pepper tree is located were fundamental to establish parameters for the obtained results.

Plant material, harvest, essential oil extraction from Schinus terebinthifolius leaves and fractioning

The leaves were collected from April to December, 2015 and the EO was obtained by hydrodistillation (two hours). For the fractioning, silica gel 60 (Merck 0.04-0.063mm) was utilized for the column chromatography in a proportion of 1/25 (EO/silica). The EO was eluded by hexane, dichloromethane, ethyl acetate and methanol (2000 mL each). The fractions were concentrated in a rotary evaporator (TE-210).

Chemical characterization of Schinus terebinthifolius fractions and essential oil from leaves

The chemical identification was done by Gas Chromatography/Mass Spectrometry (GC/MS), utilizing a chromatographer (Agilent 7890 B) coupled to a mass spectrometer (Agilent 5977 A), equipped with a capillary column of funded silica HP-5MS UI Agilent (30 m x 0.250 mm x 0.25 µm). The analysis conditions were: injector temperature at 220ºC, injection volume of 2.00 µL and injection rate in split mode 1:30, initial temperature of column at 60ºC kept for 2 minute, with heating ramp of 2ºC/min until reaching 180ºC and kept for 4 min, ramp of 10ºC/min until 260ºC, and finally a ramp of 40ºC/min until 300ºC (Cavalcante et al., 2015). The transfer line was kept at 285ºC and the ionization source and quadrupole were 230ºC and 150ºC, respectively. Helium was utilized as the carrier gas with a flow of 1 mL/min. The detection system was EM in "Scan" mode, in mass/charge (*m/z*) ratio of 40 - 550, with 3-minute Solvent Delay. The oil samples were diluted in a proportion of 1:10 with dichloromethane. The compounds found in the essential oil were identified by comparing their mass spectra to mass spectra from WILEY 275 libraries and also comparing their obtained retention indexes (RI) using a homologous series of standard n-alkanes (C7 - C26) (Adams, 2012).

Acaricidal and larvicidal activity of Schinus terebinthifolius essential oils against Rhipicephalus (B.) microplus

The acaricidal activity of *S. terebinthifolius* essential oil and fractions from leaves fresh and fractions was determined by the Adult Immersion Test (AIT) and by the Larval Packet Test (LPT), as recommended by Drummond et al. (1973) and Chagas et al. (2012).

Treatment groups

480 female *R.* (*B*) *microplus* ticks were collected from milk cattle that had not been treated against ticks for more than 60 days, belonging to the Veterinary Hospital of Paranaense University - UNIPAR, in Umuarama, northwestern region of the state of Paraná, Brazil. The ticks were transported in a container with appropriate airing to the laboratory of Natural Products of UNIPAR.

The engorged females were selected at their maximum engorgement. Dilutions of EO from *S. terebinthifolius* leaves were utilized, starting at 500.00 to 0.19 mg/mL. The EO from leaves was diluted in an aqueous solution of polysorbate 80 at 2.00% (Chagas et al*.,* 2012). For the negative control, an aqueous solution of polysorbate 80 at 2.00% (v/v) was used. A commercial solution (cipermetrine 150.00 mg/mL; chlorpyrifos 250.00 mg/mL and citronellal 10.00 mg/mL, in a solution at 0.125% (v/v)) was utilized As positive control. The treatments were done in triplicate. The Adult Immersion Test (AIT) was carried out according to the protocol described by Drummond et al. (1973).

Sensitivity of ingurgitated females in adult immersion test (AIT)

According to by Drummond et al. (1973), groups of 10 female ticks were weighed and immersed for 5 minutes in each treatment. They were dried in paper and distributed in a Petri dish, identified and labelled. The Petri dishes were stored in a styrofoam box containing moist cotton and kept for 14 days for egg laying.

After 14 days, the mass of eggs from each female tick was weighed and transferred to appropriately identified assay tubes (each female and its eggs), the tubes were placed in the styrofoam box containing moist cotton for 21 days to hatch.

After 21 days, the larvae were killed with sulfuric ether and counted using an entomologic loupe to verify the egg hatching rate. From data of female ticks' mass, egg mass and hatching percentage, the reproductive efficiency (RE) and product efficiency (PE) were determined by Equations 1 and 2, respectively (Drummond et al., 1973).

$$RE = \frac{Egg\ mass\ (g) \times Hatching\ rate\ (\%)}{Female\ tick\ mass} \times 20 \quad \text{Eq.1}$$

$$PE = \frac{RE_{\ negative\ control\ group} \times RE_{\ treated\ group}}{RE_{\ negative\ control\ group}} \times 100 \quad \text{Eq.2}$$

Larvicidal activity of Schinus terebinthifolius leaves essential oil and fractions on Larval Packet Test (LPT)

Larval Packet Test (LPT) was done with fractions and dilutions of EO from rose pepper leaves, according to the technique described by Fernandes et al*.* (2008). The engorged females without treatment were kept in a controlled environment for larval production. 100 larvae were placed on a 2.00 x 2.00 cm paper filter that had recently been moistened with fractions and dilutions of EO from rose pepper leaves, forming a sealed "sandwich", stored in a Petri dish and kept at room temperature. The reading was done after 24h when live and dead larvae were separated using an entomologic loupe.

Twenty dilutions of EO from rose pepper leaves and fractions were prepared at concentrations ranging from 112.50 to 0.00018 mg/mL, utilizing polysorbate (80) at 2.00% (v/v) as emulsifier and purified water as solvent. As negative control, purified water and polysorbate (80) at 2.00% (v/v) were utilized, whereas organophosphates from an acaricide (cipermetrine 15.00%; chlorpyrifos 25.00%; citronellal 1.00%) were the positive control. The treatments were done in triplicate and the larval mortality (LM) (Equation 3) and the average larval mortality were determined.

$$LM\ (\%) = \frac{Dead\ larvae}{Total\ of\ larvae} \times 100 \quad \text{Eq. 3}$$

Anticholinesterase activity of essential oils and fractions

The bioautographic assay was done according to the method described by Yang et al*.* (2009) to determine the anticholinesterase activity. *S. terebinthifolius* fractions and EO from leaves were tested starting at a concentration of 3.12 until 0.0001 mg/mL (in methanol). As positive standard acaricide was an organophosphate (cypermethrin 150.00 mg/mL; chlorpyrifos 250.00 mg/mL and citronellal 10.00 mg/mL, in a solution at 0.125% (v/v)).

Statistical analysis for the acaricidal and larvicidal activity

The experimental design was completely randomized (CRD). The tests were done in triplicate and the mortality percentage (%) of *R.* (*B.*) *microplus* female ticks and larvae were obtained by calculating the average ± standard error utilizing Microsoft Excel® program (Excel® Version 2010). The data were submitted to analysis of variance (ANOVA) and compared utilizing Sisvar 5.6 program by Scott-Knott's test ($p<0.05$). The values of lethal concentration (LC_{50} and $LC_{99.9}$) and their respective confidence intervals (CI) were calculated by Probitos analysis (ED 50 Plus version 1.0).

Conclusion

The EO from rose pepper leaves obtained by hydrodistillation presented hydrocarbon sesquiterpenes as the major class of compounds, showed biological activity against cattle tick, inhibiting egg hatchability and killing larvae. This action may be due to the major compounds of the essential oil, bicyclogermacrene and germacrene D. Therefore, *S. terebinthifolius* EO is a source that may

substitute as well as act in synergism with conventional chemical acaricides.

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References

Adams RP (2012) Identification of essential oil components by gas chromatography/mass spectrometry. 4 ed. Allured Publishing Corporation, Illinois, USA. 804 p.

Álvarez V, Loaiza J, Bonilla R, Barrios M (2008) Control in vitro tick (*Boophilus microplus*; Acari: Ixodidae) through plant extracts. Rev Biol Trop. 56(1):291-302.

Avelar BR, Lambert MM, Siqueira RCS, Cid YP, Chaves DSA, Coumendouros K (2016) *In vitro* activity of essential oils and extracts from *Schinus molle* L. against *Rhipicephalus microplus*. Rev Bras Med Vet. 38(13):183-187.

Benelli G, Pavela R, Canale A, Mehlhorn H (2016) Ticks repellents and acaricides of botanical origin: a green roadmap to control tick-borne diseases? Parasitol Res. 115:2545-2560.

Brasil Ministério da Agricultura Pecuária e Abastecimento. Instrução Normativa nº 2, de 9 de out. 2008. Tipos de solos. Diário Oficial, Brasília, p.71, seção 1.

Brasil Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Politica Agrícola – SPA. Brasil projeções do Agronegócio. Brasília, 2017. p.1-125.

Cavalcante AS, Alves MS, Silva LCP, Patrocinio DS, Sanches MN, Chaves DAS, Souza MAA (2015) Volatiles composition and extraction kinetics from *Schinus terebinthifolius* in *Schinus molle* leaves and fruit. Rev Bras Farmacogn. 25:356-362.

Cesario LF, Gaglianone MC (2008) Floral biology and reproductive phenology of *Schinus terebinthifolius* Raddi (Anacardiaceae) in the restinga of northern Rio de Janeiro state. Acta Bot Brasilica. 22(3):828-833.

Chagas ACS, Barros LD, Cotinquiba F, Furlan M, Giglioti R, Oliveira SMC, Bizzo HR (2012) *In vitro* efficacy of plant extracts and synthesized substances on *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae). J Parasitol Res. 110(1):295-303.

Drummond RO, Ernst SE, Trevino JL, Gladney WJ, Graham OH (1973) *Boophilus annulatus* and *Boophilus microplus* (Acarine: Ixodidae) in natural and experimental conditions. Folia Parasitol. 37:331-336.

EMBRAPA. 2006 Centro Nacional de Pesquisa de Solos. Sistema Brasileiro de Classificação de Solos. Rio de Janeiro: Embrapa Solos. 2 ed. 306 p.

Fernandes FF, Bessa PAD, Freitas EPS (2008) Evaluation of activity of the crude ethanolic extract of *Magonia pubescens* St. Hil (Sapindaceae) against larvae of the cattle tick *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini, 1887) (Acari: Ixodidae). Braz Arch Biol Technol. 51(6):1147–1152.

Furlong J, Martins JR, Prata MCA (2007) La tique des bovins et la résistance: avons-nous de quoi nous réjouir?. Hora Vet. 27:26-32.

Grisi L, Leite RC, Martins JRS, Barros ATM, Andreotti R, Cançado PHD, León AAP (2014) Reassessment of the potential economic impact of cattle parasites in Brazil. Rev Bras Parasitol Vet. 2:150-156.

Jeribi C, Karoui IJ, Hassine DB, Abderrabba M (2012) Comparative study of bioactive compounds and antioxidant activity of *Schinus terebinthifolius* Raddi fruits and leaves essential oils. Int J of Sc Res. 3(12):453-458.

Khan AF, Mujeeb F, Aha F, Farooqui A (2015) Effect of plant growth regulators on growth and essential oil content in palmarosa (*Cymbopogon martini*). Asian J Pharm Clin Res. 8(2):373-376.

Lange BM, Ahkami A (2013) Metabolic engineering of plant monoterpenes, sesquiterpenes and diterpenos-current status and future opportunities. Plant Biotechnol J. 11:169-196.

Mendes MC, Pereira JR, Prado AP (2007) Sensitivity of *Boophilus microplus* (Acari: Ixodidae) to pyrethroids and organophosphate in farms in the Vale do Paraíba region, São Paulo, Brazil. Arq Inst Biol. 74(2):81-85.

Morais LAS (2009) Influência dos fatores abióticos na composição química dos óleos essenciais. Hort Bras. 27(2):4050-4063.

Nunes JL, Mufioz-Cobefias ME, Moltedo HE (2011) *Boophilus microplus*, The Common Cattle Tick. 6 ed. Springer-Verlag Berlin Heidelberg, Argentina. 204p .

Oliveira LFM, Oliveira Junior LFG, Santos MC, Narain N, Leite Neto MTS (2014) Distillation time and volatile profile of the essential oil of brazilian pepper (*Schinus terebinthifolius*) in Sergipe, Brazil. Rev Bras Plantas Med. 16(2):243-249.

Robbertse L, Baron S, Merwe NA, Madder M, Stoltsz WH, Oliver CM (2016) Genetic diversity, acaricide resistance status and evolutionary potential of a *Rhipicephalus microplus* population from a disease-controlled cattle farming area in South Africa. Ticks Tick borne Dis. 7:595-603.

Santana JS, Sartorelli P, Guadagnin RC, Matsuo AL, Figueiredo CR, Soares MG, Lago JH (2012) Essential oils from *Schinus terebinthifolius* leaves – chemical composition and *in vitro* cytotoxicity evaluation. Pharm Biol. 50(10):1248-1253.

Santos TRB, Castro NA, Bretanha LC, Schuch LFD, Freitag RA, Nizoli LQ (2015) *In vitro* study of the effectiveness of citronella (*Cymbopogon wynterianus*) on the cattle tick *Rhipicephalus* (*Boophilus*) *microplus.* Science and Animal Health. 3:135-149.

Seidel EP, Silva SC, Silva LPE, Spaki AP (2012) Effect of cover crops on common bean yield and soil physical properties under no-till system. Acta Sci. 34(4):399-404.

Silva FC (2009) Manual de análises químicas de solos, plantas e fertilizantes. 2 ed. Embrapa Solos, Rio de Janeiro, BR. 627 p.

Silva L, Oniki GH, Agripino DG, Moreno PRH, Young MCM, Mayworm MAS, Ladeira AM (2007) Bicyclogermacrene, resveratrol and fungitoxic activity on leaves extracts of *Cissus verticillata* L. Nicolson & Jarvis (Vitaceae). Rev Bras Farmacogn. 17(3):361-367.

Singh NK, Miller RJ, Klafke GM, Goolsby JA, Thomas DB, Leon AAP (2017) *In vitro* efficacy of a botanical acaricide and its active ingredients against larvae of susceptible and acaricide-resistant strains of *Rhipicephalus* (*Boophilus*) *microplus* Canestrini (Acari: Inoxidae). Ticks Tick Borne Dis. 9(2):201-206.

Torres FC, Lucas AM, Ribeiro VLS, Martins JR, Poser G, Guala MS, Elder HV, Cassel E (2012) Influence of essential oil fractionation by vacuum distillation on acaricidal activity against the cattle tick. Braz Arch Biol Technol. 55(4):613-621.

Yang Z, Zhang X, Duan D, Song Z, Yang M, Li S (2009) Modifiel TLC bioautographic method for screening acetylcholinesterase inhibitors from plant extracts. J Sep Sci. 32:3257-3259.