Brazilian Journal of Veterinary Parasitology
ISSN 1984-2961 (Electronic)
www.cbpv.org.br/rbpv
Braz. J. Vet. Parasitol., Jaboticabal, v. 27, n. 2, p. 203-210, apr.-june 2018
Doi: http://dx.doi.org/10.1590/S1984-296120180015

Original Article

In vitro efficacy of essential oils with different concentrations of 1,8-cineole against *Rhipicephalus* (*Boophilus*) *microplus*

Eficácia *in vitro* de óleos essenciais com diferentes concentrações de 1,8-cineol contra *Rhipicephalus* (*Boophilus*) *microplus*

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Received November 22, 2017
Accepted February 15, 2018

Abstract

The aim of this study was to evaluate the acaricidal activity of essential oils from three species of plants with intermediary concentrations of 1,8-cineole against the tick species *Rhipicephalus (Boophilus) microplus*. For this purpose, five serial concentrations (100.0, 50.0, 25.0, 12.5, 6.2 mg/mL) of essential oils from *Mesosphaerum suaveolens (L.) Kuntze*, *Ocimum gratissimum* L. and *Alpinia zerumbet (Pers.) B. L. Burtt & R. M. Sm.* were used on larval packet and adult immersion tests. The essential oils were analysed by gas chromatography-mass spectrometry (GC/MS) and gas chromatography-flame ionization detection (GC-FID), being detected 35.8, 24.7 and 24.0% of 1.8-cineol in the oils of *M. suaveolens*, *O. gratissimum* and *A. zerumbet,* respectively. The lethal concentration (LC_{50}) of each oil for larvae and engorged females was calculated through Probit analysis. All essential oils showed high efficacy (≥ 95.0%) on engorged females at the 100.0 mg/mL concentration. In regards to larvae, *O. gratissimum* (LC_{50} = 11.9 mg/mL) was the most potent, followed by the *A. zerumbet* (LC_{50} = 19.7 mg/mL) and the *M. suaveolens* (LC_{50} = 51.6 mg/mL) essential oils. These results show that other compounds interfere with 1,8-cineole action.

Keywords: Acaricide, *Alpinia zerumbet*, *Mesosphaerum suaveolens*, *Ocimum gratissimum*, tick.

Resumo

O objetivo deste estudo foi avaliar a atividade acaricida de óleos essenciais de três espécies de plantas com concentrações intermediárias de 1,8-cineol contra o carrapato *Rhipicephalus (Boophilus) microplus*. Dessa forma, cinco concentrações diferentes (100,0; 50,0; 25,0; 12,5; 6,2 mg/mL) de óleos essenciais de *Mesosphaerum suaveolens (L.) Kuntze*, *Ocimum gratissimum* L. e *Alpinia zerumbet (Pers.) B. L. Burtt & R. M. Sm.* foram avaliadas pelos testes de pacote de larvas e de imersão de adultos. Os óleos essenciais foram analisados pela cromatografia gasosa acoplada a espectometria de massa (GC/MS) e cromatografia gasosa acoplada a detector de ionização de chama (GC-FID), sendo detectados 35,8, 24,7 e 24,0% de 1,8-cineol nos óleos de *M. suaveolens*, *O. gratissimum* e *A. zerumbet*, respectivamente. A concentração letal (CL_{50}) de cada óleo essencial para larvas e fêmeas ingurgitadas foi calculada por meio da análise de Probit. Todos os óleos essenciais na concentração de 100,0 mg/mL apresentaram elevada eficácia (≥ 95,0%) sobre fêmeas ingurgitadas. Com relação as larvas, o óleo essencial de *O. gratissimum* (CL_{50} = 11,9 mg/mL) foi o mais potente, seguido pelos óleos de *A. zerumbet* (LC_{50} = 19,7 mg/mL) e *M. suaveolens* (LC_{50} = 51,6 mg/mL). Estes resultados demonstram que outros compostos interferem na eficácia de 1,8-cineol.

Palavras-chave: Acaricida, *Alpinia zerumbet*, *Mesosphaerum suaveolens, Ocimum gratissimum*, carrapato.

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Introduction

Ticks are parasites with economic importance for bovine and other domestic species in tropical and subtropical countries (MUHAMMAD et al., 2008). Blood spoliation, reduced weight gain, and transmission of *Babesia* spp. and *Anaplasma marginale* cause severe economic losses for livestock farmers (GRISI et al., 2014). The control of this parasite often requires the use of intense chemical acaricides that consequently select for resistant populations (RODRÍGUEZ-VIVAS et al., 2006). Acaricide resistance in ticks has become a major problem throughout the world and has been detected in *Rhipicephalus (Boophilus) microplus* against almost all the registered pesticides indicated for use against this parasite (CASTRO-JANER et al., 2009). Aditionally, the chemical control are costly and contaminate the environment with residues harmful to the hosts and also humans (FREITAS et al., 2005). On the other hand, substances obtained from plants have a low cost, few residual effects and a low incidence of generating resistance (ROSADO-AGUILAR et al., 2010). Essential oils contain a variety of volatile secondary metabolites that are known for their significant role in plant defence mechanisms (NAZZARO et al., 2013). These biodegradable, bioactive oils could be an alternative source for controlling insects, helminths and ticks (BAGAVAN et al., 2009). The terpenes in essential oils are an alternative means to control parasites (ANTHONY et al., 2005). The chemical variability in essential oils and the relationship among the compounds play important roles in the acaricidal activity (SOARES et al., 2016). It is not possible to affirm that the most abundant chemical compounds in an essential oil are responsible for the observed biological effects (GALINDO et al., 2010). Compounds found in small proportions can act as synergists with other minor or major molecules (SOARES et al., 2016). 1,8-cineole is an oxygenated monoterpene that acts on cell membranes, mainly through its hydrophobicity (HONÓRIO et al., 2015). This terpene exists, at varied concentrations, in several essential oils with acaricidal activity (PRATES et al., 1998; CHAGAS et al., 2002; SOARES et al., 2016). Studies comparing the essential oils of plants with intermediate concentrations of 1,8-cineole (24 to 35%) are scarce, and understanding of how this compound participates in the activity of natural oils remains obscure.

The aim of the present study was to evaluate the acaricidal activity of essential oils, from aerial parts of two species of the Lamiaceae family (*Mesosphaerum suaveolens*, *Ocimum gratissimum)* and one of the Zingiberaceae family (*Alpinia zerumbet*), with intermediary concentrations of 1,8-cineole against the females and larvae of tick *R. (B.) microplus*.

Materials and Methods

Plant material

M. suaveolens is a plant native from Brazil where is known as "bamburral", while *O. gratissimum* and *A. zerumbet* are considered in this country as naturalized species and known, respectively, as "alfavaca-cravo" and "colônia". *M. suaveolens* and *O. gratissimum* were harvested during July 2013 (3°05'24.0"S, 41°46'12.5"W) and June 2014 (03°05'16.0"S, 41°47'01.5"W), respectively, at the Unidade de Execução de Pesquisa da Embrapa Meio-Norte, in Parnaíba, Piauí, Brazil. The *A. zerumbet* was collected at Fazenda Tabuleiros II of the Anidro do Brasil Extrações S.A., Parnaíba, Piauí, in July 2011 (03°01'27.5'S, 41°44'53.5"W). Reference plant specimens were deposited in the herbarium of the Embrapa Recursos Genéticos e Biotecnologia under the registration numbers 81.097 (*M. suaveolens*), 92.497 (*O. gratissimum)* and 81.103 (*A. zerumbet)*.

The essential oils were extracted at the Unidade de Execução de Pesquisa da Embrapa Meio-Norte, in Parnaíba, Piauí, from the aerial parts of *M. suaveolens* and from the leaves of *O. gratissimum* by hydro-distillation using a Clevenger apparatus. Nearly 2 kg of fresh plant was mixed with 3 L of water and boiled for 3 h. A water steam distillation method was used to extract the essential oil from the leaves of *A. zerumbet*. The essential oils obtained were dried over anhydrous sodium sulphate, filtered, and stored at 4 °C until being tested and analysed at the Embrapa Agroindústria Tropical, in Fortaleza, Ceará, by Gas Chromatography Mass Spectrometry (GC-MS) and Flame Ionization Detector (GC-FID) for determination of their chemical composition (CASTRO et al., 2016).

Essential oil analysis

The GC–MS analyses were carried out on an Agilent 7890B-GC/5977A-MS instrument equipped with a nonpolar VF-5MS fused silica capillary column (30 m × 0.25 mm ID, 0.25 µm film thickness) utilizing a split ratio of 1:30 and helium at 1.5 mL min^{-1} as the carrier gas. The injector temperature and detector temperature were set at 250 °C. The oven temperature was raised from 70 to 180 °C at 4 °C min^{-1} and afterwards to 250 °C at 10 °C min^{-1}. Mass spectra were recorded in a range of mass-to-charge ratio (m/z) between 30 and 450. GC-FID analyses were performed on a Shimadzu GC-2010 Plus chromatograph in the same aforementioned chromatographic conditions except for the carrier gas (hydrogen). The retention indices were determined by the injection of a mixture of C_7-C_{30} homologous n-alkanes (Sigma, St. Louis, MO). The identification of the volatile compounds was achieved through the comparison of the mass spectra recorded with those provided by the spectrometer database (NIST 02–287,324 compounds) along with the retention indices and mass spectra of the literature (ADAMS, 2007; NIST, 2011). The relative content of each constituent was quantified as the normalized peak area detected in the GC-FID chromatogram and expressed as a percentage.

Preparation of the dilutions

For each oil, solutions were prepared at concentrations of 6.2, 12.5, 25.0, 50.0 and 100.0 mg/mL. The negative controls consisted of the solvents used in the essential oil dilutions, which were 50% ethanol and 3% Tween 80. As the positive control, a mixture of 0.18 mg/mL cypermethrin, 0.30 mg/mL chlorpyrifos, and 0.012 mg/mL citronellal (Colosso®, Ouro Fino, São Paulo) was used. This solution was diluted to a 0.125% concentration in ultrapure water. All tests were replicated three times.

Tick preparation

Engorged *R. (B.) microplus* females were collected from naturally infected cattle, washed with water and then dried with paper towels. A number of the engorged females were kept in an incubator and maintained at 27±1 °C with relative humidity (RH) ≥ 80% until oviposition of the larvae (for the larval packet test) was finished. Acaricide tests had been performed previously that demonstrated the resistance of the tick population to amidinic and pyrethroid compounds. The analyses with ticks were performed at the Unidade de Execução de Pesquisa da Embrapa Meio-Norte, in Parnaíba, Piauí.

Larval packet test

The larval packet test was used following the method of Stone & Haydock (1962) and the Food and Agriculture Organization of the United Nations (FAO, 1971). Approximately one hundred larvae, 14-21 days old, were placed between two filter papers (2×2 cm) impregnated with the appropriate essential oil and concentration, to form a sandwich. Each "sandwich" was introduced to a filter paper envelope and then sealed, identified, and incubated at 27 °C with RH ≥ 80% for 24 h (LEITE, 1988). After this time, larvae, alive and dead, were counted. Ticks showing no movement were considered dead. Each treatment was performed in triplicate. Mortality was calculated from the average of three replicates:

$$\text{Corrected mortality}(\%) = \frac{\left(\begin{matrix}\%\text{ test mortality} - \\ \%\text{ control mortality}\end{matrix}\right)\times 100}{100 - \%\text{ control mortality}} \quad (1)$$

Adult immersion test

The adult immersion test of engorged *R. (B.) microplus* females was conducted according to the method described by Drummond et al. (1973). Engorged females were selected based on their mobility, body integrity, and size (≥ 4.5 mm). The females were then weighed and distributed into ten-specimen groups with similar weights. The weight of engorged females ranged between 170 and 210 mg (BENNETT, 1974). Each group of females was immersed in their appropriate treatment solution for 5 minutes, dried with paper towels, and stored in a chamber at 27 °C and RH ≥ 80% for 18 days. After that period, the egg mass was weighed, transferred to adapted syringes, and incubated for 20 days (27 °C and RH ≥ 80%). The experiment was performed with three replicates for each treatment.

Hatchability was estimated from the average number of eggs and larvae. The egg production index (EPI), oviposition reduction (OR), reproductive efficiency (RE), and product effectiveness (PE) were calculated according to the following formulas: EPI = (weight of eggs/weight of engorged females) × 100 (BENNETT, 1974); OR = ((control EPI – treated EPI)/control EPI) × 100 (ROULSTON et al., 1968); RE = (Egg mass weight × % of eclosion/weight of the mass of females) × 20,000; and PE = (control RE – treated RE)/(control RE × 100) (DRUMMOND et al., 1973).

This study was performed with the approval of the Ethics Committee for Animal Experimentation of the Universidade Federal do Maranhão under approval number 23115018061.

Statistical analysis

The lethal concentration of the essential oil for 50% of the population (LC_{50}) of larvae and engorged females was calculated through Probit analysis with GraphPad Prism 7.0 software. An essential oil was considered to be significantly different from another one when the 95% confidence intervals of the calculated LC_{50} did not overlap (RODITAKIS et al., 2005). The difference between the concentrations of mortality against larvae and engorged females and the index RO, hatchability and EP were analysed by a paired t-test ($p<0.05$).

Results and Discussion

The chemical compositions of the tested essential oil samples are presented in Table 1. The chemical composition of the *A. zerumbet* essential oil was previously determined by Castro et al. (2016). Twenty-three, nineteen and twenty-one components were identified in the essential oils from *M. suaveolens, O. gratissimum* and *A. zerumbet,* respectively, representing over 96% of their total compounds.

The basic components of all the oils were monoterpenes and sesquiterpenes, except for the *O. gratissimum* essential oil, which contained a short-chain alcohol (3-hexen-1-ol) and a phenylpropanoid. This latter compound (eugenol) was the major component of the essential oil, approximately 53%, followed by 1,8-cineole (24.7%). The most abundant components found in the *M. suaveolens* essential oil were 1,8-cineole (35.8%) and sabinene (19.6%), while the *A. zerumbet* essential oil presented p-cymene (32.7%), 1,8-cineole (24.0%) and terpinen-4-ol (20.2%) as the main components (Table 1).

One of the major constituents in all three species of essential oils was 1,8-cineole. This pure monoterpene caused 100% lethality in *R. (B.) microplus* larvae (PRATES et al., 1998). On the other hand, 1,8-cineole extracted from *Ocimum* species and used at a 2% concentration was not an effective acaricidal against *R. (B.) microplus* (HÜE et al., 2015). This latter result may be due to a low concentration of this compound in the evaluated solution.

1,8-Cineole is present in several essential oils that do and do not have activity against *R. (B.) microplus*. At a concentration of 10.6% in the *Melinis minutiflora* essential oil and of 85.8% in *Eucalyptus globulus* essential oil, the acaricidal action of 1,8-cineole was maximum (100%) on larvae of *R. (B.) microplus* (PRATES et al., 1998; CHAGAS et al., 2002). A change in the major components of essential oils does not always lead to significate changes in their action on ticks. Minor components of the oil mixture are just as important as major constituents, and their effects must be considered as a reflection of the interaction of all the constituents (AKHTAR et al., 2012). As an example, the essential oil from *O. gratissimum* contains 24.7% of 1,8-cineole

Table 1. Comparative chemical composition of essential oils from *Mesosphaerum suaveolens, Ocimum gratissimum* and *Alpinia zerumbet*.

Compounds	KI[a]	KI_{lit} [b]	Relative percentages (%)[c]		
			M. suaveolens	*O. gratissimum*	**A. zerumbet*
3-hexen-1-ol	855	853		0.38	
á-thujene	934	930	0.43		2.57
á-pinene	941	939	3.03	0.83	1.54
Camphene	960	954	0.31		0.29
Sabinene	979	975	19.61	0.67	5.02
â-pinene	984	979	7.59	2.11	2.37
Myrcene	993	990	0.59	0.56	0.66
ä-3-carene	1019	1011	1.46		
á-terpinene	1024	1017	0.36		0.06
p-cymene	1034	1024	1.65		32.72
Limonene	1037	1029	2.79		2.09
1,8-cineole	**1038**	**1031**	**35.77**	**24.68**	**24.05**
Cis-ocimene	1040	1037		5.30	
Trans-ocimene	1051	1050		0.34	
ã-terpinene	1063	1059	0.69	0.14	0.68
Trans-4-thujanol	1075	1070			0.15
á-terpinolene	1095	1088	2.80		0.28
Linalol	1101	1096	0.24	0.43	
Cis-â-terpineol	1102				1.01
Fenchol	1122	1116			2.73
Cis-p-menth-2-en-1-ol	1129	1121			0.65
Citronellal	1156	1153	0.48		
Terpinen-4-ol	1181	1177	1.77	0.31	20.23
á-terpineol	1193	1188		0.73	
Trans-piperitol	1214				0.23
Bornyl acetate	1293	1285			0.17
Eugenol	1362	1359		52.99	
Bourbonene	1393	1388	0.31		
â-elemene	1398	1390	0.44		
â-caryophyllene	1423	1408	3.13	2.72	0.24
á-caryophyllene	1458	1454		0.36	
Germacrene D	1484	1485	3.00	1.01	
â-selinene	1489	1490		4.22	
á-selinene	1497	1498		1.50	
Bicyclogermacrene	1505	1500	7.80		
7-*epi*-á-selinene	1521	1522		0.27	
Spathulenol	1587	1578	1.05		
Caryophyllene oxide	1593	1593	0.33		1.70
Total			96.25	99.55	99.44

[a]KI: Kovats index displayed by compounds in RTX column; [b]KI_{lit}: Literature data (ADAMS, 2007; NIST, 2011); [c]Peak relative areas determined in the GC-FID chromatogram; *Data reported by Castro et al. (2016).

and presents high acaricidal activity on tick females and larvae (Table 2 and 3), however *Ocimum canum* essential oil, which is 70.2% 1,8-cineole, does not show any action on *R. (B.) microplus* (HÜE et al., 2015). It is possible that the identified components of essential oils could have antagonistic activity towards each other and consequently reduce the acaricidal action of other major compounds (SOARES et al., 2016).

All three essential oils tested in this study exhibited significant anti-tick activity against *R. (B.) microplus.* The *M. suaveolens* essential oil had significantly lower activity (LC_{50} = 51.6 mg/mL) on larvae than both the *O. gratissimum* (LC_{50} = 11.9 mg/mL) and the *A. zerumbet* (LC_{50} = 19.7 mg mL^{-1}) essential oils. Moreover it was significantly lower (LC_{50} = 31.3 mg mL^{-1}) than the *A. zerumbet* (LC_{50} = 20.7 mg mL^{-1}) essential oil on engorged females (Table 4). The presence of a probable antagonistic compound to 1,8-cineole in the *M. suaveolens* essential oil could justify the reduced larval mortality in relation to the other species. Essential oils has complex mixtures of chemical constituents that may act together synergistically or antagonistically contributing to control of ticks by different action modes (SOARES et al., 2016).

The action of the three essential oils was dose-dependent in the two different life stages of the tick. Tables 2 and 3 present the percentages obtained for in vitro action of the three essential oils on the larvae (mortality) and engorged females (egg production index, reduction in oviposition, eggs hatched and efficiency).

In addition to having the lowest LC_{50} among the essential oils tested in the present study, the *M. suaveolens* essential oil, at 100 mg/mL, reduced larvae mortality to 87.7% and had 100% efficacy on engorged females. In Laos, the *M. suaveolens* essential oil is a strong repellent against the tick *Ixodes ricinus.* This oil also has 1,8-cineole (16.5-46.6%) and sabinene (15.0-21.2%) as major compounds, which vary in concentration according to the geographic origin of the plants (ASHITANI et al., 2015). Furthermore, the chemical differences in the genotypes of the plant may influence the acaricidal activity against *R. (B.) microplus* (SOARES et al., 2016).

Table 2. Efficacy of essential oils from *Mesosphaerum suaveolens, Ocimum gratissimum* and *Alpinia zerumbet* on larvae of *Rhipicephalus (Boophilus) microplus.*

Concentration (mg/mL)	*M. suaveolens* (%)	*O. gratissimum* (%)	*A. zerumbet* (%)
Negative control	0.5 ± 0.5	1.8 ± 0.5	0.5 ± 0.9
6.2 (0.62%)	0.4 ± 0.6	20.8 ± 6.9	0.5 ± 0.8
12.5 (1.25%)	0.4 ± 0.7	65.6 ± 6.0	0.9 ± 1.6
25.0 (2.5%)	9.6 ± 7.6	91.6 ± 8.2	94.4 ± 7.0
50.0 (5%)	39.2 ± 9.7	98.3 ± 2.9	100.0 ± 0.0
100.0 (10%)	87.7 ± 3.5	99.4 ± 1.1	100.0 ± 0.0
Positive control	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0

Negative control = 50% ethanol + 3% Tween 80; Positive control = 0.18 mg/mL cypermethrin, 0.30 mg/mL chlorpyrifos, and 0.012 mg/mL citronellal.

The *O. gratissimum* essential oil tested in our study induced high mortality (98.3%) on *R. (B.) microplus* larvae at a concentration of 5.0% (50 mg/mL) (Table 2). Nevertheless, the essential oil from this plant (undisclosed chemical composition) collected in the Minas Gerais state (Brazil) caused only 74.6% of larvae mortality, even though its concentration was five times higher (25%) (HOCAYEN & PIMENTA, 2013). *Ocimum* is also known for its large chemotypic variation depending on the geographical origin (HILTUNEN & HOLM, 1999). Environmental factors could support one expressive variation in chemical composition and consequently alter the oil's acaricidal efficacy (HÜE et al., 2015). In regards to engorged females, the *O. gratissimum* essential oil of this study at 10% (100 mg/mL) achieved 99.9% efficacy.

The proportion of the major compounds of the *A. zerumbet* essential oil (p-cymene, 1,8-cineole, 4-terpineol) in this study are similar to those reported for this species that greatly inhibited (81.2%) the egg hatch of the parasite *Haemonchus contortus* (MACEDO et al., 2013). The larvicidal effect of essential oils from *A. zerumbet* and *O. gratissimum* (100 mg/mL) was similar to that induced by chemical acaricide (0.18 mg/mL cypermethrin, 0.30 mg/mL chlorpyrifos, and 0.012 mg/mL citronellal).

In engorged females, the three essential oils, at 100 mg/mL, greatly reduced oviposition (ranging from 95.5 to 100.0%) and hatchability (0.0 a 0.8%), exhibiting an average efficiency that ranged from 99.9 to 100%, but there was no significant difference among them at this concentration ($p > 0.05$). This action on engorged females was similar to that induced by the positive control (Table 3).

The susceptibility of adult *Rhipicephalus* spp. to essential oils generally appears to be substantially lower than that of the larvae (ELLSE & WALL, 2014). As an example, in this study, the essential oils from *A. zerumbet* and *O. gratissimum* obtained an

Table 3. Egg production index (EPI), reduction in oviposition (RO), eggs hatched and efficiency (EP) of essential oil from *Mesosphaerum suaveolens, Ocimum gratissimum* and *Alpinia zerumbet* on engorged females of *Rhipicephalus (Boophilus) microplus*.

	mg/mL	EPI	RO (%)	Eggs hatched (%)	EP (%)
Negative control	-	47.2 ± 7.4	-	83.4 ± 7.9	-
M. suaveolens	6.2	51.3 ± 2.5	4.3 ± 7.5	72.1 ± 2.3	9.4 ± 14.7
	12.5	40.2 ± 5.2	15.3 ± 15.6	73.8 ± 2.7	23.4 ± 14.4
	25.0	31.9 ± 7.6	32.8 ± 8.8	75.9 ± 5.8	39.0 ± 7.1
	50.0	11.8 ± 1.6	74.6 ± 5.8	68.0 ± 9.3	79.3 ± 5.0
	100.0	0.0 ± 0.0	100.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0
O. gratissimum	6.2	45.4 ± 3.3	10.8 ± 7.8	70.6 ± 13.1	22.6 ± 21.7
	12.5	42.8 ± 4.7	17.7 ± 8.3	66.2 ± 2.5	32.2 ± 12.5
	25.0	34.2 ± 9.1	34.2 ± 17.8	56.8 ± 10.8	51.6 ± 24.4
	50.0	19.7 ± 8.0	62.2 ± 15.3	24.1 ± 12.7	90.1 ± 4.9
	100.0	2.3 ± 2.6	95.5 ± 5.2	0.8 ± 1.3	99.9 ± 0.2
A. zerumbet	6.2	50.3 ± 2.0	1.9 ± 2.3	87.6 ± 1.7	7.2 ± 6.6
	12.5	48.7 ± 3.4	2.5 ± 2.7	68.9 ± 5.1	29.0 ± 6.6
	25.0	33.3 ± 5.6	33.5 ± 7.4	52.2 ± 4.3	63.2 ± 6.6
	50.0	9.0 ± 8.3	82.1 ± 15.8	31.9 ± 12.9	92.6 ± 6.7
	100.0	0.8 ± 1.4	98.2 ± 3.1	0.0 ± 0.0	100.0 ± 0.0
Positive control		1.7 ± 2.8	96.1 ± 6.5	11.1 ± 18.4	98.6 ± 2.4

Negative control = 50% ethanol + 3% tween 80; Positive control = 0.18 mg/mL cypermethrin, 0.30 mg/mL chlorpyrifos, and 0.012 mg/mL citronellal. EPI = egg production index; RO = reduction in oviposition; EP = efficiency of the product.

Table 4. Lethal concentration of the essential oils from *Mesosphaerum suaveolens, Ocimum gratissimum* and *Alpinia zerumbet* against *Rhipicephalus (Boophilus) microplus* larvae and engorged females.

Treatment	LC_{50} (mg/mL)	CL 95%	R^2
Larvae			
M. suaveolens	51.6[c]	47.7-55.9	0.97
O. gratissimum	11.9[a]	11.0-13.0	0.97
A. zerumbet	19.7[b]	15.7-24.6	0.99
Engorged female			
M. suaveolens	31.3[b]	26.4-37.0	0.93
O. gratissimum	28.4[ab]	21.9-36.9	0.85
A. zerumbet	20.7[a]	18.8-22.8	0.98

LC_{50} = Lethal concentration 50% (mg/mL) *R. (Boophilus) microplus* larvae or engorged females; CL = 95% Confidence limit; R^2 = Regression coefficient. Values followed by the same letters do not differ significantly, alpha = 5%.

efficacy value of 99.9 to 100.0% against engorged females when used at a concentration of 100 mg/mL, while similar values of larvae mortality (98.3 to 100%) were observed at concentrations 50% lower (50 mg/mL). However, opposite results were obtained when the *M. suaveolens* essential oil was evaluated, which had a higher activity against the engorged females (100%) than the *R. (B.) microplus* larvae (87.7%) at 100 mg/mL. This variation in activity is probably related to each oil's unique mode of action against the specific life stage of the tick (SOARES et al., 2016).

In this study, the first point of contact of the essential oils is the cuticle of engorged females and larvae (DRUMMOND et al., 1973; LEITE, 1988). When crossing the integument of the tick, the substances can reach the haemolymph and act on the internal organs (REMEDIO et al., 2015). Certain substances may affect the salivary glands, consequently impairing the process of concentrating ingested blood and thus hindering the effective absorption of nutrients by the ticks (REMEDIO et al., 2016). There is an important correlation between the tick's digestive and reproductive system and, thus, digestive problems can damage their reproductive capacity (REMEDIO et al., 2016). The cattle tick has a high biotic potential (OLIVER, 1989), therefore products and substances that act on their reproductive system are of great interest in the control of these populations (OLIVEIRA et al., 2016). The ovary of *R. (B.) microplus* consists of an epithelial cell wall and oocytes (germ cells) that are at different developmental stages (OLIVEIRA et al., 2005). An injury to the oocytes decreases the female's capacity to produce viable eggs and descendents. A high reduction in oviposition and hatchability resulted in a high efficiency of the essential oils from *A. zerumbet, O. gratissimum* and *M. suaveolens,* at 100 mg/mL, against engorged females of *R. (B.) microplus* (Table 3).

Functional impairment of the reproductive system of ticks is usually a consequence of significant morphophysiological alterations that have been detected in females exposed to andiroba oil (*Carapa guianensis* Aubl.) (VENDRAMINI et al., 2012) and to neem oil (*Azadirachta indica* A. Juss.) (REMEDIO et al., 2015). Some extracts of plants, when used to treat females in the beginning or in the middle of the engorgement process, are more efficient in the control of this parasite. Thus, the substances can penetrate the cells more easily and act in their interior (OLIVEIRA et al., 2016). It is reasonable to assume that the efficacy of the three essential oils in this study would be higher in early stages of engorged females even at lower concentrations.

The tick has a central nervous system that is known as synganglion (ROMA et al., 2012). Neural morphological damage resulting from a degenerative process would be capable of affecting nerve impulse transmission in ticks, impairing locomotion and feeding mechanisms. Some oils that originate from plants promote such morphological changes (ROMA et al., 2013). Neurotoxic action can be caused also by oils and compounds that inhibit acetylcholinesterase (AChE), an enzyme essential for the transmission of action potentials (MILLS et al., 2004; LÓPEZ & PASCUAL-VILLALOBOS, 2010). The monoterpenoid 1,8-cineole inhibit AChE activity from insects (ABDELGALEIL et al., 2009) and is one of the main compounds of the three essential oils evaluated in this study whose efficacy on larvae and females was demonstrated. This result indicates the possible action of these three essential oils on the nervous system of the tick.

The possibility of reduction in risks of contamination of food and the environment by alternative products is a worldwide desire (ADENUBI et al., 2016). However, more studies should be performed to develop formulations that protect this oils and your active compounds from environmental degradation. These results, demonstrated that the essential oils evaluated may be a viable alternatives to synthetic acaricides.

Conclusion

All three essential oils evaluated show a high efficacy against engorged females at their highest concentration and produced similar results to those induced by chemical acaricide. In regards to larvae, *O. gratissimum* was the most efficient, followed by the *A. zerumbet* and the *M. suaveolens* essential oils. Our results indicated that minor compounds interfere on the action of 1,8-cineole present in essential oils.

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