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ORIGINAL ARTICLE

In vitro effect of seven essential oils on the reproduction of the cattle tick *Rhipicephalus microplus*

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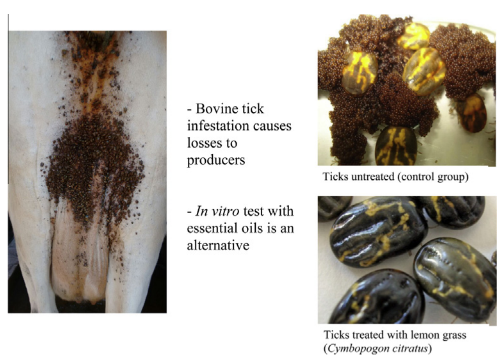
GRAPHICAL ABSTRACT



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ABSTRACT

The acaricidal effect of seven essential oils was examined *in vitro* against the cattle tick (*Rhipicephalus microplus*). Engorged female ticks were manually collected in farms of Southern Brazil and placed into petri dishes ($n = 10$) in order to test the following oils: juniper (*Juniperus communis*), palmarosa (*Cymbopogon martinii*), cedar (*Cedrus atlantica*), lemon grass (*Cymbopogon citratus*), ginger (*Zingiber officinale*), geranium (*Pelargonium graveolens*) and bergamot (*Citrus aurantium var bergamia*) at concentrations of 1%, 5%, and 10% each. A control group was used to validate the tests containing Triton X-100 only. Treatment effectiveness was measured considering inhibition of tick oviposition (partial or total), egg's weight, and hatchability. *C. martinii*, *C. citratus* and *C. atlantica* essential oils showed efficacy higher than 99% at all concentrations tested. In addition, *J. communis*, *Z. officinale*, *P. graveolens*, and *C. aurantium var bergamia* oils showed efficiency ranging from 73% to 95%, depending on the concentration tested, where higher concentrations showed greater efficacy. It was concluded that essential oils can affect tick reproduction *in vitro* by inhibiting oviposition and hatchability.

Introduction

The cattle tick *Rhipicephalus microplus* stands out as the most harmful pest for cattle, causing animal stress, lower growth, and poor performance, in addition to higher production costs due to constant anti-parasitic treatments [1,2]. The economic impact caused by cattle ticks in Brazil is of approximately $3.24 billion dollars a year [1] since climatic conditions are favorable to their survival and development [3], increasing control costs with synthetic acaricides [4]. Moreover, restrictions on the use of insecticides and acaricides, such as organophosphates due to their effects on human and animal health [5], and the environment [6] have enhanced the development of effective alternatives for control, including essential oils.

The essential oils used in this study have exhibited several biological activities as previously described in the literature. Essential oils from *Cymbopogon citratus* (Poaceae family), *Cymbopogon martinii* (Gramineae family) and *Juniperus communis* (*Cupressaceae* family) have showed antioxidant [7], antimicrobial, antifungal and anthelmintic properties [8,9]. *Cedrus atlantica* (Pinaceae family) is the plant with fewer studies, even though its analgesic property has been described [10]. *In vitro, Zingiber officinale* (Zingiberaceae family) extract was able to reduce *Streptococcus mutans* and *Streptococcus sanguinis* growth with minimum inhibitory concentration of 0.02 mg/mL and 0.3 mg/mL, respectively [11]. The *Pelargonium graveolens* essential oil has been used due to its hypoglycemic and antioxidant [12] properties, and exhibits also antifungal and insecticidal activities against *Rhizoctonia solani* and *Rhysopertha dominica,* respectively [13]. The use of *Citrus aurantium* essential oil by Homa et al. [14] revealed the antifungal activity against different isolates of *Fusarium keratitis,* antibacterial activity against Vibrio species [15], as well as insecticidal activity against *Aedes aegypti* and *Anopheles dirus* [16]. As mentioned above, there are many properties of these essential oils, but there are only few studies on their acaricidal effects despite the great interest on finding alternative control methods. Therefore, this study aimed to evaluate the *in vitro* effect of essential oils (*C. martinii*, *C. citratus*, *C. atlantica, J. communis*, *Z. officinale*, *P. graveolens*, and *Citrus aurantium var bergamia*) on cattle tick *R. microplus.*

Material and methods

Essential oils

Seven essential oils were used to test the reproduction of engorged *R. microplus* females. The oils used were as follows: juniper (*J. communis*), palmarosa (*C. martinii*), cedar (*C. atlantica*), lemon grass (*C. citratus*), ginger (*Z. officinale*), geranium (*P. graveolens*), and bergamot (*C. aurantium var bergamia*). Three concentrations (1%, 5%, and 10%, i.e. 1v/99v, 5v/95v, and 10v/90v, respectively) were evaluated and Triton X-100 (Sigma Aldrich®, São Paulo, Brazil) was used as surfactant (1v/v), in addition to distilled water [17]. The essential oils of juniper, palmarosa, and lemon grass were acquired from BioEssencia® (São Paulo, Brazil), while essential oils of cedar, ginger, geranium, and bergamot were acquired from Phytoterápica® (São Paulo, Brazil).

Gas chromatography-flame ionization detector (GC-FID) of essential oils

The gas chromatography (GC) analyses were carried out using an 6890N GC-FID system equipped with DB-5 capillary column (30 m × 0.25 mm; film thickness of 0.25 mm) (Agilent Technologies, Santa Clara, United States) connected to an FID detector. The injector and detector temperatures were set at 280 °C at a rate of 5 °C/min. The carrier gas was helium (> 99.2% purity) at a flow rate of 1.3 mL/min. All samples were analyzed in duplicate. Relative component concentrations were calculated based on GC peak areas without using correction factors [18].

Gas chromatography–mass spectrometry (GC–MS)

GC–MS analyses were performed on Agilent Technologies AutoSystem XL GC–MS operated in the EI mode at 70 eV

(Hewlett Packard, Palo Alto, CA, USA) equipped with a splitless injector (250 °C). The transfer line temperature was 280 °C. Helium was used as the carrier gas (1.3 mL/min) and the capillary columns were DB-5 and HP5 MS (30 m × 0.25 mm; film thickness of 0.25 mm). Column temperature was programmed on 40–220 °C at 3 °C/min. The oils were diluted in hexane (1:5, v/v) and 1 μL was injected.

Identification of the constituents was performed on the basis of retention index (RI) on DB-5 capillary column, determined in relation to homologous series of *n*-alkanes (C_7–C_{30}) with those reported in the literature. Fragmentation patterns in the mass spectra library search (NIST and Wiley) were compared with those stored on databases [19]. The quantification of the compounds was performed on the basis of their relative peak areas on DB-5.

Ticks

The ticks were collected from dairy cows naturally infested in farms located in Quilombo city, Santa Catarina State, Southern Brazil. These animals did not receive any acaricidal treatment in the last 50 days prior to the beginning of the study in order to avoid any negative interference. The engorged female ticks were stored in plastic bottles, packed in a cooler (±15 °C), and immediately transported to the laboratory where the bioassays were conducted.

Bioassays

In the laboratory, engorged females ticks with similar weights were randomly distributed, placed into covered petri dishes during the incubation period. The experimental design was completely randomized with three replicates per oil concentration, and 10 ticks for each petri dish (total of 30 ticks per oil tested). The tests were performed according to the methodology described by Drummond et al. [20], where ticks were immersed for five minutes in the test solutions with essential oils at concentrations of 1%, 5%, and 10%. After that, they were dried and incubated under controlled conditions (25 °C; 75% relative humidity (RH)) for 14 days. Subsequently, oviposition was recorded as total, partial or absent and their eggs were weighted. Laid eggs were placed into glass tubes and incubated for 30 days in order to verify hatchability, which was measured considering the number of remaining eggs that did not hatch and the number of shells, versus the number of larvae (active or inactive) [21].

A control group containing only the diluents (water + Triton X-100) at concentration of 10% of Triton was used. The results were tabulated and reproductive efficiency (RE) and effectiveness of the treatment (ET) were calculated as described by Drummond et al. [20] [RE = egg weight × % of hatchability × 20,000/weight of engorged female ticks; ET = (RE control − RE treatment) × 100/RE control].

Table 1 Mean and standard deviation of the weight of engorged tick, number of postures by treatment, egg weight, and hatchability after treatment with essential oils of juniper (*J. communis*), palmarosa (*C. martinii*), cedar (*C. atlantica*), lemon grass (*C. citratus*), ginger (*Z. officinale*), geranium (*P. graveolens*) and bergamot (*C. aurantium bergamia*).

Treatment	Engorged tick weight (g)	Number posture by treatment* (n = 10)	Weighing eggs per treatment (g)	Hatchability (%)
Control	0.190 ± 0.016	$10.0^{a} \pm 0.0$	$0.96^{a} \pm 0.03$	90
Juniper 1%	0.198 ± 0.021	$8.0^{b} \pm 1.1$	$0.35^{c} \pm 0.01$	38
Juniper 5%	0.201 ± 0.011	$7.0^{bc} \pm 1.5$	$0.28^{d} \pm 0.03$	10
Juniper 10%	0.187 ± 0.018	$7.0^{bc} \pm 0.2$	$0.25^{de} \pm 0.02$	08
Palmarosa 1%	0.177 ± 0.019	$5.0^{d} \pm 1.1$	$0.14^{ef} \pm 0.01$	03
Palmarosa 5%	0.203 ± 0.013	$2.0^{e} \pm 1.0$	$0.06^{g} \pm 0.01$	00
Palmarosa 10%	0.192 ± 0.022	$0.3^{f} \pm 0.5$	$0.06^{g} \pm 0.01$	00
Cedar 1%	0.196 ± 0.016	$8.7^{ab} \pm 1.1$	$0.51^{b} \pm 0.04$	00
Cedar 5%	0.184 ± 0.020	$6.6^{bc} \pm 0.5$	$0.35^{c} \pm 0.05$	00
Cedar 10%	0.188 ± 0.012	$4.6^{d} \pm 1.1$	$0.06^{g} \pm 0.01$	00
Lemon grass 1%	0.204 ± 0.018	$8.6^{ab} \pm 1.1$	$0.27^{d} \pm 0.02$	00
Lemon grass 5%	0.179 ± 0.015	$5.6^{cd} \pm 1.5$	$0.27^{d} \pm 0.03$	00
Lemon grass 10%	0.192 ± 0.017	$4.3^{d} \pm 1.2$	$0.12^{f} \pm 0.01$	00
Ginger 1%	0.185 ± 0.014	$8.6^{ab} \pm 1.5$	$0.42^{bc} \pm 0.06$	15
Ginger 5%	0.194 ± 0.016	$7.0^{bc} \pm 1.7$	$0.13^{f} \pm 0.04$	06
Ginger 10%	0.205 ± 0.019	$4.3^{d} \pm 0.6$	$0.20^{e} \pm 0.01$	05
Geranium 1%	0.191 ± 0.013	$9.0^{ab} \pm 1.0$	$0.42^{bc} \pm 0.04$	13
Geranium 5%	0.188 ± 0.017	$6.3^{cd} \pm 2.0$	$0.16^{e} \pm 0.02$	09
Geranium 10%	0.199 ± 0.015	$5.3^{d} \pm 1.2$	$0.09^{fg} \pm 0.01$	05
Bergamot 1%	0.178 ± 0.010	$7.3^{bcd} \pm 1.1$	$0.36^{c} \pm 0.05$	20
Bergamot 5%	0.197 ± 0.014	$6.3^{cd} \pm 0.6$	$0.26^{d} \pm 0.03$	11
Bergamot 10%	0.180 ± 0.013	$6.3^{cd} \pm 1.5$	$0.29^{cd} \pm 0.04$	08

Note: Means followed by the same letter in the same column do not differ statistically among themselves, the significance level of 5% ($P > 0.05$).

* Number of engorged females (ticks) that perform posture (partial or total) per treatment, and "*n*" by repeating 10 specimens (test performed in triplicate).

Statistical analysis

The data collected were subjected to normality test which showed normal distribution. Then, the data were analyzed statistically by analysis of variance (one-way ANOVA) and Duncan's test. The results were considered significant when $P < 0.05$.

Results

In vitro test

The number of ticks that had partial or total oviposition, as well as egg weight, and percentage of hatched larvae is shown in Table 1. All results were compared to the control group that showed total oviposition and 86.3% of hatchability. The use of *J. communis* oil caused partial oviposition of smaller eggs ($P = 0.0032$) in all concentrations tested, even though it was unable to inhibit hatchability. On the contrary, the use of *C. martinii* oil (1%) led to lower egg hatchability ($P = 0.0012$), in addition to lower oviposition and egg weight, on a dose-dependent effect. *C. atlantica*, *C. citratus*, *Z. officinale*, and *P. graveolens* essential oils tested at 1% were unable to reduce the number of ticks that showed oviposition, i.e. these oils did not cause any effect on reproduction ($P = 0.142$), which was not observed at concentrations of 5 and 10% ($P = 0.092$). *C. atlantica* and *C. citratus* oils were able to inhibit hatchability, an effect not seen for *Z. officinale* and *P. graveolens* oils. *C. aurantium var bergamia* oil was able to reduce the number of ticks that performed oviposition and the weight of eggs at all concentrations, but did not inhibit hatchability.

Data on tick reproductive efficiency and oil treatment efficacy are shown in Table 2. Oil treatment was able to significantly reduce tick reproductive efficiency compared to the control group ($P = 0.0001$). Regarding *C. atlantica* and *C. citratus* oils, all concentrations tested interfered with the reproduction of cattle ticks (100% efficacy) similar to *C. martinii* oil at 5% and 10%. The *C. aurantium var bergamia*, *Z. officinale*, *J. communis*, and *P. graveolens* oils at concentration 10%, exhibited an approximate efficiency of 90%, 94%, 96%, and 97%, respectively.

Table 2 Reproductive efficiency and effectiveness of treatment of seven essential oils against cattle tick *Rhipicephalus microplus*.

Treatment	Reproductive efficiency (%)	Treatment efficacy (%)
Control	81.0	0.0
Juniper 1%	23.3	73.8
Juniper 5%	4.2	95.2
Juniper 10%	3.6	96.3
Palmarosa 1%	1.8	99.7
Palmarosa 5%	0.0	100.0
Palmarosa 10%	0.0	100.0
Cedar 1%	0.0	100.0
Cedar 5%	0.0	100.0
Cedar 10%	0.0	100.0
Lemon grass 1%	0.0	100.0
Lemon grass 5%	0.0	100.0
Lemon grass 10%	0.0	100.0
Ginger 1%	5.7	85.7
Ginger 5%	2.9	92.6
Ginger 10%	2.5	94.0
Geranium 1%	5.6	85.9
Geranium 5%	3.3	91.6
Geranium 10%	1.2	97.0
Bergamot 1%	5.1	84.9
Bergamot 5%	5.3	86.6
Bergamot 10%	3.7	90.5

Oil composition

The major components found in each oil were as follows: linalool (*J.* communis; 18.07%), geraniol (*C.* martini; 35.27%), α-himachalene (*C. atlantica*; 19.74%), geranial (*C. citratus*; 46.51%), α-zingiberene (*Z. officinale*; 26.47%), citronellol (*P. graveolens*; 31.37%), and limonene (*C. aurantium var bergamia*; 30.17%) (Suppl. Table 1).

Discussion

In this study, it was observed that the *J. communis* oil was able to partially inhibit oviposition, and therefore, reduce tick reproductive efficiency. Carrol et al. [22] reported repellent action of juniper oil against two species of ticks (*Amblyomma americanum* and *Ixodes scapularis*). Studies conducted by Dietrich et al. [23] and Dolan et al. [24] have reported that the *J. communis* oil is a rich source of anti-tick compounds with well-known repellent and insecticidal activities. Researchers also found 43.2% of repellent effect for juniper oil against *A. aegypti* after 210 min of application [25].

Additionally, *C. citratus* oil showed 100% efficacy against *R. microplus*, similar to those findings reported by other authors [26,27]. The effectiveness of *C. citratus* oil on ticks, according to Tchoumbougnang et al. [28] may be due to its geraniol content, measured as 47%. *C. martinii* oil at 5% and 10% showed 100% efficacy against adult ticks in this current study, and this oil has been studied for its repellent activity to insects [29,30] and antifungal actions [31], but it had not been tested on cattle ticks yet.

Z. Officinale belongs to Zingiberceae family, an aromatic plant used as spice and in medicine. According to the literature, the *Z. officinale* oil showed bactericidal effect on *Staphylococcus aureus* [32], repellent activity against mosquitoes of the species *Culex quinquefasciatus* [33], as well as repellent effect against *Leptotrombidium deliense* larvae, a species of mite [34], similar to the cattle tick used in this study.

The *C. aurantium var bergamia* oil negatively affected the reproduction of cattle tick. According to the literature, some compounds present in *Citrus* sp. essential oils showed repellant effect against mosquitoes and ticks [35]. Already, the *Cedrus deodara* oil demonstrated strong effect against cattle ticks [36], similar to what was observed in this study, even though a different kind of *C. atlantica* was used in this current study. Another study also reported efficacy to control cattle tick using a herbal preparation containing extracts of *C. deodara, Azadirachta indica,* and *Embelia ribes* [37], and according to these authors, these extracts have acaricidal effect against larvae, nymphs, and adult stages of ticks.

The *P. graveolens* oil showed some effect on tick oviposition (inhibited or reduced), but it did not interfere on hatchability. Tabanca et al. [38] tested ten essential oils of *P. graveolens* and demonstrated repellent activities against nymphs of the medically important lone star tick, *A. americanum*. Researchers described that *P. graveolens* oil showed 100% repellency against host-seeking nymphs of *Ixodes ricinus* [39].

Conclusions

Based on these *in vitro* results it is possible to conclude that *C. martinii*, *C. citratus*, and *C. atlantica* oils may interfere on cattle tick reproduction. The essential oils of *J. communis*, *Z. officinale*, *P. graveolens*, and *C. aurantium var bergamia* also caused a negative effect on tick reproduction, but they were unable to inhibit hatchability. The use of essential oils in the control of *R. microplus* shows great potential for the future as an alternative method besides chemical products. Note that more tests, especially *in vivo*, are needed, in order to conclude whether such oils could be used as an alternative for the control of cattle ticks, and this is the main perspective of our research group.

Conflict of Interest

The authors have declared no conflict of interest.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jare.2016.05.003.

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