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Short communication

## Acaricidal potential of volatile oils from *Croton* species on *Rhipicephalus microplus*

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### ABSTRACT

The objective of this study was to evaluate the acaricidal activity of the volatile oils of three species of *Croton*, Euphorbiaceae, against the cattle tick *Rhipicephalus microplus*. The volatile oils were obtained by hydrodistillation, analyzed by GC–MS and GC-FID and their acaricidal activity was evaluated by the larval packet test and adult immersion test. The volatile oils from *Croton conduplicatus* Kunth, *Croton pulegioidorus* Baill., and two different collections of *Croton grewiooides* Baill. (CG1 and CG2) showed eucalyptol (24.09%), *p*-cymene (23.13%) and methyl chavicol (83.59% and 95.38%) as the major compounds, respectively. All the volatile oils tested in this study showed efficacy against larvae and engorged females of *Rhipicephalus microplus*. Therefore, *Croton pulegioidorus* volatile oil is promising for a potential acaricidal formulation because of the best activity against both stages of the cattle tick.

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### Introduction

*Rhipicephalus microplus* (Canestrini 1888) is responsible for significant economic losses in cattle production (Grisi et al., 2014). Chemical control has been the most used method for controlling ticks. However, populations of these parasites are resistant to most of the commercially available acaricides in the world (Graf et al., 2004). The use of plants with acaricide compounds have been proposed to reduce the environmental and financial impact of synthetic acaricides (Castrejon, 2003).

Brazil possesses a great diversity of medicinal plants; therefore, their extracts have been tested against parasites. Significant results *in vitro* have been obtained against tick with some extracts from *Piper tuberculatum* and *Lippia gracilis* (Chagas et al., 2012; Cruz et al., 2013). Advantages of acaricides plant-based preparations are their generally lower toxicity for host animals, rapid degradation and slow development of resistance in acari (Chungsamarnyart et al., 1991).

The Caatinga is an exclusively Brazilian biome, one of the most diverse in the world and an almost unexplored source of biologically active substances (Albuquerque and Oliviera, 2007). Various species of the genus *Croton*, Euphorbiaceae, are common plants of this biome. Extracts of *Croton sphaerogynus* Baill. and *C. joufra* Roxb. have been shown to be effective against *R. microplus* (Righi et al., 2013). Following up these investigations, the objective of the study reported here was to make a quantitative evaluation of acaricidal activity on *R. microplus* using volatile oils from three native species of *Croton* from the Caatinga biome in Piauí state, northeast Brazil.

### Materials and methods

Specimens of *Croton conduplicatus* Kunth. (8°20'43.8"S, 42°19'34.3"W), *C. pulegioidorus* Baill. (8°20'38.9" S, 42°19'35.1" W) and *C. grewiooides* Baill., Euphorbiaceae (08°20'15.2"S, 042°17'57.8"W) were collected in April 2015 in the municipality of São João do Piauí, Piauí state, northeast Brazil. The latter species was also collected in May 2015 in the municipality of Caxingó, Piauí state (3°21'41.25", 41°48'09.5"W). Reference specimens of *C. conduplicatus*, *C. pulegioidorus* and *C. grewiooides* (CG1) collected in São João do Piauí were deposited at the Herbar-

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**Table 1**  
Comparative chemical composition of volatile oils from *Croton conduplicatus*, *C. pulegioides* and *C. grewoides* (CG1 and CG2).

| Compounds                 | KI <sup>a</sup> | KI <sub>lit</sub> <sup>b</sup> | Relative percentages (%) <sup>c</sup> |                       |        |        |
|---------------------------|-----------------|--------------------------------|---------------------------------------|-----------------------|--------|--------|
|                           |                 |                                | <i>C. conduplicatus</i>               | <i>C. pulegioides</i> | CG1    | CG2    |
| α-Thujene                 | 937             | 930                            | 1.50                                  | 1.09                  |        |        |
| α-Pinene                  | 947             | 939                            | 5.46                                  | 1.33                  | 0.13   |        |
| Camphene                  | 963             | 954                            | 0.33                                  | 0.83                  |        |        |
| Sabinene                  | 985             | 975                            | 6.49                                  | 0.41                  | 0.57   | 0.17   |
| β-Pinene                  | 990             | 979                            | 0.52                                  | 0.75                  |        |        |
| Myrcene                   | 996             | 990                            | 0.59                                  | 3.14                  | 0.50   | 0.79   |
| α-Phellandrene            | 1012            | 1002                           | 9.88                                  | 1.65                  | 0.12   |        |
| α-Terpinene               | 1027            | 1017                           |                                       | 9.32                  |        |        |
| p-Cymene                  | 1034            | 1024                           | 6.40                                  | 23.13                 |        |        |
| Limonene                  | 1039            | 1029                           |                                       | 2.16                  |        |        |
| o-Cymene                  | 1039            | 1026                           |                                       |                       | 0.11   |        |
| Eucalyptol                | 1044            | 1031                           | 24.09                                 | 1.57                  | 4.27   | 0.98   |
| E-β-Ocimene               | 1054            | 1050                           |                                       |                       | 0.41   | 0.18   |
| γ-Terpinene               | 1068            | 1059                           | 0.52                                  | 5.88                  |        |        |
| Terpinolene               | 1097            | 1088                           |                                       | 0.59                  |        |        |
| Chrysanthenone            | 1137            | 1127                           | 0.79                                  |                       |        |        |
| Limonene oxide            | 1148            | 1136                           |                                       | 0.80                  |        |        |
| Camphor                   | 1159            | 1146                           |                                       | 8.26                  |        |        |
| β-Pinene oxide            | 1167            | 1159                           |                                       | 0.46                  |        |        |
| Isoborneol                | 1170            | 1160                           |                                       | 2.33                  |        |        |
| cis-Chrysanthenol         | 1172            | 1164                           | 2.00                                  |                       |        |        |
| Borneol                   | 1178            | 1169                           | 0.57                                  | 0.57                  |        |        |
| Terpinen-4-ol             | 1183            | 1177                           | 1.78                                  | 2.22                  | 0.19   |        |
| α-Terpineol               | 1199            | 1188                           | 3.89                                  | 0.77                  |        | 0.18   |
| trans-Isocarveol          | 1196            | 1189                           |                                       | 0.30                  |        |        |
| Myrtenol                  | 1206            | 1195                           | 0.26                                  | 0.30                  |        |        |
| Methyl chavicol           | 1204            | 1196                           |                                       |                       | 83.59  | 95.38  |
| Ascaridole                | 1252            | 1237                           |                                       | 22.50                 |        |        |
| trans-Piperitone epoxide  | 1266            | 1256                           |                                       | 0.45                  |        |        |
| cis-Chrysanthenyl acetate | 1270            | 1265                           | 1.05                                  |                       |        |        |
| Tymol                     | 1297            | 1290                           |                                       | 0.37                  |        |        |
| Carvacrol                 | 1308            | 1299                           |                                       | 0.35                  |        |        |
| Eugenol                   | 1368            | 1359                           |                                       |                       | 4.34   |        |
| α-Copaene                 | 1387            | 1376                           | 0.37                                  |                       |        |        |
| β-Elemene                 | 1401            | 1390                           | 1.69                                  | 0.74                  | 0.13   | 0.22   |
| Methyl eugenol            | 1411            | 1403                           |                                       |                       | 1.44   |        |
| β-Caryophyllene           | 1434            | 1419                           | 5.97                                  |                       | 0.85   | 0.62   |
| α-Caryophyllene           | 1468            | 1454                           | 1.09                                  | 0.27                  | 0.09   |        |
| allo-Aromadendrene        | 1475            | 1460                           | 1.24                                  |                       |        |        |
| γ-Murolene                | 1494            | 1479                           | 1.53                                  |                       |        |        |
| Germacrene D              | 1494            | 1481                           |                                       |                       | 0.32   | 0.43   |
| β-Selinene                | 1499            | 1490                           | 0.74                                  |                       |        |        |
| Bicyclgermacrene          | 1511            | 1500                           | 5.93                                  |                       | 2.14   | 0.94   |
| Germacrene A              | 1517            | 1509                           | 0.02                                  | 0.02                  |        |        |
| γ-Cadinene                | 1528            | 1513                           | 0.93                                  |                       |        |        |
| δ-Cadinene                | 1536            | 1523                           | 0.77                                  |                       | 0.08   |        |
| cis-Calamenene            | 1536            | 1529                           |                                       |                       |        | 0.10   |
| Spathulenol               | 1593            | 1578                           | 6.23                                  |                       | 0.48   |        |
| Caryophyllene oxide       | 1599            | 1583                           |                                       |                       | 0.23   |        |
| β-Copaen-4-α-ol           | 1598            | 1590                           | 3.41                                  |                       |        |        |
| α-Murolol                 | 1655            | 1646                           | 3.64                                  |                       |        |        |
| Valerianol                | 1670            | 1658                           | 0.30                                  |                       |        |        |
| Total                     |                 |                                | 100.00                                | 92.57                 | 100.00 | 100.00 |

<sup>a</sup> KI: Kovats index displayed by compounds in RTX column.<sup>b</sup> KI<sub>lit</sub>: Literature data (Adams, 2007).<sup>c</sup> Peak relative areas determined in the GC-FID chromatogram.

ium of the Embrapa Recursos Genéticos e Biotecnologia (CEN) (registration numbers 92493, 92494, and 92492, respectively), and the specimens of *C. grewoides* collected in Caxingó (CG2) was deposited at the Herbário Prisco Bezerra (EAC) under the registration number 56771. This study was registered in the Sisgen database (Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado) under protocol A0981DB. The study has been approved by the Ethics Committee on Animal Use of the Universidade Federal do Maranhão under protocol 23115018061.

Volatile oils from leaves of the three species were extracted by hydrodistillation using a Clevenger apparatus. The chemical analyses were carried out using gas chromatography–mass spectroscopy

(GC–MS) and gas chromatography flame ionization detector (GC–FID) methodology. The experimental protocol for extraction and chemical characterization are described by Castro et al. (2019). Five concentrations, ranging from 6.2–100.0 mg/ml, of each volatile oil were used for the larval packet test and adult immersion test. A solution of 50% ethanol + 1% Tween 80 was used as the negative control, and a mixture of 0.18 mg/ml cypermethrin, 0.30 mg/ml chlorpyrifos, and 0.012 mg/ml citronellal (Colosso®, Ouro Fino, São Paulo) diluted in ultrapure water (0.125%), was used for the positive control. The experiment was performed with three replicates for each treatment.

Engorged females and larvae of *R. microplus* were collected in August 2015 from naturally infected cattle. Larvae aged between

**Table 2**Action of *Croton conduplicatus*, *C. pulegioidorus* and *C. grewioides* (CG1 and CG2) on larvae (mortality) and engorged female (egg production index, reduction of oviposition, hatchability and product efficacy) of *Rhipicephalus microplus*.

| Treatment               | Concentration (mg/ml) | Larvae (%)                 |                         | Engorged females (%)     |                            |                            |
|-------------------------|-----------------------|----------------------------|-------------------------|--------------------------|----------------------------|----------------------------|
|                         |                       | Mortality                  | Eggs production index   | Reduction of oviposition | Hatchability               | Product efficacy           |
| Negative control        | –                     | 0.0 ± 0.0 <sup>a</sup>     | 51.5 ± 4.0 <sup>a</sup> | –                        | 91.1 ± 3.8 <sup>a</sup>    | –                          |
| <i>C. conduplicatus</i> | 6.2                   | 2.5 ± 2.7 <sup>d</sup>     | 49.3 ± 1.0 <sup>a</sup> | 4.2 ± 2.0 <sup>a</sup>   | 74.8 ± 7.6 <sup>d</sup>    | 21.6 ± 7.0 <sup>a</sup>    |
|                         | 12.5                  | 11.4 ± 1.6 <sup>d</sup>    | 45.8 ± 6.6 <sup>d</sup> | 11.1 ± 12.9 <sup>d</sup> | 60.8 ± 10.7 <sup>a,b</sup> | 39.7 ± 18.2 <sup>d</sup>   |
|                         | 25.0                  | 16.2 ± 3.2 <sup>d</sup>    | 27.7 ± 9.8 <sup>b</sup> | 46.2 ± 19.1 <sup>b</sup> | 15.4 ± 12.0 <sup>b,c</sup> | 89.2 ± 11.8 <sup>d</sup>   |
|                         | 50.0                  | 17.2 ± 2.0 <sup>a</sup>    | 11.5 ± 6.3 <sup>c</sup> | 77.6 ± 12.3 <sup>c</sup> | 31.0 ± 45.3 <sup>a,c</sup> | 94.7 ± 6.1 <sup>d</sup>    |
|                         | 100.0                 | 75.8 ± 11.5 <sup>b</sup>   | 3.0 ± 4.2 <sup>c</sup>  | 94.2 ± 8.2 <sup>c</sup>  | 0.0 ± 0.0 <sup>c,d</sup>   | 100.0 ± 0.0 <sup>d</sup>   |
| <i>C. pulegioidorus</i> | 6.2                   | 8.7 ± 4.2 <sup>d</sup>     | 46.1 ± 2.7 <sup>d</sup> | 10.4 ± 5.3 <sup>d</sup>  | 88.8 ± 4.4 <sup>d</sup>    | 12.7 ± 8.2 <sup>d</sup>    |
|                         | 12.5                  | 28.8 ± 10.9 <sup>d,b</sup> | 41.5 ± 0.9 <sup>d</sup> | 19.4 ± 1.7 <sup>d</sup>  | 68.8 ± 16.6 <sup>d</sup>   | 39.3 ± 14.5 <sup>b</sup>   |
|                         | 25.0                  | 81.4 ± 5.5 <sup>b,c</sup>  | 30.5 ± 4.7 <sup>b</sup> | 40.7 ± 9.1 <sup>b</sup>  | 40.1 ± 16.4 <sup>b</sup>   | 74.9 ± 7.4 <sup>c</sup>    |
|                         | 50.0                  | 96.1 ± 5.5 <sup>c</sup>    | 3.4 ± 2.1 <sup>c</sup>  | 93.5 ± 4.0 <sup>c</sup>  | 0.0 ± 0.0 <sup>d</sup>     | 100.0 ± 0.0 <sup>d</sup>   |
|                         | 100.0                 | 100.0 ± 0.0 <sup>c</sup>   | 0.0 ± 0.0 <sup>c</sup>  | 100.0 ± 0.0 <sup>c</sup> | 0.0 ± 0.0 <sup>d</sup>     | 100.0 ± 0.0 <sup>d</sup>   |
| CG1                     | 6.2                   | 2.9 ± 0.9 <sup>d</sup>     | 53.2 ± 4.8 <sup>d</sup> | 0.0 ± 9.3 <sup>d</sup>   | 88.8 ± 2.4 <sup>d</sup>    | 0.0 ± 11.6 <sup>d</sup>    |
|                         | 12.5                  | 0.0 ± 0.0 <sup>a</sup>     | 52.1 ± 2.1 <sup>d</sup> | 0.0 ± 4.2 <sup>d</sup>   | 68.6 ± 7.5 <sup>b,c</sup>  | 23.7 ± 11.5 <sup>d,b</sup> |
|                         | 25.0                  | 24.2 ± 6.1 <sup>d</sup>    | 47.6 ± 3.4 <sup>d</sup> | 7.5 ± 6.6 <sup>d</sup>   | 71.7 ± 7.2 <sup>b</sup>    | 27.0 ± 12.1 <sup>b,c</sup> |
|                         | 50.0                  | 62.9 ± 10.5 <sup>d</sup>   | 46.9 ± 5.1 <sup>d</sup> | 8.9 ± 9.8 <sup>d</sup>   | 59.9 ± 2.7 <sup>c</sup>    | 40.2 ± 7.2 <sup>b,c</sup>  |
|                         | 100.0                 | 74.5 ± 25.4 <sup>b</sup>   | 46.3 ± 3.3 <sup>d</sup> | 10.1 ± 6.4 <sup>d</sup>  | 52.0 ± 1.2 <sup>c</sup>    | 48.7 ± 4.2 <sup>c</sup>    |
| CG2                     | 6.2                   | 0.0 ± 0.0 <sup>a</sup>     | 48.8 ± 4.8 <sup>d</sup> | 5.2 ± 9.4 <sup>d</sup>   | 84.3 ± 6.7 <sup>d</sup>    | 12.4 ± 9.9 <sup>d</sup>    |
|                         | 12.5                  | 0.0 ± 0.0 <sup>a</sup>     | 42.9 ± 7.3 <sup>d</sup> | 16.6 ± 14.1 <sup>d</sup> | 65.9 ± 7.6 <sup>b</sup>    | 39.0 ± 17.0 <sup>b</sup>   |
|                         | 25.0                  | 59.1 ± 15.5 <sup>b</sup>   | 18.0 ± 8.1 <sup>b</sup> | 65.0 ± 15.8 <sup>b</sup> | 31.7 ± 13.3 <sup>c</sup>   | 86.3 ± 11.7 <sup>d</sup>   |
|                         | 50.0                  | 75.9 ± 4.6 <sup>b</sup>    | 0.5 ± 0.9 <sup>c</sup>  | 99.0 ± 1.7 <sup>c</sup>  | 1.6 ± 2.7 <sup>d</sup>     | 99.9 ± 0.1 <sup>d</sup>    |
|                         | 100.0                 | 84.5 ± 7.1 <sup>b</sup>    | 0.0 ± 0.0 <sup>c</sup>  | 100.0 ± 0.0 <sup>c</sup> | 0.0 ± 0.0 <sup>d</sup>     | 100.0 ± 0.0 <sup>d</sup>   |
| Positive control        | –                     | 100.0 ± 0.0 <sup>c</sup>   | 1.0 ± 1.0 <sup>c</sup>  | 98.0 ± 1.9 <sup>c</sup>  | 0.0 ± 0.0 <sup>d</sup>     | 100.0 ± 0.0 <sup>d</sup>   |

Values represent average ± standard deviation. Negative control (ultrapure water and 50% ethanol + 1% tween 80). Positive control (ultrapure water with 0.18 mg/ml of Cypermethrin, 0.30 mg/ml chlorpyrifos and 0.012 mg/ml citronellal (Colosso®, Ouro fino, São Paulo). Different letters in the same column represent a difference statistically significant ( $p \leq 0.05$ ).

14 and 21 days after hatching were used in the larval packet test. The larval packet test was performed according to Stone and Haydock (1962) as modified by Food and Agriculture Organization of the United Nations (FAO, 1984). The adult immersion test was performed as described by Drummond et al. (1973). Hatchability was estimated from the average numbers of eggs and larvae. The egg production index (EPI), oviposition reduction (OR), reproductive efficiency (RE), and product efficacy (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). Lethal concentrations for 50% of the population (LC<sub>50</sub>) of larvae and engorged females were calculated using GraphPad Prism 6.0 by Probit analysis. Formulations were considered significantly different when the 95% confidence intervals of LC<sub>50</sub> did not overlap (Roditakis et al., 2005). The differences among the concentrations of mortality against larvae, EPI, OR, and hatchability were analyzed by the F test of ANOVA followed by Tukey test ( $p < 0.05$ ).

## Results and discussion

The largest yield of volatile oil was obtained from *C. grewioides* (5.0%), in both CG1 and CG2 collections, followed by *C. pulegioidorus* (1.1%) and *C. conduplicatus* (0.8%). The chemical composition of the tested volatile oils, along with the retention indices and percentages, are shown in Table 1. A total of thirty-two, twenty-nine, nineteen and eleven components were identified in the volatile oils from *C. conduplicatus*, *C. pulegioidorus*, and *C. grewioides* (CG1 and CG2), respectively, representing over 92% of their total compounds.

The most abundant compound in *C. conduplicatus* volatile oils was eucalyptol (24.09%). Eucalyptol or 1,8-cineol is present in volatile oils of *Mesosphaerum suaveolens* (35.77%), *Ocimum gratissimum* (24.68%) and *Alpinia zerumbet* (24.05%). Their volatile oils have shown high efficacy against larvae and engorged females of *R. microplus* in previous study (Castro et al., 2018).

Analysis of *C. pulegioidorus* volatile oils showed that *p*-cymene (23.13%) and ascaridole (22.50%) were the main components. The volatile oil of *C. pulegioidorus* had maximum efficacy on larvae (100%) and engorged females (100%) of *R. microplus* at concentrations of 50 and 100 mg/ml, respectively (Table 2). The volatile oil of *A. zerumbet* also contained the compound *p*-cymene (32.72%) and demonstrated efficacy against both stages of tick life (Castro et al., 2018).

Methyl chavicol was the predominant component in *C. grewioides* CG1 (83.59%) and CG2 (95.38%). *C. grewioides* CG1 and CG2 were collected in the same state (Piauí), but their localities were 530 km apart, which could explain the significant difference obtained in their volatile oil. Since different factors as genotype, herbivorous attack, and edaphoclimatic conditions can influence the production of secondary metabolites, including terpenes, the acaricidal activity of the volatile oils may be affected (Cruz et al., 2013; Soares et al., 2016).

The low product efficacy (48.7%) on engorged females induced by *C. grewioides* CG1 volatile oils at 100 mg/ml was due to low reduction of oviposition (10.1%) and high hatchability (52.0%), which maintained the egg production index unchanged at all concentrations evaluated (Table 2). This result differs substantially from that obtained with *C. grewioides* CG2 volatile oils, that reached maximum product efficacy (100%). This remarkable difference between the results obtained by two volatile oils originating from the same species can be related to its chemical profile, and geographical origin as discussed (Cruz et al., 2013). Methyl chavicol was the major constituent of the volatile oils of both population samples, mainly in *C. grewioides* CG2. This result suggests that methyl chavicol is one of the main active compounds responsible for acaricidal action on engorged females, even though its synergism with minor chemical constituents should also be taken into consideration in the overall biological effect of the volatile oil on the cattle tick (Soares et al., 2016). This major compound, also known as estragole, showed fast insecticidal activity against adult fruit flies (Chang et al., 2009).

All the volatile oils tested in this study showed efficacy against larvae and engorged females of *R. microplus* (Table 2). The volatile

**Table 3**

Lethal concentration, confidence limit and R<sup>2</sup> values for the volatile oils from *Croton conduplicatus*, *C. pulegioidorus* and *C. grewioides* (CG1 and CG2) against *Rhipicephalus microplus* larvae and engorged females.

|                            | LC <sub>50</sub> (mg/ml) | Confidence limit | R <sup>2</sup> |
|----------------------------|--------------------------|------------------|----------------|
| Larvae                     |                          |                  |                |
| <i>C. conduplicatus</i>    | 49.35 <sup>d</sup>       | 17.38–81.32      | 0.21           |
| <i>C. pulegioidorus</i>    | 17.52 <sup>a</sup>       | 16.08–19.09      | 0.98           |
| <i>C. grewioides</i> (CG1) | 30.91 <sup>c</sup>       | 25.40–38.24      | 0.90           |
| <i>C. grewioides</i> (CG2) | 22.16 <sup>b</sup>       | 19.50–24.82      | 0.96           |
| Engorged female            |                          |                  |                |
| <i>C. conduplicatus</i>    | 16.52 <sup>b</sup>       | 13.56–19.48      | 0.93           |
| <i>C. pulegioidorus</i>    | 17.41 <sup>b</sup>       | 15.19–19.97      | 0.96           |
| <i>C. grewioides</i> (CG1) | 57.69 <sup>a</sup>       | 41.04–80.79      | 0.82           |
| <i>C. grewioides</i> (CG2) | 15.73 <sup>b</sup>       | 13.72–18.19      | 0.95           |

LC<sub>50</sub> = Lethal concentration 50% (mg/ml) R. microplus larvae or engorged females; Confidence limit = 95%; R<sup>2</sup> = Regression coefficient. Values down a column with different letters are significantly different.

oils of *C. conduplicatus*, *C. grewioides* (CG2) (25–100 mg/ml) and *C. pulegioidorus* (50 and 100 mg/ml) showed statistically similar ( $p \leq 0.05$ ) efficacy against engorged females when compared to commercial acaricides (positive control). The same was true for *C. pulegioidorus* against larvae at concentrations of 25–100 mg/ml. The results also show that volatile oils of *C. pulegioidorus* and *C. grewioides* (CG2) completely inhibit egg production at 100 mg/ml, while those of *C. conduplicatus* at 100 mg/ml and *C. pulegioidorus* at 50 mg/ml resulted in the ticks laying infertile eggs and consequently produced similar hatchability inhibition in regard to the positive control ( $p \leq 0.05$ ) (Table 2).

In relation to the lethal concentration reached in each tick stage, the *C. pulegioidorus* volatile oils had significantly higher activity (LC<sub>50</sub> = 17.52 mg/ml) on larvae, being followed by those of *C. grewioides* CG2 (LC<sub>50</sub> = 22.16 mg/ml), CG1 (LC<sub>50</sub> = 30.91 mg/ml) and *C. conduplicatus* (LC<sub>50</sub> = 49.35 mg/ml). For engorged females, the oils of *C. grewioides* CG2 (LC<sub>50</sub> = 15.73 mg/ml), *C. conduplicatus* (LC<sub>50</sub> = 16.52) and *C. pulegioidorus* (LC<sub>50</sub> = 17.51 mg/ml) did not differ statistically but showed higher activity than those of *C. grewioides* CG1 (LC<sub>50</sub> = 57.69 mg/ml) (Table 3).

Our findings showed that *C. pulegioidorus* volatile oils at 50 mg/ml obtained the highest acaricidal effect on *R. microplus* larvae (96.1 %), when compared to the other volatile oils at the same concentration. Additionally, the volatile oils of this species also presented high effectiveness on *R. microplus* engorged females (100.0%) at 50 mg/ml, similar to the volatile oils of *C. conduplicatus* (94.7%) and *C. grewioides* CG2 (99.9%). It should not be forgotten that cattle are parasitized by both larvae and females and therefore an acaricide which is active against different stages of the parasite tends to be more effective (Soares et al., 2016).

The action of the volatile oils of these three analyzed plant species against the cattle tick *R. microplus* is demonstrated here for the first time. Among these volatile oils studied, that one from *C. pulegioidorus* exhibited the best results against both larvae and engorged females of *R. microplus*. Nanoparticles synthesis studies to mosquitoes control already has been highly promising to improve the efficacy of botanical pesticides extending the stability while preserving their environmental and health safety (Benelli et al., 2018). In regards to tick, further research is needed to determine more precisely the compounds responsible for the activity of these volatile oils and the development of nanoformulations that have the required stability for *in vivo* usage. This study is thus a contribution to food safety in supporting the development of new acaricidal molecules for the treatment of cattle that produce both milk and meat.

## Conflict of interest

The authors declare no conflicts of interest.

## Authors' contribution

KNCC, ACSC, LMCJ and IMA made the study conception, design and acquisition and interpretation of data. THSR, KMC and ESB contributed on chemical analysis and interpretation of data. All the authors made important contributions in the accomplishment of the work and approved the final manuscript to be submitted for publication.

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