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Original Article

Anthelmintic activity of *Eucalyptus citriodora* essential oil and its major component, citronellal, on sheep gastrointestinal nematodes

Atividade anti-helmíntica do óleo essencial de *Eucalyptus citriodora* e seu componente majoritário, citronelal, sobre nematoides gastrintestinais de ovinos

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Abstract

This study aimed to evaluate the anthelmintic activity of *Eucalyptus citriodora* essential oil and citronellal on sheep gastrointestinal nematodes. Essential oil composition was determined by gas chromatography mass spectrometry. The substances were evaluated *in vitro* using adult worm motility test (AWMT) and transmission electron microscopy (TEM). The acute toxicity test in mice and the fecal egg count reduction test (FECRT) in sheep were performed. Citronellal was confirmed as the essential oil major constituent (63.9%). According to the AWMT, 2 mg/mL of essential oil and citronellal completely inhibited *Haemonchus contortus* motility at 6 h post exposure. *H. contortus* exposed to essential oil and citronellal exhibited internal ultrastructural modifications. The lethal dose 50 values in mice were 5,000 and 2,609 mg/kg for essential oil and citronellal, respectively. *E. citriodora* essential oil reduced sheep epg at 14 days post treatment by 69.5% ($P<0.05$). No significant differences were observed in epg between the citronellal and negative control groups ($P>0.05$). The interaction between citronellal and other constituents in the essential oil may be relevant for its *in vivo* anthelmintic activity. Thus, *E. citriodora* essential oil and citronellal pharmacokinetic studies may help elucidate the anthelmintic activity of these compounds.

Keywords: *Haemonchus contortus*, Phytotherapy, alternative control, monoterpenoid.

Resumo

Este trabalho objetivou avaliar a atividade anti-helmíntica do óleo essencial de *Eucalyptus citriodora* e citronelal sobre nematoides gastrintestinais de ovinos. A composição do óleo essencial foi determinada por cromatografia gasosa acoplada à espectrometria de massas. As substâncias foram avaliadas *in vitro* utilizando-se teste de motilidade de vermes adultos (AWMT) e microscopia eletrônica de transmissão (TEM). Teste de toxicidade aguda em camundongos e teste de redução da contagem de ovos fecais (FECRT) em ovinos foram realizados. Citronelal foi confirmado como componente majoritário do óleo essencial (63,9%). No AWMT, 2 mg/mL de óleo essencial e citronelal inibiram completamente a motilidade de *H. contortus* 6 h pós-exposição. *H. contortus* expostos ao óleo essencial e citronelal exibiram modificações ultraestruturais internas. Os valores da dose letal 50 em camundongos foram 5.000 e 2.609 mg/kg para óleo essencial

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e citronelal, respectivamente. Óleo essencial de *E. citriodora* reduziu opg de ovinos 14 dias pós-tratamento em 69,5% (*P*<0,05). Não houve diferença significativa de opg entre grupo controle negativo e citronelal (*P*>0,05). A interação entre citronelal e outros constituintes do óleo essencial pode ser relevante na atividade anti-helmíntica *in vivo*. Portanto, avaliação farmacocinética do óleo essencial de *E. citriodora* e citronelal pode auxiliar a elucidar a atividade anti-helmíntica desses compostos.

Palavras-chave: *Haemonchus contortus*, fitoterapia, controle alternativo, monoterpenoide.

Introduction

Gastrointestinal nematodes are considered one of the greatest threats to goat and sheep farming, causing reduced weight gain, diarrhea, anemia and, in severe cases, death. *Haemonchus contortus* is a parasite that is highly prevalent in Brazilian herds and pathogenic due to its hematophagous feeding habit (FERREIRA et al., 2013; ASHRAF & PRICHARD, 2014).

The control of small ruminant helminthiasis is almost exclusively realized with synthetic anthelmintics. However, reports of resistant nematode populations are becoming more common all over the world. Low efficacy was reported even for monepantel and derquantel plus abamectin, the two anthelmintic most recently launched on the market. Thus, anthelmintic resistance is currently considered one of the main challenges for goat and sheep parasite control (KOTZE & PRICHARD, 2016; SALES & LOVE, 2016).

The search for alternative control methods is essential, and studies have been carried out for this purpose. Many of these studies have demonstrated the potential promising of the phytotherapy. Thus, plant extracts, decoctions, essential oils and isolated bioactive compounds have been widely evaluated for their anthelmintic activity (KEARNEY et al., 2016; ANDRÉ et al., 2016; RIBEIRO et al., 2017).

Essential oils are complex mixtures of volatile secondary metabolites produced by aromatic plants and often obtained by hydrodistillation. Although essential oils can contain a high range of constituents, two or three are present in greater quantity. Generally, the major constituent or the synergic interaction between constituents determines the biological properties of the essential oils (BAKKALI et al., 2008; BALLHORN et al., 2009; BENELLI & PAVELA, 2018).

The genus *Eucalyptus* belongs to the family Myrtaceae, which comprises approximately 900 species and subspecies. In addition to exploitation of its wood, *Eucalyptus* spp. is well known for its essential oils, widely marketed and used in the fragrance and pharmaceutical industries (BATISH et al., 2008). The essential oil of *E. citriodora* (Lemon Scented *Eucalyptus*) is one of the most popular in Brazil, together with *E. staigeriana* and *E. globulus* essential oils. The main constituent of *E. citriodora* essential oil is a monoterpenoid, citronellal (60-80%) (VITTI & BRITO, 2003; HASEGAWA et al., 2008).

E. citriodora essential oil has been reported to possess different biological activities, such as antibacterial and antifungal (BRITO et al., 2012; ELAISSI et al., 2012), anti-inflammatory, analgesic (GBENOU et al., 2013) and insecticidal (MACIEL et al., 2010). Citronellal demonstrated antifungal activity (NAKAHARA et al., 2003) and effect on insects (KIM et al., 2005).

E. citriodora essential oil (71.77% citronellal) was evaluated *in vitro* against *H. contortus*. This oil showed an efficacy of 98.8% on egg hatching inhibition and 99.71% on larval development inhibition at 5.3 and 10.6 mg/mL, respectively. The same oil was evaluated in goats using the fecal egg count reduction test (FECRT). The animals received 500 mg/kg for three consecutive days, and after 15 days, there was a 60.3% reduction in the egg count (MACEDO et al., 2011).

The *in vitro* effects of *E. citriodora* essential oil and its main constituent, citronellal, on *H. contortus* eggs, larvae and adult motility have been evaluated; both synthetic anthelmintic-susceptible and resistant *H. contortus* isolates were tested. Both products showed effectiveness against all life stages of the tested isolates, and it was suggested that citronellal was mainly responsible for the anthelmintic efficacy of the essential oil. However, no data are available on the *in vivo* anthelmintic activity of citronellal (ARAÚJO-FILHO et al., 2018).

The aim of this study was to evaluate the anthelmintic activity of *E. citriodora* essential oil and citronellal, its major constituent, *in vivo* against sheep gastrointestinal nematodes.

Material and Methods

This study was approved by the Ethics Committee of the Universidade Estadual do Ceará and registered under the number 2836026/2017.

Eucalyptus citriodora essential oil and citronellal

E. citriodora essential oil was purchased from Ferquima® (São Paulo, Brazil), and its chemical composition was determined by gas chromatography-mass spectrometry (GC-MS) using a GCMS-QP2010S (Shimadzu®, Japan). The following experimental conditions were employed: RTX-5 (30 m x 0.25 mm) capillary column; helium carrier gas; injector temperature of 250 °C; detector temperature of 260 °C; column temperature of 50-150 °C at 2.5 °C/min and then 150-250 °C at 25°C/min. The running time was 50 min. For mass spectrometry, the electron impact was 70 eV.

The components of *E. citriodora* oil were identified according to their GC retention time, as expressed by Kovats index, which was calculated using the Van den Dool and Kratz equation (ADAMS, 2007). Additionally, compound mass spectra were compared to spectra from the National Institute for Standard Technology computer database and published spectra. The quantification of the compounds of *E. citriodora* essential oil was performed on the basis of the relative percentage of peak areas of the chromatogram. Citronellal was purchased from Sigma-Aldrich® (product code 27470, ≥ 95% GC).

Acute oral toxicity test

The acute oral toxicity test in mice was performed to define the safe dose of *E. citriodora* essential oil and citronellal for administration in sheep. Therefore, 14 female Swiss albino mice (n=7 for each group) with an average weight of 26.2 ± 1.8 g were allowed to acclimate to the experimental conditions (cycle of 12 h light/dark and a temperature of 25°C) for seven days, during which they were kept in polypropylene boxes. Commercial feed (Nuvilab®, Brazil) and filtered water were provided *ad libitum* to the rodents.

The acute toxicity test was in accordance with the Organization for Economic Cooperation and Development guideline number 425, the "Up-and-Down" method (OECD, 2008). *E. citriodora* essential oil and citronellal were administered orally in a single dose progression (175; 440; 1,100; 2,800 and 5,000 mg/kg). Each animal was carefully evaluated for up to 48 h prior to deciding the dose to be given to the next animal. Dose values and estimation of the lethal dose to 50% (LD50) of animals were obtained using the AOT425StatPgm software (OECD, 2008).

Adult Worm Motility Test (AWMT)

AWMT was performed based on the methodology described by Hounzangbe-Adote et al. (2005). Briefly, adult *H. contortus* were collected from a sheep naturally infected with gastrointestinal nematodes at eggs per gram of feces (epg) greater than 15,000. Immediately after euthanasia, the abomasum was removed, opened and placed in saline solution at 37°C. Females of *H. contortus* were rapidly collected and placed into 24-well plates at a ratio of 3 worms per well in 1 ml of phosphate-buffered saline (PBS) enriched with 4% penicillin/streptomycin (Sigma-Aldrich®) at 37°C. After 1 hour of incubation (37°C, 5% carbon dioxide), 1 ml of *E. citriodora* essential oil or citronellal at 2, 1.75, 1.5, 1.25, 1 or 0.75 mg/mL was added to the wells. PBS with 4% penicillin/streptomycin and 3% Tween® 80 and 100 µg/mL ivermectin were used as negative and positive controls, respectively. After 3, 6 and 12 h of incubation, the motility of adult worms was observed under an inverted microscope. Eight replicates were performed for each treatment and for each control.

Transmission Electron Microscopy (TEM)

H. contortus specimens from AWMT were fixed in 2.5% glutaraldehyde, 4% paraformaldehyde and 0.1 M pH 7.1 sodium cacodylate buffer for 48 h. The samples were then washed in sodium cacodylate buffer, post fixed in 1% osmium tetroxide for 1 h and washed again in the same buffer. The samples were dehydrated in an increasing series of acetone (30, 40, 50, 70, 90 and 100%) and embedded in Spurr polymerized resin in an oven at 60°C. Ultrathin slices of 70 nm were obtained and collected in 400 mesh copper grids and contrasted with 1% uranyl acetate and 5% lead citrate. The visualization was performed using a JEOL 1400 PLUS transmission electron microscope at 60 kV. The methodology was adapted from Sant'Anna et al. (2013).

Fecal Egg Count Reduction Test (FECRT)

Sixty sheep from 7 to 16 months old and with an average weight of 24 ± 5.3 kg naturally infected with gastrointestinal nematodes with epg greater than 1,000 were selected and randomly divided (n=15) into four groups: G1, 500 mg/kg *E. citriodora* essential oil; G2, 250 mg/kg citronellal; G3, 2.5 mg/kg monepantel (Zolvix®, Novartis Animal Health, New Zealand); and G4, distilled water (COLES et al., 1992). The treatments were administered orally in a single dose. Fecal samples were collected on days 0, 7 and 14 post treatment to estimate epg reduction. Larval culture using a pool of feces from each group was performed to identify nematode genera. The identification of third stage larvae (L3) was based on Ueno & Gonçalves (1998).

Statistical analysis

The AWMT experimental design was a factorial arrangement with time (3; 6 and 12 h) and treatments (*E. citriodora* essential oil; citronellal; ivermectin and PBS) as the factors capable of causing worm motility inhibition, calculated as the number of motionless worms/total number of worms per well ×100. Then, we performed a two-way ANOVA followed by comparison with the Bonferroni test to detect significant differences ($P<0.05$) using GraphPad Prism 5.0. The results were expressed as the mean ± standard deviation (SD) ($P<0.05$). The effective concentration to inhibit 50% (EC50) of worm motility was determined by probit regression using the SPSS 17.0 program.

The FECRT experimental design was a factorial arrangement with time (0; 7 and 14 days post treatment) and treatments (*E. citriodora* essential oil; citronellal; monepantel and distilled water) as the factors capable of causing epg variation. The anthelmintic efficacy of *E. citriodora* essential oil and citronellal was interpreted by the FECRT based on each group arithmetic mean fecal egg count using the following formula: FECRT = 100× (1 – [T2/T1] [C1/C2]), in which the arithmetic fecal egg count means in controls (C) and treated (T) animals before (T1 and C1) and 7 or 14 days after (T2 and C2) deworming were compared (DASH et al., 1988), using BootStreat 1.0 software (CABARET, 2014). The epg was log-transformed (log10[x + 1]), submitted to ANOVA and compared using Tukey's test using GraphPad Prism® 5.0 software ($P<0.05$).

Prior to ANOVA, AWMT and FECRT data were submitted to the Shapiro-Wilk test for normality analysis ($P>0.05$).

Results

The chemical composition of *E. citriodora* essential oil is shown in Table 1. Citronellal was confirmed as the essential oil major constituent (63.9%). The presence of other constituents, including neo-isopulegol (8.2%), citronellol (5.2%) and iso-isopulegol (4.7%), was also revealed.

The effects of *E. citriodora* essential oil and citronellal on *H. contortus* motility inhibition are presented in Table 2. In the AWMT, the interactions between factors (treatments and times) were statistically significant, except for essential oil at 12 h ($F = 2.055$; $P = 0.09$), essential oil at 1.75 mg in all times ($F = 1.815$; $P = 0.1875$),

Table 1. Composition of *Eucalyptus citriodora* essential oil as determined by gas chromatography-mass spectrometry (GC-MS).

Constituents	KI_{lit}	KI_{exp}	Percentage (%)
Alpha-pinene	942	940	0.46
Beta-pinene	981	980	0.87
Limonene	1030	1029	0.34
Eucalyptol	1033	1032	1.67
Bergamal	1052	1053	0.30
Linalool	1098	1099	0.34
Rose Oxide	1109	1111	0.27
Neo-isopulegol	1147	1149	**8.23**
Citronellal	1154	1157	**63.94**
Iso-isopulegol	1159	1162	**4.72**
Neoiso-isopulegol	1170	1173	0.39
Citronellol	1224	1228	**5.24**
Menthol<8-hydroxy-neo>	1327	1333	0.59
CytronellylAcetate	1339	1346	3.27
Beta-caryophyllene	1408	1417	0.63
Total identified	-	-	91.26

KI_{lit}: Kovats index found in the literature; KI_{exp}: Kovats index for the experiment. The values in bold highlight the chemical constituents found in higher percentages in the essential oil.

Table 2. Mean efficacy (percentage ± standard deviation) of *Eucalyptus citriodora* essential oil and citronellal on motility inhibition of *Haemonchus contortus*.

Concentrations	***E. citriodora* essential oil**			**Citronellal**		
	Exposure time (hours)			**Exposure time (hours)**		
	3 h	**6 h**	**12 h**	**3 h**	**6 h**	**12 h**
2 mg/ml	100.00 ± 0.00^{Aa}	100.00 ± 0.00^{Aa}	100.00 ± 0.00^{Aa}	91.67 ± 15.43^{Aa}	100.00 ± 0.00^{Aa}	100.00 ± 0.00^{Aa}
1.75 mg/ml	87.50 ± 17.26^{Aa}	91.67 ± 15.43^{ABa}	100.00 ± 0.00^{Aa}	87.50 ± 17.26 ^{Aa}	95.83 ± 11.79^{Aa}	100.00 ± 0.00^{Aa}
1.5 mg/ml	62.49 ± 11.78^{Ba}	79.16 ± 17.26 ^{Bab}	95.83 ± 11.79 ^{ABb}	54.16 ± 17.25 ^{Ba}	83.33 ± 17.82^{ABb}	95.83 ± 11.79 ^{Ab}
1.25 mg/ml	45.83 ± 17.25 ^{BCa}	75.00 ±15.43 ^{Bb}	91.67 ± 15.43 ^{ABc}	41.66 ± 15.43 ^{BCa}	70.83 ± 21.36^{BCb}	95.83 ± 11.79 ^{Ac}
1 mg/ml	45.83 ± 17.25 ^{BCa}	62.50 ± 21.36 ^{Ba}	91.67 ± 15.43 ^{ABb}	37.50 ± 11.78 ^{Ca}	54.16 ± 17.25^{Ca}	83.33 ± 17.82 ^{ABb}
0.75 mg/ml	29.16 ± 11.78 ^{Ca}	41.66 ± 15.43 ^{Ca}	83.33 ± 17.82 ^{Bb}	20.83 ± 17.25 ^{Ca}	37.50 ± 11.78^{Da}	66.66 ± 25.20 ^{Bab}
Ivermectin (0.10 mg/ml)	95.83 ± 11.79^{Aa}	100.00 ± 0.00 ^{Aa}	100.00 ± 0.00^{Aa}	95.83 ± 11.79 ^{Aa}	100.00 ± 0.00^{Aa}	100.00 ± 0.00^{Aa}
PBS + TW	0.00 ± 0.00 ^{Da}	8.33 ± 15.43 ^{Dab}	20.83 ± 17.25^{Cb}	0.00 ± 0.00 ^{Da}	8.33 ± 15.43^{Eab}	20.83 ± 17.25 ^{Cb}

Capital letters compare means in the columns and small letters compare means in the rows. Different letters indicate significantly different values (P< 0.05).

citronellal at 2 mg in all times (F = 2.333; P = 0.1216), citronellal at 1.75 mg in all times (F = 2.227; P = 0.1327) and ivermectin in all times (F = 1; P = 0.3847). The highest concentration of both treatments (2 mg/mL) completely inhibited the motility of *H. contortus* at 6 and 12 h post exposure. The lowest concentration of essential oil and citronellal (0.75 mg/mL) demonstrated 41.66 and 37.50% (at 6 h post exposure) and 83.33 and 66.66% (at 12 h post exposure) of efficacy on motility inhibition of *H. contortus*, respectively. No statistically significant differences were observed between the positive control and the 2 mg/mL and 1.75 mg/mL concentrations for both treatments (P>0.05). The effects on *H. contortus* motility were dose-dependent and greater at 12 h post exposure. The EC50s of the essential oil and citronellal at 12 h post exposure were 0.41 mg/mL (y = 1.28 + 2.29*x; R^2 = 0.905) and 0.64 mg/mL (y = 1.04 + 4.73*x; R^2 = 0.934), respectively.

The adult *H. contortus* exposed to *E. citriodora* essential oil and citronellal exhibited internal ultrastructural modifications (Figure 1). Contact with citronellal induced internal damage, with formation of vacuoles and tissue disorganization. Nematodes exposed to *E. citriodora* essential oil showed high disorganization in the muscle layer with degradation of the muscular fibrils and vacuole formation.

In the acute toxicity test, four mice died, three from the citronellal group and one animal from the *E. citriodora* essential oil group. These animals received the highest dose (5,000 mg/kg). The estimated LD50 values for *E. citriodora* essential oil and citronellal were 5,000 mg/kg and 2,609 mg/kg, respectively.

The FECRT results are presented in Table 3. In the FECRT, statistical differences between factors (treatments and times) were observed, except for citronellal at 0, 7 and 14 days (F = 0.028; P = 0.9719) and distilled water at 0, 7 and 14 days (F = 0.4926;

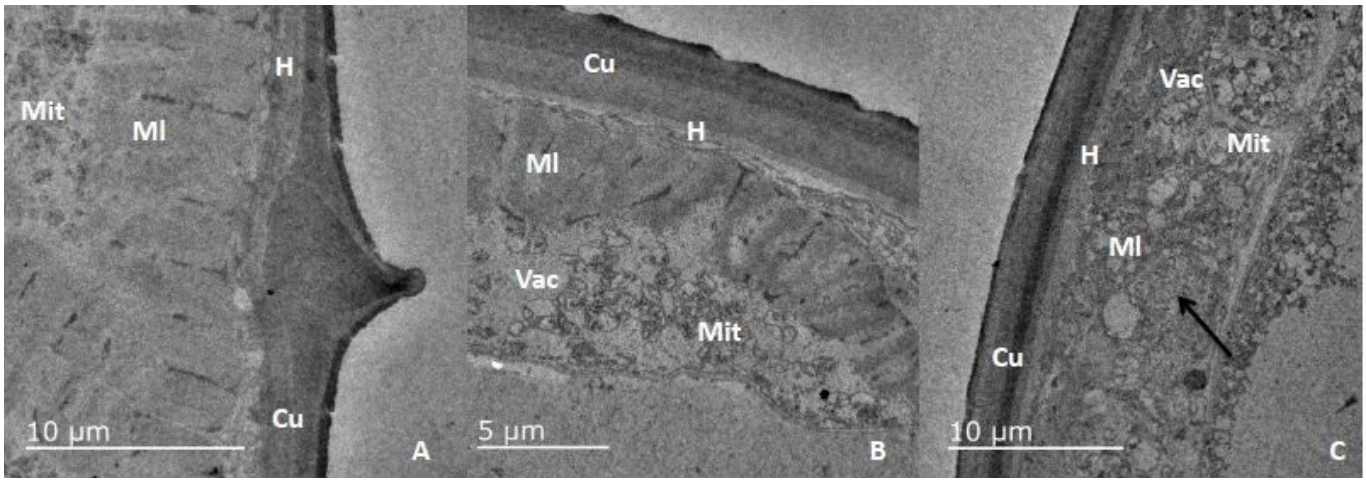

Figure 1. Transmission electron microscopy images of adult *Haemonchus contortus* after incubation in either PBS + Tween 80 (A), citronellal (B) or *Eucalyptus citriodora* essential oil. The following structures are shown: cuticle (Cu), hypodermis (H), muscular layer (Ml), mitochondria (Mit) and vacuole (Vac). Note the vacuole formation and changes in the mitochondrial profile. Marked muscular disorganization is shown (black arrow) after exposure to essential oil. Scale bars: 10 µm in A and C, 5 µm in B.

Table 3. Eggs per gram of feces (mean EPG ± standard deviation) and efficacy of *Eucalyptus citriodora* essential oil and citronellal on fecal egg count reduction test (FECRT).

Treatments	Day0	Day7	Day 14
E. citriodora			
Mean EPG	1,835 ±1,104^{Aa}	1,010±872.7^{Ab}	675 ± 600.3^{Ac}
Efficacy (%)	-	41.8	69.5
Citronellal			
Mean EPG	1,800 ±1,442^{Aa}	1,665 ±1,233^{Ba}	1,755 ±1,169^{Ba}
Efficacy (%)	-	2.8	0.7
Monepantel (Positive Control)			
Mean EPG	1,805 ±1,262^{Aa}	155 ± 206.1^{Cb}	105 ±140.3^{Cb}
Efficacy (%)		90.9	95.1
Distilledwater (Negative Control)			
Mean EPG	1,825 ±1,068^{Aa}	1,770 ± 971.9^{Ba}	2,040 ±1,392^{Ba}

Capital letters compare means in the columns and small letters compare means in the rows. Different letters indicate significantly different values (P<0.05).

P = 0.6164). *E. citriodora* essential oil and citronellal reduced epg by 69.5% and 0.7%, respectively, at day 14 post treatment. The epg reduction in the monepantel group (95.1%) was significantly higher than those of essential oil and citronellal (P<0.05). There were no statistically significant differences in epg between the citronellal group and the negative control group at days 7 and 14 post treatment (P>0.05).

The larvae recovered by fecal cultures of sheep before treatment were identified as *Haemonchus* spp. (73%), *Trichostrongylus* spp. (23%), *Oesophagostomum* spp. (2%) and *Strongyloides* spp. (2%). The larvae recovered 14 days after treatment with *E. citriodora* essential oil were *Haemonchus* spp. (71%), *Trichostrongylus* spp. (25%), *Oesophagostomum* spp. (3%) and *Strongyloides* spp. (1%) while those recovered after treatment with citronellal were *Haemonchus* spp. (69%), *Trichostrongylus* spp. (27%) and *Oesophagostomum* spp. (4%). The group that received monepantel showed *Haemonchus* spp. (56%), *Trichostrongylus* spp. (33%) and *Oesophagostomum* spp. (11%).

Discussion

Natural products derived from plants have been suggested as promising alternatives to be used in the control of gastrointestinal nematodes of small ruminants. The use of phytotherapics with anthelmintic activity would reduce the use of synthetic (such as thiabendazole and levamisole) and semisynthetic (such as ivermectin and derquantel) anthelmintics and consequently delay the development and dissemination of anthelmintic resistance (KEARNEY et al., 2016; LOPES et al., 2018).

Since the geographic origin of the nematodes, as well as the anthelminthic resistance pattern, are indicated as factors that cause variation in the anthelmintic activity of natural products, we chose to use a regional isolate obtained from a farm of the municipality of Caucaia, Ceará, Northeast Brazil, in this work, therefore representing the reality for breeders of small ruminants (CHAN-PÉREZ et al., 2016; GAÍNZA et al., 2016).

In AWMT, citronellal and essential oil had similar adulticidal effects (P>0.05), completely inhibiting the motility of *H. contortus* in the highest concentrations tested (2 mg/kg and 1.75 mg/kg). These results corroborate the hypothesis that the biological properties of the essential oil may be due to its major compounds (BAKKALI et al., 2008). Additionally, these results corroborate those of Araújo-Filho et al. (2018), who used *H. contortus* isolates that were susceptible (*Inbred-Susceptible Edinburgh*) and multiresistant (*Kokstad*) to synthetic anthelmintics, and both isolates were sensitive to natural products.

The essential oil of *Thymus vulgaris* and its major constituent, thymol, demonstrated *in vitro* efficacy against *H. contortus* eggs, larvae and adults. In the AWMT, *T. vulgaris* essential oil (50 mg/mL) and thymol (25 mg/mL) completely inhibited the *H. contortus* motility within 8 h post exposure (FERREIRA et al., 2016). In the present study, statistical differences were not observed in the efficacy of *E. citriodora* essential and citronellal at the concentration of 2 mg/mL on the adult motility. Ferreira et al. (2016) demonstrated that the lowest concentration of thymol (0.25 mg/mL) showed 91% of efficacy on motility inhibition of *H. contortus* (8 h post exposure). In the present study, the citronellal at the concentration of 0.75 mg/mL presented 66.66% of efficacy on motility inhibition of this nematode (12 h post exposure).

The images of *H. contortus* exposed to citronellal and essential oil demonstrated ultrastructural alterations, such as the disorganization of the muscular layer, formation of vacuoles and alteration in the mitochondrial profile. These changes observed by transmission electron microscopy demonstrate the loss of homeostasis of the parasites exposed to the treatments and can explain the loss of motility, such as the destruction of the muscular layer after exposure to *E. citriodora* essential oil (BRUNET et al., 2011).

The anthelmintic activity of the essential oil could be attributed to its main compound. Therefore, the isolated major constituent should be more effective than the essential oil at the same concentration (BAKKALI et al., 2008). However, as observed in the *in vitro* tests the efficacy between *E. citriodora* essential oil and citronellal demonstrated no statistical difference. Thus, the other constituents in the essential oil composition (34.1%) did not promote significant variation in the *in vitro* anthelmintic activity of citronellal.

The citronellal was found to be nearly twice as toxic as the *E. citriodora* essential oil, as demonstrated by their LD50 values of 2,609 mg/kg and 5,000 mg/kg, respectively. Both substances are considered slightly toxic agents because they have LD50 values of 1,000 mg/kg to 5,000 mg/kg (NIESINK et al., 1996).

E. citriodora essential oil promoted reductions of 41.8% and 69.5% of epg in sheep at 7 and 14 days post treatment, respectively. These results differ from those of Ribeiro et al. (2014), who achieved 55.9% epg reduction in sheep at 10 days post treatment with *E. citriodora* essential oil at 250 mg/kg. The composition of the oil used by Ribeiro et al. (2014) was slightly different from that used in this study for citronellal (67.5%) and citronellol (6.9%) contents. On the other hand, the menthol content (6.1%) in their oil was more than 10-fold ours (0.59%). This monoterpenoid was recently shown to be a positive allosteric modulator of *Oesophagostomum dentatum* levamisole-sensitive nicotinic acetylcholine receptor. Thus, its use is suggested in combination with cholinomimetic drugs to increase anthelmintic effectiveness (CHOUDHARY et al., 2019).

Goats treated with 500 mg/kg of *E. citriodora* essential oil for three consecutive days showed reduced epg by 66.2% and 60.3% on day 8 and 15 post treatment, respectively (MACEDO et al., 2011). Pharmacokinetics studies for synthetic anthelmintics have shown that goats have greater metabolization capacity than sheep, resulting in low bioavailability and, therefore, less efficacy than in sheep at the same dose, which explains the need for an increase in the number of treatments (LESPINE et al., 2012; SINGH et al., 2018).

In the present study, sheep harboring gastrointestinal nematodes were treated with citronellal (250 mg/kg) and *E. citriodora* essential oil (500 mg/kg). The choice of different doses was made considering previous studies (RIBEIRO et al., 2014) and the percentage of citronellal present in the oil (63.9%). Curiously, citronellal showed no anthelmintic activity *in vivo*, causing only a 0.7% epg reduction. Therefore, as occurred in other studies evaluating natural products derived from plants, *in vitro* anthelmintic efficacy was not reproduced *in vivo* (EGUALE et al., 2007; KATIKI et al., 2017).

Citronellal is described as a compound of unstable nature, being subject to reduction reactions for citronellol through the action of isolated enzymes and microorganisms, as well as oxidation reactions generating citronellic acid. It appears that isolated citronellal is more vulnerable to these reactions than citronellal in association with the other constituents that form the essential oil *E. citriodora*, and perhaps the ruminal microbiota influences this bioconversion. Therefore, pharmacokinetic studies would be important to allow a better understanding of the results of this test (ODA et al., 1996; LENARDÃO et al., 2007; COBELLIS et al., 2016).

The L3 from coprocultures of the animals submitted to FECRT demonstrated a high frequency of *Haemonchus* spp. (59% - 79%) and *Trichostrongylus* spp. (17% - 34%), corroborating the results of other authors whose experiments were carried out in northeast Brazil (ANDRÉ et al., 2016; RIBEIRO et al., 2017). *H. contortus* is a parasite well stablished under tropical and temperate conditions. In addition the daily egg output (5,000-15,000) and the nematode life cycle ensure the contamination of the pasture and then reinfection of the grazing animals (EMERY et al., 2016). No significant difference of the nematode genera found before and after treatment with the *E. citriodora* essential oil was observed. Considering that the essential oil promoted the reduction of 69.5% of epg, it probable affects similarly the nematodes found in abomasum, small intestine and large intestine. Camurça-Vasconcelos et al. (2008) demonstrated that the *Lippia sidoides* essential oil reduced epg of sheep naturally infected with the genera *Haemonchus* and *Trichostrongylus*. Therefore, the efficacy of essential oils against different nematodes genera have been documented.

Conclusion

E. citriodora essential oil and citronellal inhibited motility and induced ultrastructural damage to adult *H. contortus in vitro*. However, only the essential oil was effective in reducing epg in sheep harboring gastrointestinal nematodes. Therefore, the essential oil would be a better candidate to be included as an

alternative method for nematode control. In addition, further studies assessing the interactions between essential oil constituents and their pharmacokinetic profiles may help in understanding the anthelmintic activity of natural products.

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