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

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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 nematoïdes 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. 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/mL (F = 2.055; P = 0.09), essential oil at 1.5 mg/mL (F = 2.055; P = 0.09), essential oil at 1.25 mg/mL (F = 2.055; P = 0.09), essential oil at 1 mg/mL (F = 2.055; P = 0.09) and essential oil at 0.75 mg/mL (F = 2.055; P = 0.09).
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 *E. citriodora* essential oil group and one animal from the citronellal 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; *P* > 0.9719). No statistically significant 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; *P* > 0.9719).
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%).

**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<sup>a</sup> | 1,010±872.7<sup>b</sup> | 675 ± 600.3<sup>c</sup> |
| Efficacy (%)                  |               | 41.8          | 69.5           |
| **Citronellal**               |               |               |                |
| Mean EPG                      | 1,800 ±1,442<sup>a</sup> | 1,665 ±1,233<sup>b</sup> | 1,755 ±1,169<sup>b</sup> |
| Efficacy (%)                  |               | 2.8           | 0.7            |
| **Monepantel (Positive Control)** |             |               |                |
| Mean EPG                      | 1,805 ±1,262<sup>a</sup> | 155 ± 206.1<sup>c</sup> | 105 ±140.3<sup>c</sup> |
| Efficacy (%)                  |               | 90.9          | 95.1           |
| **Distilled water** (Negative Control) |             |               |                |
| Mean EPG                      | 1,825 ±1,068<sup>a</sup> | 1,770 ± 971.9<sup>b</sup> | 2,040 ±1,392<sup>b</sup> |

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 (\(P>0.05\)).

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**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 phytotherapeutics 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 anthelmintic 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 monoterprenoid 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 (MACHEDO 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, citronellol 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 citronelic acid. It appears that isolated citronellol 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 established 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 probably 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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References

Adams RP. Identification of essential oil components by gas chromatography/ quadrupole mass spectrometry. 4th ed. Illinois: Allured; 2007.

André WPP, Ribeiro WLC, Cavalcante GS, Santos JML, Macedo ITF, Paula HCB, et al. Comparative efficacy and toxic effects of carvacyl acetate and carvacrol on sheep gastrointestinal nematodes and mice. Vet Parasitol 2016; 218: 52-58. http://dx.doi.org/10.1016/j.vetpar.2016.01.001. PMid:26872928.

Araújo-Filho JV, Ribeiro WLC, André WPP, Cavalcante GS, Guerra MCM, Muniz CR, et al. Effects of Eucalyptus citriodora essential oil and its major component, citronellol, on Haemonchus contortus isolates susceptible and resistant to synthetic anthelmintics. Ind Crops Prod 2018; 124: 294-299. http://dx.doi.org/10.1016/j.indcrop.2018.07.059.

Asfar S, Prichard RK. Haemonchus contortus microtubules are cold resistant. Mol Biochem Parasitol 2014; 193(1): 20-22. http://dx.doi.org/10.1016/j.molbiopara.2014.01.006. PMid:24525483.

Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils - a review. Food Chem Toxicol 2008; 46(2): 446-475. http://dx.doi.org/10.1016/j.fct.2007.09.106. PMid:17996351.

Ballhorn DJ, Kaurz S, Heil M, Hegeman AD. Cyanogenesis of wild lime bean (Phatulus lunatus L.) is an efficient direct defence in nature. Plаs One 2009; 4(5): 1-7. http://dx.doi.org/10.1371/journal.pone.0005450. PMid:19424497.

Batish DR, Singh HP, Kohli RK, Kaur S. Eucalyptus essential oil as a natural pesticide. For Ecol Manage 2008; 256(12): 2166-2174. http://dx.doi.org/10.1016/j.foreco.2008.08.008.

Benelli G, Pavela R. Beyond mosquitos - Essential oil toxicity and repellency against bloodsucking insects. Ind Crops Prod 2018; 117: 382-392. http://dx.doi.org/10.1016/j.indcrop.2018.02.072.

Brito DR, Ootani MA, Ramos ACC, Serrató WC, Aguiar RWS. Efeito dos óleos de citronela, eucalipto e composto citronelol sobre microflora e desenvolvimento de plantas de milho. J Biotechnol Biodivers 2012; 3(4): 184-192. http://dx.doi.org/10.20873/jbb.uft.cemaf.v3n4.brito.

Brunet S, Fourquaux I, Hoste H. Ultrastructural changes in the third-stage, infective larvae of ruminant nematodes treated with sainfoin (Onobrychis vicifolia) extract. Parasitol Int 2011; 60(4): 419-424. http://dx.doi.org/10.1016/j.parint.2010.09.011. PMid:21787880.

Cabaret J. Reliable phenotypic evaluations of anthelmintic resistance in herbivores: how and when should they be done? In: Quick W. Anthelmintics: clinical pharmacology uses in veterinary medicine and efficacy. New York: Nova Science Publisher; 2014. p. 1-26.

Camurça-Vasconcelos ALF, Bevilacqua CML, Morais SM, Maciel MV, Costa CTC, Macedo ITF, et al. Anthelmintic activity of Lippia sidoides essential oil on sheep gastrointestinal nematodes. Vet Parasitol 2008; 154(1-2): 167-170. http://dx.doi.org/10.1016/j.vetpar.2008.02.023. PMid:18423877.

Chan-Pérez JL, Torres-Acosta JFJ, Sandoval-Castro CA, Hoste H, Castañeda-Ramírez GS, Vilarem G, et al. In vitro susceptibility of ten Haemonchus contortus isolates from different geographical origins towards acetone-water extracts of two tannin rich plants. Vet Parasitol 2016; 217: 53-60. http://dx.doi.org/10.1016/j.vetpar.2015.11.001. PMid:26827861.

Choudhary S, Marijanovic DS, Wong CR, Zhang X, Abongwu M, Coats JR, et al. Menthol acts as a positive allosteric modulator on nematode levamisole sensitive nicotinic acetylcholine receptors. Int J Parasitol Drugs Drug Resist 2019; 9: 44-53. http://dx.doi.org/10.1016/j.ijpddr.2018.12.005. PMid:30682641.

Cabellis G, Trabalza-Marinucci M, Yu Z. Critical evaluation of essential oils as rumen modifiers in ruminant nutrition: A review. Sci Total Environ 2016; 545-546: 556-568. http://dx.doi.org/10.1016/j.scitotenv.2015.12.103. PMid:26760275.

Coles GC, Bauer C, Borgsteede FHM, Geerts S, Klei TR, Taylor MA, et al. World association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for detection of anthelmintic resistance in nematodes of veterinary importance. Vet Parasitol 1992; 44(1-2): 35-44. http://dx.doi.org/10.1016/0304-4017(92)90141-U. PMid:1441190.

Dash K, Hall K, Barger IA. The role of arithmetic and geometric worm egg counts in faecal egg count reduction test and in monitoring strategic drenching programs in sheep. Aust Vet J 1988; 65(2): 66-68. http://dx.doi.org/10.1111/j.1751-0813.1988.tb07359.x. PMid:33554548.

Egualé T, Tilahun G, Debella A, Feleke A, Makonnen E. In vitro and in vivo anthelmintic activity of crude extracts of Coriandrum sativum against Haemonchus contortus. J Ethnopharmacol 2007; 110(3): 428-433. http://dx.doi.org/10.1016/j.jep.2006.10.003. PMid:17113738.

Elaiissi A, Rouis Z, Mabrouk S, Salah KB, Aouni M, Khoua ML, et al. Correlation between chemical composition and antibacterial activity of essential oils from fifteen Eucalyptus species growing in the Korbous Province of Tunisia. Food Chem Toxicol 2012; 17(3): 3044-3057. http://dx.doi.org/10.1016/j.fct.2011.11.010. PMid:22410416.

Emery DL, Hunt PW, Le Jambre LF. Haemonchus contortus: the then and now, and where to from here? Int J Parasitol 2016; 46(12): 755-769. http://dx.doi.org/10.1016/j.ijpara.2016.07.001. PMid:27620133.

Ferreira LE, Benincasa BI, Fachin AL, França SC, Contini SS, Chagas ACS, et al. Thymus vulgaris L. essential oil and its main component thymol: anthelmintic effects against Haemonchus contortus from sheep. Vet Parasitol 2016; 228: 70-76. http://dx.doi.org/10.1016/j.vetpar.2016.08.011. PMid:27692335.

Ferreira LE, Castro PMN, Chagas ACS, França SC, Beleboni RO. In vitro anthelmintic activity of aqueous leaf extract of Annona muricata L. (Annonaceae) against Haemonchus contortus from sheep. Exp Parasitol 2013; 134(3): 327-332. http://dx.doi.org/10.1016/j.exppara.2013.03.032. PMid:23583362.

Gainza YA, Fantatto RR, Chaves FCM, Bizzo HR, Esteves SN, Chagas ACS. Piper aduncum against Haemonchus contortus isolates: cross resistance
and the research of natural bioactive compounds. Rev Bras Parasitol Vet 2016; 25(4): 383-393. http://dx.doi.org/10.1590/s1984-296120160673. PMid:27925067.

Ghenou JD, Ahounou JF, Akakpo HB, Laley A, Yayi E, Bgaguidi F, et al. Phytochemical composition of Cymbopogon citratus and Eucalyptus citriodora essential oils and their anti-inflammatory and analgesic properties on wistar rats. Mol Biol Rep 2013; 40(2): 1127-1134. http://dx.doi.org/10.1007/s11033-012-2155-1. PMid:23065287.

Hasegawa T, Takano F, Takata T, Niyama M, Ohra T. Bioactive monoterpene glycosides conjugated with gallic acid from the leaves of Eucalyptus globulus. Phytochemistry 2008; 69(3): 747-753. http://dx.doi.org/10.1016/j.phytochem.2007.08.030. PMid:17936865.

Hounzangbe-Adote MS, Paolini V, Fournste I, Moutairou K, Hoste H. In vitro effects of four tropical plants on three life-cycle stages of the parasitic nematode, Haemonchus contortus. Res Vet Sci 2005; 78(2): 155-160. http://dx.doi.org/10.1016/j.rvsc.2004.05.009. PMid:15536923.

Katki LM, Gomes ACP, Barbieri AME, Pacheco PA, Rodrigues L, Verissimo CJ, et al. Terminalia catappa: chemical composition, in vitro and in vivo effects on Haemonchus contortus. Vet Parasitol 2017; 246: 118-123. http://dx.doi.org/10.1016/j.vetpar.2017.09.006. PMid:28969774.

Kearney PE, Murray PJ, Hoy JM, Hohenhaus M, Kotze A. The 'Toolbox' of strategies for managing Haemonchus contortus in goats: what's in and what's out. Vet Parasitol 2016; 220: 93-107. http://dx.doi.org/10.1016/j.vetpar.2016.02.028. PMid:26995278.

Kim JK, Kang CS, Lee JK, Kim YR, Han HY, Yun HK. Evaluation of repellency effect of two natural aroma mosquito repellent compounds, citronella and citronellal. Enzontol Res 2005; 35(2): 117-120. http://dx.doi.org/10.1111/j.1748-5967.2005.tb00146.x.

Kotze A, Prichard R. Anthelmintic resistance in Haemonchus contortus: history, mechanisms and diagnosis. Adv Parasitol 2016; 93: 397-428. http://dx.doi.org/10.1016/bs.apar.2016.02.012. PMid:27238009.

Lenardão EJ, Botteselle GV, Azambuja F, Perin G, Jacob RG. Citronellal as key compound in organic synthesis. Tetrahedron 2007; 63(29): 6671-6712. http://dx.doi.org/10.1016/j.tet.2007.03.159.

Lespine A, Chartier C, Hoste H, Alvinerie M. Endectocides in goats: pharmacology, efficacy and use conditions in the context to anthelmintics resistance. Small Rumin Res 2012; 103(1): 10-17. http://dx.doi.org/10.1016/j.smallrumres.2011.10.013.

Lopes LG, Silva MH, Figueiredo A, Canuto KM, Brito ES, Ribeiro PRV, et al. The intake of dry cashew apple fiber reduced fecal egg counts in Haemonchus contortus-infected sheep. Exp Parasitol 2018; 195: 38-43. http://dx.doi.org/10.1016/j.exppara.2018.10.004. PMid:30393118.

Macedo ITF, Bevilaqua CML, Oliveira LMB, Camurça-Vasconcelos ALF, Vieira LS, Amôra SSA. Evaluation of Eucalyptus citriodora essential oil on goat gastrointestinal nematodes. Rev Bras Parasitol Vet 2011; 20(3): 223-227. http://dx.doi.org/10.1590/S1984-2961201100030009. PMid:21961753.

Maciel MV, Morais SM, Bevilaqua CML, Silva RA, Barros RS, Sousa RN, et al. Chemical composition of Eucalyptus spp. essential oils and their insecticidal effects on Lutzomyia longipalpis. Vet Parasitol 2010; 167(1): 1-7. http://dx.doi.org/10.1016/j.vetpar.2009.09.053. PMid:1996276.

Nakahara K, Alzoreky NS, Yoshihashi T, Nguyen HT, Trakootivakorn G. Chemical composition and antifungal activity of essential oil from Cymbopogon nardus (Citronella Grass). Jpn Agric Res Q 2003; 37(4): 249-252. http://dx.doi.org/10.6090/jarq.37.249.

Niesink RJM, de Vries J, Hollandiner MA. Toxicology: principles and practice. Boca Raton: CRC Press; 1996.

Oda S, Inada Y, Kobayashi A, Kato A, Matsudomi N, Ohra H. Coupling of metabolism and bioconversion: microbial esterification of citronellol with acetyl coenzyme a produced via metabolism of glucose in an interface bioreactor. Appl Environ Microbiol 1996; 62(7): 2216-2220. PMid:16535347.

Organisation for Economic Co-operation and Development – OECD. Acute Oral Toxicity: Up-and-Down Procedure (UDP) [online]. Paris: OECD; 2008 (Test Guideline; 425). [cited 2019 Jan 11]. Available from: https://ntp.niehs.nih.gov/iccvam/suppdocs/teddocs/oecd/425.pdf

Ribeiro JC, Ribeiro WL, Camurça-Vasconcelos AL, Macedo IT, Santos JM, Paula HC, et al. Efficacy of free and nanoencapsulated Eucalyptus citriodora essential oil on sheep gastrointestinal nematodes and toxicity for mice. Vet Parasitol 2014; 204(3-4): 243-248. http://dx.doi.org/10.1016/j.vetpar.2014.05.026. PMid:24929446.

Ribeiro WLC, André WPP, Cavalcante GS, Araújo-Filho JV, Santos JM, Macedo ITF, et al. Effects of Spigelia anthelmia decoction on sheep gastrointestinal nematodes. Small Rumin Res 2017; 153: 146-152. http://dx.doi.org/10.1016/j.smallrumres.2017.06.001.

Sales N, Love S. Resistance of Haemonchus sp. to monopanel and reduced efficacy of a derquant/abamectin combination confirmed in sheep in NSW, Australia. Vet Parasitol 2016; 228: 193-196. http://dx.doi.org/10.1016/j.vetpar.2016.08.016. PMid:27692326.

Sant’anna V, Vommaro RC, de Souza W. Caeorhabditis elegans as a model for the screening of anthelmintic compounds: ultrastructural study of the effects of albendazole. Exp Parasitol 2013; 135(1): 1-8. http://dx.doi.org/10.1016/j.exppara.2013.05.011. PMid:23727123.

Singh P, Scott I, Jacob A, Storillo VM, Pomroy WE. Pharmacokinetics of abamectin in sheep, goat and deer. Small Rumin Res 2018; 165: 30-33. http://dx.doi.org/10.1016/j.smallrumres.2018.06.009.

Ueno H, Gonçalves PC. Manual para diagnóstico das helmintose de ruminantes. Tokyo: Japan International Cooperation Agency; 1998.

Vitti AMS, Brito JO. Óleo essencial de eucalipto. Documentos Florestais 2003; 17: 1-35.