Brazilian Journal of Veterinary Parasitology
ISSN 1984-2961 (Electronic)
www.cbpv.org.br/rbpv
Braz. J. Vet. Parasitol., Jaboticabal, v. 28, n. 3, p. 522-527, july.-sept. 2019
Doi: https://doi.org/10.1590/S1984-29612019065

Short Communication

Anthelmintic effect of *Cymbopogon citratus* essential oil and its nanoemulsion on sheep gastrointestinal nematodes

Efeito anti-helmíntico do óleo essencial de *Cymbopogon citratus* e sua nanoemulsão sobre nematoides gastrintestinais de ovinos

Iara Tersia Freitas Macedo[1]; Lorena Mayana Beserra de Oliveira[1]; Weibson Paz Pinheiro André[1]; José Vilemar de Araújo Filho[1]; Jéssica Maria Leite dos Santos[1,2]; Fernanda Cristina Macedo Rondon[1]; Wesley Lyeverton Correia Ribeiro[1,3]; Ana Lourdes Fernandes Camurça-Vasconcelos[1,4]; Erick Falcão de Oliveira[5]; Haroldo César Beserra de Paula[5]; Claudia Maria Leal Bevilaqua[1]*

[1] Programa de Pós-graduação em Ciências Veterinárias, Laboratório de Doenças Parasitárias, Faculdade de Veterinária, Universidade Estadual do Ceará – UECE, Fortaleza, CE, Brasil

[2] Centro Universitário – INTA-Uninta, Sobral, CE, Brasil

[3] Departamento de Fisiologia e Farmacologia, Faculdade de Medicina, Universidade Federal do Ceará – UFC, Fortaleza, CE, Brasil

[4] Faculdade Terra Nordeste – FATENE, Fortaleza, CE, Brasil

[5] Departamento de Química Analítica e Físico-Química, Laboratório de Biopolímeros, Universidade Federal do Ceará – UFC, Fortaleza, CE, Brasil

Received January 22, 2019
Accepted May 24, 2019

Abstract

The anthelmintic resistance stimulated the search for strategies for controlling gastrointestinal nematodes, including the use of free essential oils or its nanoemulsion. This study evaluated the anthelmintic efficacy of *Cymbopogon citratus* essential oil (CcEO) and *C. citratus* essential oil nanoemulsion (CcEOn). Pysicochemical analyses were performed. The *in vitro* effect was determined using the egg hatch test (EHT) on *Haemonchus contortus* and *in vivo* effect was evaluated in sheep infected with gastrointestinal nematodes. The animals were treated with CcEO (500 mg/kg) or CcEOn (450 mg/kg) for the fecal egg count (FEC) and the determination of worm burden. The main component of CcEO was citral. The CcEO content in the nanoemulsion was 20% (v/v), and the mean particle size was 248 nm. In EHT, CcEO and CcEOn (1.25 mg/mL) inhibited larval hatching by 98.4 and 97.1%, respectively. Three animals treated with CcEO died whereas in the group treated with CcEOn one animal died. The FEC and total worm burden of the treated groups did not differ from the negative control (p>0.05). The CcEOn showed efficacy only on *H. contortus* (p<0.05). In conclusion, nanoencapsulation reduced toxicity and increased efficacy on *H. contortus*.

Keywords: Phytotherapy, nanotechnology, *Haemonchus contortus,* small ruminants.

Resumo

A resistência anti-helmíntica estimulou a busca por estratégias de controle de nematoides gastrintestinais, incluindo óleos essenciais livres ou em nanoemulsão. Este estudo avaliou a eficácia anti-helmíntica do óleo essencial de *Cymbopogon citratus* (OECc) e da nanoemulsão do óleo essencial de *C. citratus* (nOECc). Análises físico-químicas foram realizadas. O efeito *in vitro* foi determinado no teste de eclosão de ovos (TEO) sobre *Haemonchus contortus* e o efeito *in vivo* foi avaliado em ovinos infectados com nematoides gastrintestinais. Os animais foram tratados com OECc (500 mg/kg) ou nOECc (450 mg/kg) para determinação do número de ovos por grama de fezes (OPG) e carga parasitária. O principal constituinte do OECc foi citral. O conteúdo de OECc na nanoemulsão foi 20% e o tamanho médio de partícula foi 248 nm. No TEO, OECc e nOECc (1,25 mg/mL) inibiram 98,4 e 97,1% da eclosão larvar, respectivamente. Três animais tratados com o OECc morreram, enquanto um animal do grupo tratado com a nOECc morreu. O OPG e a carga parasitária total dos grupos tratados não diferiram do controle negativo (p>0.05). A nOECc apresentou eficácia somente sobre *H. contortus* (p<0.05). Como conclusão, o nanoencapsulamento reduziu a toxicidade e aumentou a eficácia sobre *H. contortus*.

Palavras-chave: Fitoterapia, nanotecnologia, *Haemonchus contortus,* pequenos ruminantes.

***Corresponding author:** Claudia Maria Leal Bevilaqua. Programa de Pós-graduação em Ciências Veterinárias, Laboratório de Doenças Parasitárias, Faculdade de Veterinária, Universidade Estadual do Ceará – UECE, Av. Dr. Silas Munguba, 1700, Campus do Itaperi, CEP 60714-903, Fortaleza, CE, Brasil. e-mail: claudiamlb@yahoo.com.br.

Introduction

Gastrointestinal nematode is one of the factors that reduce productivity of livestock worldwide (ROEBER et al., 2017). Control measures for parasitic diseases of livestock have relied heavily on the use of commercial anthelmintics. However, the injudicious use of these drugs resulted in nematode resistance to anthelmintics in different parts of the world (KOTZE & PRICHARD, 2016). The intensification of animal production has led to an increasing demand for more effective and low cost anthelmintics (ADEMOLA et al., 2004). These concerns have stimulated the search for alternative control methods, such as the use of medicinal plants (RIBEIRO et al., 2015).

Cymbopogon citratus is a tropical plant that belongs to the Poaceae (Gramineae) family. An ethnoveterinary study carried out on the Colares island, Pará state, eastern Amazon, Brazil, reported that *C. citratus* has anthelmintic indication (RITTER et al., 2012). Several studies have showed that the *C. citratus* essential oil (CcEO) has a number of biological properties including activity against *Haemonchus contortus* in *Meriones unguiculatus* (MACEDO et al., 2015). Nevertheless, the anthelmintic activity of CcEO has not yet been assessed in small ruminants.

Essential oils are complex mixtures of active substances of plant origin which exhibit a variety of properties. However there are some limitations related to the stability of these active substances that hinder their preservation. These substances may change rapidly due volatilization and oxidation of their chemical constituents (GONSALVES et al., 2009). In order to overcome these drawbacks, carrier systems are used which increase the stability of these compounds and modulate substance release (TIWARI et al., 2010). Chitosan is a non-toxic polymer that may be used as an encapsulating agent as it has biocompatible, biodegradable properties and is also capable of forming films, gels, beads, and particles (WORANUCH & YOKSAN, 2013). To protect and maximize the nematicidal effect of oils, encapsulation techniques employing chitosan have been investigated (RIBEIRO et al., 2013, 2015).

The objective of this study was to evaluate the anthelmintic activity of CcEO and *C. citratus* essential oil nanoemulsion (CcEOn) using chitosan by egg hatching test and on sheep naturally infected with gastrointestinal nematodes.

Materials and Methods

Chemical analysis of Cymbopogon citratus essential oil

A commercial form of CcEO prepared by the company Phytoterápica (State of São Paulo, Brazil, lot 185) was used. The chemical composition of the CcEO used in this study was determined by gas chromatography and mass spectrometry (ADAMS, 2001).

Nanoemulsion of Cymbopogon citratus essential oil and physicochemical characterization

Chitosan powder (Polymar®) was used as encapsulant biopolymer. To promote encapsulation, a mixture of CcEO and tween 80 at the ratio of 2:1 was added to a solution of 1% chitosan (v/v) under stirring at 188.5 rad/s for 5 min in a mechanical stirrer. The resulting emulsion was evaluated according to the macroscopic characteristics of CcEOn stability which were observed over 72 h at 27°C. The size and distribution of nanoparticles in solution were determined using a beam of red light (ZetaSizer 3600, Malvern, United Kingdom).

Animal welfare

The protocol was approved by the ethics committee for animal use of Universidade Estadual do Ceará (number 09657334-1).

Egg hatch test

The egg hatch test (EHT) was performed according to the method described by Coles et al. (1992). For this, sheep experimentally infected with *H. contortus* was used as a source of fresh eggs, which were recovered according to Hubert & Kerboeuf (1992). An suspension (250 μL) containing approximately 100 fresh eggs was incubated with 250 μL of essential oil or nanoemulsion at the concentrations of 0.07, 0.15, 0.31, 0.62, and 1.25 mg/mL for 48 h at 27°C. The CcEOn concentrations were adjusted according to the volume of essential oil present in the nanoemulsion. After this time, drops of Lugol´s iodine solution were added. The eggs and first-stage larvae (L1) were counted under a light microscope. This test had two controls: a negative and a positive. The negative control containing 3% tween 80 and 1% chitosan. The tween 80 concentration was the same used to dilute the oil and its nanuelmusion. The positive control containing 0.025 mg/mL thiabendazole. Two repetitions with five replicates for each concentration and for each control were performed.

In vivo test

Thirty sheep of both sexes between the ages of 10 to 16 months with an average weight of 22 kg were kept in paddocks and fed with fresh grass, salt-mineral supplement, and water *ad libitum*. Individual fecal samples were collected to determine the level of natural infection by gastrointestinal nematode using a modified McMaster technique (UENO & GONÇALVES, 1998). Sheep were divided into three homogeneous groups according to the fecal egg count (FEC). Each group (n = 10) received orally the following treatments for three days: G1 - 500 mg/kg CcEO; G2 - 450 mg/kg CcEOn; and G3 (control) - 3% tween 80 and 1% chitosan. The CcEOn dose was adjusted according to the volume of essential oil present in the nanoemulsion. The animals of G2 received the smallest dose because the nanoencapsulation may potentiate the anthelmintic effect of CcEO. For the fecal egg count reduction test (FECRT), samples from each animal were collected before and after 7 and 14 days of treatment to determine the FEC using the McMaster technique (UENO & GONÇALVES, 1998) and to perform fecal cultures according to Roberts & O'Sullivan (1950).

The controlled test was performed 15 days after treatment when five sheep of each group (G1, G2 and G3) were euthanized for parasite count and estimate parasite load. The selection of animals for euthanasian was aleatory. The gastrointestinal tract of each necropsied animal was removed after applying double ligatures at the abomasum, small intestine, and large intestines, and were then examined separately. Each of these segments of the gastrointestinal tract was opened and their contents thoroughly washed separately (WOOD et al., 1995). A sample (5%) of the contents from each of these parts of the gastrointestinal tract was collected individually for parasitological analysis. Samples were stored in AFA solution (alcohol, formol, acetic acid, and water). Worms recovered from the gastrointestinal contents were identified using a stereomicroscope (UENO & GONÇALVES, 1998).

Statistical Analysis

The efficacy of each treatment in EHT was determined based on the following formula:

$$EHT = \frac{\text{number of eggs}}{\text{number of hatched larvae} + \text{number of eggs}} \times 100 \quad (1)$$

To evaluate the difference between the concentrations of a single product, data were analyzed using the one-way analysis of variance (ANOVA), and were compared using the Tukey's test ($p<0.05$). To compare the results of the same concentration of CcEO and CcEOn was used the two-way ANOVA followed by the Bonferroni's test ($p<0.05$). The effective concentration to inhibit 50% (EC_{50}) of larvae hatching was determined by linear regression (ANDRÉ et al., 2017).

The anthelmintic efficacy of CcOE and CcOEn was interpreted through the FECRT based on each group arithmetic mean FEC using the following formula:

$$FECRT = 100 \times \left(1 - \frac{T2}{T1} \times \frac{C1}{C2}\right) \quad (2)$$

In this formula, the arithmetic FEC 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). This software was only used to calculate the anthelmintic efficacy of CcOE and CcOEn. The FEC data were log transformed ($\log_{10}[x + 1]$), submitted to ANOVA and compared using Newman–Keuls's test. The significance level was $p<0.05$.

In the controlled test, the percentage of reduction in parasitic burden in the treated sheep group was estimated using the following formula:

$$\text{Controlled test} = \frac{\text{control group worm burden} - \text{treated group worm burden}}{\text{control group worm burden}} \times 100 \quad (3)$$

The worm burden data were log transformed ($\log_{10}[x + 1]$), analyzed by ANOVA, and compared with the Newman–Keuls's test ($p<0.05$).

The ANOVA and Tukey, Bonferroni and Newman–Keuls's tests were realized using the GraphPad Prism® 5.0 software (GraphPad Software Inc., EUA). The EC_{50} of larvae hatching was determined using IBM SPSS Statistic version 17 for Windows (IBM, USA).

Results

The main components of CcEO were 3,7-nonadien-2-one, 4,8-dimethyl (24.86%), geranial (18.98%), neral (17.77%), nerol (9.31%), 1-heptadec-1-ynyl-cyclopentanol (9.63%), 7,7-dimethyl-bicycloheptan-2-ol (7.96%) and d-limonene (2.44%).

To obtain a stable nanoemulsion (no phase separation), 20.0% (v/v) of the CcEO content was used. Analysis of the resulting nanoemulsion particles demonstrated a mean size of 248 nm with bimodal distribution. The nanoemulsion was white, with a milky consistency and could flow through an oral dosing pistol. No phase separation was visually observed after 72 h.

The mean efficacy of CcEO and CcEOn obtained in the EHT is presented in Table 1. CcEO and CcEOn showed ovicidal activity at all tested concentrations, and for the higher concentration (1.25 mg/mL), the effect was similar to that of thiabendazole ($p>0.05$). The data of Table 1 indicate that the CcEO and CcEOn had dose-dependent effect. The EC_{50} values in the EHT were 0.15 and 0.16 mg/mL for CcEO and CcEOn, respectively.

Three animals treated with CcEO died whereas in the group treated with CcEOn one animal died. The FEC of sheep at 8 and 15 days post-treatment with CcEO and CcEOn is expressed in Table 2. Reduction in treated group with CcEO and CcOEn varied from 19.5 to 23.9% and 51.7 to 47.0%, respectively. Although the numerical results of mean FEC were different, there was no significant difference between the treated groups and the control group ($p>0.05$). The most prevalent nematode genus in fecal cultures was *Haemonchus* followed by *Trichostrongylus* (Table 2).

The mean efficacy of CcEO and CcEOn on sheep worm burden is summarized in Table 3. The species found were *H. contortus*, *Trichostrongylus colubriformis*, *Oesophagostomum columbianum* and *Trichuris ovis*. However, due to the small number of parasites found, the worm burden of the large intestine was not statistically analyzed. The total worm burden of the groups treated with CcEO and CcEOn and control group was similar ($p>0.05$). CcEO and CcEOn were more effective against *H. contortus* because reductions in adult worm burden were 66.4 and 83.1%, respectively. *H. contortus* adult worm burden of sheep treated with CcEOn was different in the treated group with CcEO and the control group ($p<0.05$). The effect on *T. colubriformis* was not statistically different between treated and control groups ($p>0.05$).

Table 1. Efficacy percentage (mean ± standard deviation) of *Cymbopogon citratus* essential oil (CcEO) and *C. citratus* essential oil nanoemulsion (CcEOn) on egg hatching of *Haemonchus contortus*.

Concentrations (mg/mL)	CcEO	CcEOn
1.25	98.4 ± 2.4^{Aa}	97.1 ± 4.7^{Aa}
0.62	96.0 ± 2.8^{Ab}	73.2 ± 10.1^{Ba}
0.31	74.8 ± 14.9^{Bb}	58.1 ± 9.4^{Ca}
0.15	37.1 ± 7.2^{Ca}	49.4 ± 7.1^{Ca}
0.07	27.5 ± 8.0^{Ca}	34.9 ± 16.3^{Da}
Negative control	9.8 ± 6.0^{Da}	9.8 ± 6.0^{Ea}
Positive control	97.1 ± 1.6^{Aa}	97.1 ± 1.6^{Aa}

Capital letters compare mean in the columns and lowercase letters compare mean in the rows. Different letters indicate significantly different values ($p<0.05$).

Table 2. Fecal egg count (mean FEC ± standard deviation) and gastrointestinal nematode larvae (%) in coprocultures of sheep before and after treatment with *Cymbopogon citratus* essential oil (CcEO) and *C. citratus* essential oil nanoemulsion (CcEOn).

Treatment	Day 0	Day 8	Day 15
CcEO (500 mg/kg)			
Mean FEC	3,720 ± 1,941.4^{Aa}	1,900 ± 2,002^{Aa}	1,729 ± 2,072^{Aa}
Efficacy (%)	-	19.6	23.9
Haemonchus spp.	81	-	77
Trichostrongylus spp.	15	-	20
Oesophagostomum spp.	4	-	3
CcEOn (450 mg/kg)			
Mean FEC	3,969 ± 2,083^{Aa}	1,219 ± 877.1^{Ab}	1,288 ± 972.4^{Ab}
Efficacy (%)	-	51.7	47.0
Haemonchus spp.	80	-	64
Trichostrongylus spp.	19	-	27
Oesophagostomum spp.	1	-	9
Negative control			
Mean FEC	3,381 ± 1,623^{Aa}	2,150 ± 1,230^{Aa}	2,069 ± 831.5^{Aa}
Haemonchus spp.	70	-	68
Trichostrongylus spp.	28	-	26
Oesophagostomum spp.	2	-	6

Capital letters compare mean in the columns and lowercase letters compare mean in the rows. Different letters indicate significantly different values ($p<0.05$).

Table 3. Worm burden (mean ± standard deviation) of sheep after treatment with *Cymbopogon citratus* essential oil (CcEO) and *C. citratus* essential oil nanoemulsion (CcEOn).

Treatment	*Haemonchus contortus*	*Trichostrongylus colubriformis*	Total
CcEO (500 mg/kg)			
Mean worm burden	200 ± 156.8^{A}	844 ± 752.6^{A}	1,044 ± 788.2^{A}
Efficacy (%)	66.4	38.4	46.9
CcEOn (450 mg/kg)			
Mean worm burden	100 ± 52.9^{B}	988 ± 842.1^{A}	1,088 ± 837^{A}
Efficacy (%)	83.1	27.9	44.7
Negative control			
Mean worm burden	596 ± 657.1^{A}	1,372 ± 721.6^{A}	1,968 ± 422^{A}

Different letters indicate significantly different values ($p<0.05$).

Discussion

The use of medicinal plants with anthelmintic activity has been regarded as an efficient control measure against gastrointestinal parasitism (ANDRÉ et al., 2018). Essential oils from plants consist of an important group of substances that may be used as an alternative or adjunct antiparasitic therapy (ANTHONY et al., 2005). These oils interfere with helminth metabolism by inhibiting or disorganizing vital functions of the nematode from its early stages of development onwards, and can also induce nervous system derangement of nematodes inhibiting the motility of these worms (OKA et al., 2000; ANDRÉ et al., 2017).

The anthelmintic properties of CcEO are mainly attributed to its major component which is citral. Citral is a natural combination of two isomeric aldehydes, neral (cis-citral) and geranial (trans-citral), that together correspond to approximately 65-85% of the total oil composition of *C. citratus* (SADDIQ & KHAYYAT, 2010). In the present study, citral concentration was 36.75%. In another study, CcEO consisted of 97.7% of citral and was able to reduce 38.6% of the parasite load of *M. unguiculatus* experimentally infected with *H. contortus* (MACEDO et al., 2015). Studies have demonstrated that citral is capable of combating several nematodes including *Anisakis simplex*, *Bursaphelenchus xylophilus* and *Meloidogyne incognita* (BAUSKE et al., 1994; HIERRO et al., 2006; CHOI et al., 2007). Many factors including genetic variation, plant ecotype or variety, plant nutrition, application of fertilizers, geographic location of the plants, surrounding climate, seasonal variations, stress during growth or maturity and also the post harvest drying and storage, affect the chemistry of essential oils. In addition, type of plant material used and the method of extraction determine the yield and composition (constituents) of an essential oil, and thereby decides its characteristic biological properties (RAUT & KARUPPAYIL, 2014). Besides the lower concentration of citral in the essential oil used in our study, other factors such as synergistic interactions among essential oil constituents may have affected the anthelmintic activity.

In the present study, CcEO and CcEOn presented a statistically similar efficacy in the EHT. Previous studies have demonstrated that the inhibition of larvae hatching by *Eucalyptus citriodora* and

Eucalyptus staigeriana essential oils was enhanced by nanoencapsulation with chitosan 1% (RIBEIRO et al., 2014, 2015). In these studies, the concentration of essential oils in nanoemulsions was 36.4%. In the present experimental trial, the concentration of the essential oil of *C. citratus* in the nanoemulsion was 20%. Possibly, the higher concentration of the encapsulating matrix in relation to the essential oil retained in the polymer system influenced the controlled release process in the aqueous environment of EHT. The diffusion of retained drugs in nanoemulsions occurs when water enters the polymer system resulting in swelling and subsequent release of the encapsulated drugs (DASH et al., 2011).

The essential oil of *E. staigeriana* (500 mg/kg) was able to reduce egg excretion in the feces of the goats in 76.5% (MACEDO et al., 2010). In goats treated with the essential oil of *E. citriodora* (500 mg/kg), there was a decrease of 60.3% in the FEC (MACEDO et al., 2011). In sheep, the essential oil of *L. sidoides* (283 mg/kg) reduced the FEC in 54% (CAMURÇA-VASCONCELOS et al., 2008). In the present study, on day 15 post-treatment, efficacy of CcEO (500 mg/kg) and CcEOn (450 mg/kg) on FEC was 23.9 and 47.0%, respectively. However, the FEC in the control and treated groups did not differ significantly. Similar results were observed when the anthelmintic efficacy of the free and nanoencapsulated essential oil of *E. citriodora* (250 mg/kg) was evaluated (RIBEIRO et al., 2014). This fact can be explained by the high values of standard deviation found in mean FEC. Besides the egg fecal excretion is a variable measure which is influenced not only by worm burden (FORTES & MOLENTO, 2013).

Haemonchus contortus is a blood-sucking abomasal nematode with high prevalence and intensity, and is considered the most pathogenic gastrointestinal helminth of small ruminants. In the presented study, CcEOn was able to significantly reduce the parasite load of *H. contortus* in the controlled test. However, the anthelmintic activity of CcEOn on *T. colubriformis* was lower in comparison with *H. contortus*. Similar results were obtained in studies conducted by Mesquita et al. (2013) using *E. staigeriana* essential oil nanoencapsulated (4% chitosan). There was a reduction of 83.7% on *H. contortus* parasite load while there was a decrease of 22.3% in the parasite burden of small intestine nematodes. These results can be explained by the bioadhesive properties of chitosan on the abomasal mucosa (SOSNIK et al., 2014). Benefits associated with the bioadhesive property of this polysaccharide include retention of the nanoemulsion in the gastrointestinal tract which improves drug absorption, increases drug bioavailability, and increases drug concentrations in tissues (HEJAZI & AMIJI, 2003; RIBEIRO et al., 2015).

A previous study was performed to evaluate the toxicity of CcEO in rats, verifying that until the dose of 1.000 mg/kg presented no toxicity (FANDOHAN et al., 2008). In another study, the administration of 800 mg/kg of CcEO to *M. unguiculatus* also presented no toxicity (MACEDO et al., 2015). In the group treated with CcEO, three animals died whereas in the group treated with CcEOn one animal died. The explanation for the toxicity found in present study can be associated to different chemical composition of the essential oil. However, before death, the sheep presented sialorrhea. The animals may have aspirated the CcEO or CcEOn and died due to wrong-route administration. Nevertheless, the death cause cannot be determined, since necropsy was not performed. In addition, the encapsulation technique used in the present experiment allowed the formation of nanoparticles of chitosan matrix that enhance the CcEO activity on *H. contortus* and decrease its toxicity in sheep. In mice, the nanoemulsion of the *E. citriodora* essential oil was less toxic to the experimental animals in comparison with free oil of this plant (RIBEIRO et al., 2014).

To our knowledge, this study is the first to assess the anthelmintic activity of *C. citratus* oil essential and its nanoemulsion in sheep. The results indicate that *C. citratus* may affect *H. contortus* and suggest potential use of CcEOn as a promising to control gastrointestinal infections in sheep. However further investigation with different doses and formulations are necessary to improve the efficacy and reduce toxicity.

Acknowledgements

This work received financial support from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP). Dr. Bevilaqua has a grant from CNPq.

References

Adams RP. *Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy*. 3rd ed. Illinois: Allured; 2001.

Ademola IO, Fagbemi BO, Idowu SO. Evaluation of the anthelmintic activity of *Khaya senegalensis* extract against gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies. *Vet Parasitol* 2004; 122(2): 151-164. http://dx.doi.org/10.1016/j.vetpar.2004.04.001. PMid:15177720.

André WPP, Cavalcante GS, Ribeiro WLC, Santos JML, Macedo ITF, Paula HCB, et al. Anthelmintic effect of thymol and thymol acetate on sheep gastrointestinal nematodes and their toxicity in mice. *Rev Bras Parasitol Vet* 2017; 26(3): 323-330. http://dx.doi.org/10.1590/s1984-29612017056. PMid:28977246.

André WPP, Ribeiro WLC, Oliveira LMB, Macedo ITF, Rondon FCM, Bevilaqua CML. Óleos essenciais e seus compostos bioativos no controle de nematoides gastrintestinais de pequenos ruminantes. *Acta Sci Vet* 2018; 46: 1522. http://dx.doi.org/10.22456/1679-9216.81804.

Anthony JP, Fyfe L, Smith H. Plant active components – a resource for antiparasitic agents? *Trends Parasitol* 2005; 21(10): 462-468. http://dx.doi.org/10.1016/j.pt.2005.08.004. PMid:16099722.

Bauske EM, Rodríguez-Kábana R, Estaún V, Kloepper JW, Robertson DG, Weaver CF, et al. Management of *Meloidogyne incognita* on cotton by use of botanical aromatic compounds. *Nematropica* 1994; 24(2): 143-150.

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, Bevilaqua 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.

Choi IH, Kim J, Shin SC, Park IK. Nematicidal activity of monoterpenoids against the pine wood nematode (*Bursaphelenchus xylophilus*). *Russ J Nematol* 2007; 15(1): 35-40.

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 the 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 KM, Hall K, Barger IA. The role of arithmetic and geometric mean worm egg counts in faecal egg count reduction tests 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:3355458.

Dash M, Chiellini F, Ottenbrite RM, Chiellini E. Chitosan - a versatile semi-synthetic polymer in biomedical applications. *Prog Polym Sci* 2011; 36(8): 981-1014. http://dx.doi.org/10.1016/j.progpolymsci.2011.02.001.

Fandohan P, Gnonlonfin B, Laleye A, Gbenou JD, Darboux R, Moudachirou M. Toxicity and gastric tolerance of essential oils from *Cymbopogon citratus, Ocimum gratissimum* and *Ocimum basilicum* in Wistar rats. *Food Chem Toxicol* 2008; 46(7): 2493-2497. http://dx.doi.org/10.1016/j.fct.2008.04.006. PMid:18511170.

Fortes FS, Molento MB. Resistência anti-helmíntica em nematoides gastrintestinais de pequenos ruminantes: avanços e limitações para seu diagnóstico. *Pesq Vet Bras* 2013; 33(12): 1391-1402. http://dx.doi.org/10.1590/S0100-736X2013001200001.

Gonsalves JKMC, Costa AMB, Sousa DP, Cavalcanti SCH. Microencapsulação do óleo essencial de *Citrus sinensis* (L) Osbeck pelo método da coacervação simples. *Scientia Plena* 2009; 5(11): 1-8.

Hejazi R, Amiji M. Chitosan-based gastrointestinal delivery systems. *J Control Release* 2003; 89(2): 151-165. http://dx.doi.org/10.1016/S0168-3659(03)00126-3. PMid:12711440.

Hierro I, Valero A, Navarro MC. In vivo larvicidal activity of monoterpenic derivatives from aromatic plants against L_3 larvae of *Anisakis simplex* s.l. *Phytomedicine* 2006; 13(7): 527-531. http://dx.doi.org/10.1016/j.phymed.2005.05.001. PMid:16785045.

Hubert J, Kerboeuf D. A microlarval development assay for the detection of anthelmintic resistance in sheep nematodes. *Vet Rec* 1992; 130(20): 442-446. http://dx.doi.org/10.1136/vr.130.20.442. PMid:1621342.

Kotze AC, Prichard RK. 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.

Macedo ITF, Bevilaqua CML, Oliveira LMB, Camurça-Vasconcelos ALF, Vieira LS, Amóra 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/S1984-29612011000300009. PMid:21961753.

Macedo ITF, Bevilaqua CML, Oliveira LMB, Camurça-Vasconcelos ALF, Vieira LS, Oliveira FR, et al. Anthelmintic effect of *Eucalyptus staigeriana* essential oil against goat gastrointestinal nematodes. *Vet Parasitol* 2010; 173(1-2): 93-98. http://dx.doi.org/10.1016/j.vetpar.2010.06.004. PMid:20609526.

Macedo ITF, Oliveira LMB, Ribeiro WLC, Santos JML, Silva KC, Filho JVA, et al. Anthelmintic activity of *Cymbopogon citratus* against *Haemonchus contortus. Rev Bras Parasitol Vet* 2015; 24(3): 268-275. http://dx.doi.org/10.1590/S1984-29612015059. PMid:26444058.

Mesquita MA, Silva Júnior JB, Panassol AM, Oliveira EF, Camurça-Vasconcelos ALF, Paula HCB, et al. Anthelmintic activity of *Eucalyptus staigeriana* encapsulated oil on sheep gastrointestinal nematodes. *Parasitol Res* 2013; 112(9): 3161-3165. http://dx.doi.org/10.1007/s00436-013-3492-2. PMid:23783400.

Oka Y, Nacar S, Putievsky E, Ravid U, Zohara Y, Spiegel Y. Nematicidal activity of essential oils and their components against the root-knot nematode. *Nematology* 2000; 90(7): 710-715. http://dx.doi.org/10.1094/PHYTO.2000.90.7.710. PMid:18944489.

Raut JS, Karuppayil SM. A status review on the medicinal properties of essential oils. *Ind Crops Prod* 2014; 62: 250-264. http://dx.doi.org/10.1016/j.indcrop.2014.05.055.

Ribeiro JC, Ribeiro WLC, Camurça-Vasconcelos ALF, Macedo ITF, Santos JML, Paula HCB, et al. Efficacy of free and nanoencapsulated *Eucalyptus citriodora* essential oils 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, Camurça-Vasconcelos ALF, Macedo ITF, Santos JML, Araújo-Filho JV, Ribeiro JC, et al. In vitro effects of *Eucalyptus staigeriana* nanoemulsion on *Haemonchus contortus* and toxicity in rodents. *Vet Parasitol* 2015; 212(3-4): 444-447. http://dx.doi.org/10.1016/j.vetpar.2015.07.019. PMid:26233731.

Ribeiro WLC, Macedo ITF, Santos JML, Oliveira EF, Camurça-Vasconcelos ALF, Paula HCB, et al. Activity of chitosan-encapsulated *Eucalyptus staigeriana* essential oil on *Haemonchus contortus. Exp Parasitol* 2013; 135(1): 24-29. http://dx.doi.org/10.1016/j.exppara.2013.05.014. PMid:23748159.

Ritter RA, Monteiro MVB, Monteiro FOB, Rodrigues ST, Soares ML, Silva JCR, et al. Ethnoveterinary knowledge and practices at Colares island, Pará state, eastern Amazon, Brazil. *J Ethnopharmacol* 2012; 144(2): 346-352. http://dx.doi.org/10.1016/j.jep.2012.09.018. PMid:23000170.

Roberts FHS, O'Sullivan PJ. Methods for egg counts and larval cultures for strongyles infesting the gastrointestinal tract of cattle. *Aust J Agric Res* 1950; 1(1): 99-102. http://dx.doi.org/10.1071/AR9500099.

Roeber F, Morrison A, Casaert S, Smith L, Claerebout E, Skuce P. Multiplexed-tandem PCR for the specific diagnosis of gastrointestinal nematode infections in sheep: an European validation study. *Parasit Vectors* 2017; 10(1): 226. http://dx.doi.org/10.1186/s13071-017-2165-x. PMid:28482924.

Saddiq A, Khayyat S. Chemical and antimicrobial studies of monoterpene: citral. *Pestic Biochem Physiol* 2010; 98(1): 89-93. http://dx.doi.org/10.1016/j.pestbp.2010.05.004.

Sosnik A, Neves J, Sarmento B. Mucoadhesive polymers in the design of nano-drug delivery systems for administration by non-parenteral routes: a review. *Prog Polym Sci* 2014; 39(12): 2030-2075. http://dx.doi.org/10.1016/j.progpolymsci.2014.07.010.

Tiwari SK, Tzezana R, Zussman E, Venkatraman SS. Optimizing partition-controlled drug release from electrospun core–shell fibers. *Int J Pharm* 2010; 392(1-2): 209-217. http://dx.doi.org/10.1016/j.ijpharm.2010.03.021. PMid:20227472.

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

Wood IB, Amaral NK, Bairden K, Duncan JL, Kassai T, Malone JB Jr, et al. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) second edition of guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine, ovine, caprine). *Vet Parasitol* 1995; 58(3): 181-213. http://dx.doi.org/10.1016/0304-4017(95)00806-2. PMid:7571325.

Woranuch S, Yoksan R. Eugenol-loaded chitosan nanoparticles: I. Thermal stability improvement of eugenol through encapsulation. *Carbohydr Polym* 2013; 96(2): 578-585. http://dx.doi.org/10.1016/j.carbpol.2012.08.117. PMid:23768603.