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In vitro efficacy of *Carapa guianensis* Aublet (Meliaceae) oil on *Damalinia* (*Bovicola*) *ovis* Schrank (1781)

Eficácia in vitro do óleo de Carapa guianensis *Aubl. (Meliaceae) sobre* Damalinia (Bovicola) ovis *Schrank (1781)*

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ABSTRACT: Productivity in small ruminants can be compromised due to lice infestations. This pest infestation is controlled by the administration of insecticides, and their excessive use causes resistance. New control alternatives are being investigated, especially the use of phytotherapeutics. Research using the oil from *Carapa guianensis* (andiroba) has shown insecticidal action on feline and goat lice specimens. The present study aimed to evaluate the efficacy of *C. guianensis* seed oil, *in vitro*, on *Damalinia* (*Bovicola*) ovis. Nine hundred specimens of *D.* (*Bovicola*) *ovis* were randomly assigned into groups and were subjected to different treatments: (G1) 1,000 to 15.6 mg/mL of *C. guianensis* oil; (G2) 3% Tween 80 (negative control); and (G3) amitraz 1.25 g/mL (positive control). Data presentation was expressed as mean efficacy ± standard deviation using the statistical program SPSS version 23.0 and statistical differences of the experimental groups were obtained by Kruskal-Wallis and Friedman. Probit Regression was performed to obtain CI_{50} and CI_{90}, with a significance level of $p < 0.05$. The oil with a concentration of 1,000 mg/mL had an efficacy of 94% after 24 hours of exposure, differing statistically from the positive and negative control ($p < 0.05$). The IC_{50} and IC_{90} values were 439.21 mg/mL and 807.09 mg/mL, respectively. The action of the oil on the specimens was independent whether it was an adult female, male, or nymph. It is concluded that *C. guianensis* oil showed *in vitro* insecticidal effect against *D.* (*Bovicola*) *ovis*.

KEYWORDS: Phytotherapics; Pediculosis; Ovinocaprinoculture.

RESUMO: A produtividade em pequenos ruminantes pode ser comprometida devido as infestações por piolhos. O controle dessa infestação é realizado com a administração de inseticidas e seu uso excessivo causam resistências. Novas alternativas de controle estão sendo estudadas, destacando-se o uso de fitoterápicos. Pesquisas realizadas usando o óleo da *Carapa guianensis* (andiroba) mostraram ação inseticida sobre espécimes de piolhos de felinos e caprinos. O presente estudo teve como objetivo avaliar a eficácia do óleo da semente de *C. guianensis*, *in vitro*, sobre *Damalinia* (*Bovicola*) *ovis*. 900 exemplares de D. (Bovicola) ovis foram distribuídos aleatoriamente em grupos e submetidos a diferentes tratamentos: (G1) 1.000 a 15,6 mg/ml de óleo de *C. guianensis*; (G2) Tween 80 a 3% (controle negativo), e: (G3) Amitraz 1,25 g/mL (controle positivo). A apresentação dos dados foi expressa em eficácia média ± desvio padrão usando o programa estatístico SPSS versão 23.0 e diferenças estatísticas dos grupos experimentais foram obtidas por Kruskal-Wallis e Friedman. Para obtenção da CI50 e CI90 foi realizada Regressão de Probit, com nível de significância de p< 0,05. O óleo com concentração de 1.000 mg/ml teve uma eficácia de 94%, após 24 horas de exposição, diferindo estatisticamente do controle positivo e negativo (p< 0,05). Os valores da CI50 e CI90 foram de 439,21 mg/mL e 807,09 mg/mL, respectivamente. A ação do óleo sobre os espécimes foi independente se era adulto fêmea, macho ou ninfa. Conclui-se que o óleo de *C. guianensis* apresentou efeito inseticida in vitro contra *D.* (*Bovicola*) *ovis*.

PALAVRAS-CHAVE: Fitoterapia; Pediculose; Ovinocaprinocultura.

INTRODUCTION

In Brazil, sheep and goat farming is considered an important activity from a social and economic point of view. The Northeast region stands out as the largest national producer of caprine and ovine animals, concentrating 93.2% and 64.2% of the Brazilian herd, respectively (IBGE, 2017). The economic

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importance of these animals in the region is significant, despite the low productive and reproductive performance, which leads to financial losses for producers (ALENCAR et al., 2010; PEREIRA et al., 2013). Sheep production in Brazil has grown significantly, standing out among the agribusiness sectors and is currently considered one of the most important activities for the Brazilian northeastern semi-arid region, both in the cultural and economic contexts.

However, parasitic diseases are the main diseases that affect small ruminants, thus becoming one of the limiting factors of sheep farming, by causing high production losses, weight loss of animals with consequent reduction in meat and milk production, and increased costs with drugs and technical assistance (OLIVEIRA et al., 2017).

Traditionally, *D.* (*Bovicola*) *ovis* infestations have been controlled by administering chemicals with insecticidal effect (LEVOT, 2012). However, by their indiscriminate use and without guidance on dosages, the effectiveness of these drugs has been compromised by the development of parasite populations resistant to them (TAYLOR, 2012). Currently, few ectoparasiticides that have insecticidal effect on lice are available for commercialization, due to the development of resistance. In this context, the search for alternative ways to control *D.* (*Bovicola*) *ovis* is necessary to minimize this problem, highlighting the study of the effectiveness of herbal medicines as an important method in lice control (JAMES; CALLANDER, 2012).

The species of the *Meliaceae* family, including *Carapa guianensis Aubl.* (andiroba) have been occupying a prominent place as bioactive plants. The oil obtained from andiroba is composed mainly of saponifiable material, its major composition being fatty acids, such as palmitic, stearic, oleic, and linoleic acids, and glycerin in smaller concentrations. In the constitution there are also triglycerides and the unsaponifiable portion, represented by limonoids, which are related to the biological effect of the oil and are present in about 2 to 5% of the constitution (AMBROZIN et al., 2006; PEREIRA; TONINI, 2012; MILHOMEM-PAIXÃO et al., 2016).

Its use in the form of extracts and oils has been prioritized for its effects on different organisms; therefore, it is an alternative source for its control (BENELLI et al., 2018). The anthelmintic, acaricidal, repellent, and insecticidal properties of the *C. guianensis* oil are attributed to the presence of limonoids, and for this factor, several studies on the biological activity of this oil have been conducted (FARIAS et al., 2010; ROMA et al.,2015; VOLPATO et al. 2015; KLAUCK et al., 2014; KLAUCK et al., 2015). In studies, this oil has demonstrated ectoparasiticidal activity on cat and goat lice, showing that it can be used as an alternative way to control infestations caused by them (BARROS et al., 2012; FARIAS et al., 2017). The present research aimed to evaluate the efficacy of *C. guianensis* oil, *in vitro*, on *D.* (*Bovicola*) *ovis*.

MATERIAL AND METHODS

The project was submitted to the Ethics Committee on the use of animals in research at UFERSA (CEUA-UFERSA) and approved (Process No. 23091.006031/2019-25 under Opinion No. 09/2019). All the handling procedures to which the animals were submitted during this research followed the specific guidelines of the commission. The oil from *Carapa guianensis* Aubl. (andiroba) was obtained commercially from Néctar Plus® (Registered at the Ministry of Agriculture under number PB/ER-0025).

We used 900 specimens of Phthiraptera *Damalia* (*Bovicola*) *ovis* Schrank (1971). The specimens were collected from naturally infested *Ovis aries* Linnaeus (1758) sheep of both sexes and of undefined breed or age, belonging to the Nucleus for the Generation and Transfer of Technology in Animal Production in the Semiarid Region (NUTESA - *Núcleo de Geração e Transferência de Tecnologia em Produção Animal do Semiárido*) of the Universidade Federal Rural do Semiárido (UFERSA).

The sheep were mechanically contained and then subjected to body inspection for lice. Specimens were removed by combing with a fine-toothed comb. After removal, the Phthiraptera were transferred to flasks and sent for identification and sexing at the Animal Parasitology Laboratory (LPA - *Laboratório de Parasitologia Animal*) of UFERSA, using the morphology described by Guimarães et. al. (2001).

The 900 specimens of *D.* (*Bovicola*) *ovis* were distributed in Petri dishes (25 lice per plate) and submitted to different treatments, according to Farias et al. (2017). (G1): oil of *Carapa guianensis* Aubl. (andiroba) at concentrations 1000 mg/mL (G.1.1), 500 mg/mL (G.1.2), 250 mg/mL (G.1.3), 125 mg/mL (G.1.4), 62.5 mg/mL (G.1.5), 31.2 mg/mL (G.1 .6), and 15.6 mg/mL (G.1.7), diluted in a 3% Tween 80 solution; (G2): 3% Tween 80 solution (negative control); and (G3): Amitraz 1.25 g/mL (positive control). Four replicates were performed for each concentration and each control.

The lice were immersed completely in 1 mL of each of the concentrations for 60 seconds, following the methodology described by Heukelbach (2008). After the time had elapsed, the specimens were removed from the solutions and transferred to Petri dishes lined with filter paper (2x2cm). During the entire evaluation, the set was kept in a laboratory environment with pre-established physical conditions such as temperature (max: 30.10° and min: 26.14°C) and relative humidity (max: 63.34% and min: 40.33%). To quantify and record the number of lice that died and that remained alive, observations were made using a stereomicroscope and a simple magnifying glass 1, 3, 6, 24, 48, and 72 hours after the beginning of the test. Lice mortality was defined according to Heukelback (2008), who cites the absence of any vital signs, such as movement of antennae and legs, with or without stimulation using tweezers, among the criteria for lice mortality.

Oil efficacy was calculated according to the formula: lice mortality (%) = [no. of dead lice/ (no. of live lice + no. of dead lice) x 100]. Data were presented as mean efficacy ± standard deviation using the statistical program SPSS version 23.0. After analyzing the parametric assumptions, the statistical differences within and between the experimental groups at the different analyzed times (1 to 72 hours) were obtained using the Kruskal-Wallis and Friedman tests, respectively. The values of the inhibitory concentrations to inhibit 50% (CI_{50}) and 90% (CI_{90}) of the population, with respective confidence intervals, were calculated by Probit Regression. A significance level of $p < 0.05$ was considered.

RESULTS AND DISCUSSION

The inhibitory concentrations of *Carapa guianensis* oil were 439.21 to inhibit 50% (CI_{50}) and 807.09 mg/ml to inhibit 90% (CI_{90}). The results of the evaluation of andiroba oil against *D.* (*Bovicola*) *ovis*, considering the time and all concentrations evaluated, are presented in Table 1. In this research, it is observed that the mortality of the specimens was increasing over some of the tested times and concentrations. The concentration of 1000 mg/ml caused 94% mortality of the lice, 1 h post-exposure. These results are similar to those found by Barros et al. (2012) and Farias et al. (2017), when testing the same oil on chewing lice at 100% concentration, observed total mortality of *Felicola subrostratus* and *Damalinia caprae*, respectively, after starting the evaluation.

It was found that after 3, 6, and 24h, the 1000 mg/mL concentration continued to have good efficacy showing high lice mortality ($p<0.05$); however, after 48 and 72h, the treatment with 1000 mg/mL and amitraz showed similar pediculicidal effect, with lice mortality rate ranging between 96% and 76%, respectively (Table 1). Similar results were obtained by Farias et al. (2017), who, when using high concentrations of andiroba oil and monosulfiram (positive control), obtained 100% efficacy. Barros et al. (2012) obtained similar results when working with lice specimens (*F. subrostratus*).

The lowest concentrations used, 31.2 mg/mL and 15.6 mg/mL, showed lower mean lice mortality ($p < 0.05$) (Table 1). These results differed from those obtained by Farias et al. (2017), because when using the same oil on *D. caprae*, they observed that the lowest tested concentrations of 10%, 5%, and 2.5% killed 100%, 100%, and 95%, respectively, of the lice. Barros et al. (2012) also obtained 100% lice mortality when using lower concentrations of the oil. Essential oils are composed of a complex combination of volatile substances extracted from plants that may contain compounds with diverse chemical structures (CASTRO et al., 2020). The chemical content and composition can be influenced by factors such as age, stage of plant development, environmental and climatic conditions (OLIVEIRA et al., 2012), and part of the plant from which it was extracted, such as seeds, leaves, and flowers (CHAHAL et al., 2017). Possibly, in this study, the difference in the insecticidal action of *C. guianensis* essential oil and its efficacy on lice mortality can be attributed to possible variations in the composition of the oil used in the tests. Compared to the negative control (Tween 80 at 3%), there was an increase in lice mortality after 24h of experiment. FARIAS et al. (2017) and BARROS et al. (2012) also verified a mortality of the negative control lice after 48h of experiment. The possible justification for the mortality of these lice is the lack of food and the toxicity of the diluent (tween 80).

Some research has been conducted using the oil extracted from andiroba as a phytotherapeutic agent and the results reported have been promising regarding its use to compose ectoparasiticidal formulations. In experiments conducted by Farias et al. (2007, 2009) using a 100% concentration of oil obtained from andiroba in the control of *Rhipicephalus* (*Boophilus*) *microplus*, *Dermacentor* (*Anocentor*) *nitens*, and *Rhipicephalus sanguineus*, it was observed total mortality of

Table 1. Mean efficacy ± standard deviation of *Carapa guianensis* oil against *Damalinia* (*Bovicola*) *ovis* Schrank (1781) and mean values ± standard deviation of the number of dead lice according to sex.

Experimental Groups	Time						Sexing		
	1h	3h	6h	24h	48h	72h	Male	Female	Nymph
1000mg/mL	94.0 ± 5.16^{Aa}	94.0 ± 5.16^{Aa}	94.0 ± 5.16^{Aa}	94.0 ± 5.16^{Aa}	96.0 ± 3.26^{Aa}	96.0 ± 3.26^{Aa}	7 ± 3.9^{A}	7.2 ± 3.4^{A}	10.7 ± 5.6^{A}
500mg/mL	24.0 ± 13.46^{Bc}	27.0 ± 15.79^{Bc}	28.0 ± 16.97^{Bc}	46.0 ± 14.04^{Bb}	54.0 ± 12.43^{Bab}	70.0 ± 5.16^{ABa}	6.7 ± 2.5^{A}	7.5 ± 5^{A}	10.7 ± 6.2^{A}
250mg/mL	6.0 ± 6.92^{Bb}	12.0 ± 4.98^{Bb}	9.0 ± 7.68^{Bb}	22.0 ± 7.21^{Cb}	48.0 ± 15.74^{Ba}	57.0 ± 16.58^{Ba}	7 ± 0^{A}	9.5 ± 2.3^{A}	8.5 ± 2.3^{A}
125mg/mL	6.0 ± 4.47^{Bb}	7.0 ± 5.19^{Bb}	9.0 ± 7.68^{Bb}	12.0 ± 9.79^{Cb}	34.0 ± 8.71^{Ca}	50.0 ± 11.48^{Ba}	8.5 ± 1.9^{A}	7.25 ± 1.8^{A}	9.25 ± 0.5^{A}
62.5mg/mL	8.0 ± 9.38^{Bc}	11.0 ± 9.53^{Bc}	15.0 ± 9.94^{Bc}	25.0 ± 11.09^{Cb}	28.0 ± 2.82^{Cab}	46.0 ± 17.54^{Ba}	8 ± 1.4^{A}	10.2 ± 1.5^{A}	6.7 ± 2.8^{A}
31.2mg/mL	1.0 ± 1.73^{Cc}	4.0 ± 6.92^{Cc}	7.0 ± 8.18^{Bc}	21.0 ± 11.09^{Cb}	35.0 ± 14.79^{Ca}	35.0 ± 9.94^{Ca}	6.7 ± 0.5^{A}	8 ± 1.1^{A}	10.5 ± 1.5^{A}
15.6mg/mL	1.0 ± 1.73^{Cc}	3.0 ± 1.73^{Cc}	6.0 ± 6.0^{Bb}	15.0 ± 11.44^{Cb}	25.0 ± 12.12^{Ca}	42.0 ± 11.48^{Ca}	8 ± 2.7^{A}	8 ± 1.4^{A}	9 ± 1.4^{A}
Tween	0	2.0 ± 3.46^{Cc}	7.0 ± 4.35^{Bc}	30.0 ± 7.21^{BCb}	58.0 ± 4.47^{ABa}	65.0 ± 7.68^{Ba}	6 ± 2.7^{A}	6±3.2A	13 ± 4.5^{B}
Amitraz	4.0 ± 6.92^{Cc}	7.0 ± 5.91^{Bc}	9.0 ± 4.35^{Bc}	46.0 ± 14.0^{Bb}	76.0 ± 6.32^{Aa}	88.0 ± 6.32^{Aa}	5 ± 3.4^{A}	11.2 ± 0.9^{B}	7.5 ± 3.3^{A}

A,BC Averages followed by distinct upper case letters in the column and distinct lower case letters a,b,c in the row show statistical difference (Two-way ANOVA followed by Tukey - $p<0.05$).

engorged females and reduction of spawning between the second and fourth day after treatment. Other authors also obtained effective results when using the same concentrations in their tests using the oil (FARIAS et al. 2010; BARROS et al., 2012; FARIAS et al., 2017).

It is suggested that the insecticidal effect of some essential oils, such as andiroba oil, are the result of their penetration into tissues and subsequent modification of some physiological functions (KLAUCK et al., 2014; OZAKI et al., 2003;). Roma et al. (2013a,b) and Roma et al. (2015) reported that andiroba oil has an action on the nervous system of ticks, causing ultrastructural changes, affecting the synganglion, compromising its neural functions and causing tissue death, suggesting that the oil, like many other synthetic acaricides, has neurotoxic action. Such knowledge on the action mechanism of the oil on ticks, suggests that similar effects may occur on lice species, such as *D.* (*Bovicola*) *ovis*. When considering the effects of different concentrations of *C. guianensis* oil on the sex and life stage of *D.* (*Bovicola*) *ovis*, it was observed that the oil at different dilutions, acted equally on all *D.* (*Bovicola*) *ovis* lice, causing their mortality regardless of life stage (adult and nymph) and sex (male and female) (Table 1).

Similar results show that the oil used acts independently of the life stage, a fact that was verified in evaluations carried out with flies and ticks (AGUIAR et al., 2014; KLAUCK et al., 2014; OLIVEIRA, 2018).

Although there is a difference in the mortality of *D.* (*Bovicola*) *ovis* at the different concentrations evaluated in this research, the insecticidal activity of *C. guianensis* oil can be considered promising, indicating the possibility that the oil can be applied in the future to produce insecticides that will be used to control lice infestations.

CONCLUSION

Carapa guianensis (andiroba) oil showed insecticidal effect on *Damalinia* (*Bovicola*) *ovis*; however, it did not cause difference in the number of dead lice, when considering the different life stages and sex. There was a difference in the number of dead lice within and between some concentrations of the oil tested and times evaluated. It is suggested that further *in vitro* and *in vivo* studies be carried out to establish the analyzed oil as a phytotherapeutic agent to be used in the alternative control of pediculosis in small ruminants in the studied of the geographic locality.

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