# Chemical composition and acaricidal activity of seed oils of the palms Mauritia

# flexuosa and Mauritiella armata in Rhipicephalus microplus (Ixodidae)

Composição química e atividade acaricida dos óleos de sementes das palmeiras Mauritia flexuosa e

Mauritiella armata em Rhipicephalus microplus (Ixodidae)

Composición química y actividad acaricida de los aceites de semillas de las palmas Mauritia

flexuosa y Mauritiella armata en Rhipicephalus microplus (Ixodidae)

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#### Abstract

*Rhipicephalus microplus* is responsible for considerable economic losses in tropical and subtropical regions. Plant compounds have been utilized as alternative to conventional acaricids. The objective in this study was to evaluate the effects of fixed oils extracted from seeds of *Mauritia flexuosa* and *Mauritiella armata*, palm trees typical of the Brazilian Cerrado on tick *R. microplus*. The fixed oils were used against engorged females and larvae by biocarrapaticidograms and larval mortality by larval pack tests (LPT). Chemical compositions of the oils were evaluated by gas chromatography using an electron impact ionization detector and showed the presence of the compounds: palmitoleic acid, linoleic acid, palmitic acid, and myristic acid. In the biocarrapaticidogram, the oil from *M. flexuosa* and *M. armata* at 5% and 10% concentrations showed efficacies > 80%. Regarding the effect of fixed oils from palm trees on *R. microplus* larvae, mortality above 80% was observed at all concentrations tested. These bioproducts are a promising alternative for controlling this tick and may be adapted into current integrated control methods for cattle farming. **Keywords:** Alternative control; Fixed oils; Arecaceae; Ticks; Acaricidal resistance.

# Resumo

*Rhipicephalus microplus* é responsável por consideráveis perdas econômicas nas regiões tropicais e subtropicais. Compostos vegetais têm sido utilizados como alternativa aos acaricidas convencionais. O objetivo deste estudo foi

avaliar os efeitos de óleos fixos extraídos de sementes de *Mauritia flexuosa* e *Mauritiella armata*, palmeiras típicas do Cerrado brasileiro, sobre o carrapato *R. microplus*. Os óleos fixos foram usados contra fêmeas ingurgitadas e larvas por biocarrapaticidogramas e larvas mortalidade por testes de embalagem larval (TEL). As composições químicas dos óleos foram avaliadas por cromatografia gasosa com detector de ionização por impacto de elétrons e mostraram a presença dos compostos: ácido palmitoléico, ácido linoléico, ácido palmítico e ácido mirístico. No biocarrapaticidograma, o óleo de *M. flexuosa* e *M. armata* nas concentrações de 5% e 10% apresentou eficácias > 80%. Em relação ao efeito dos óleos fixados de palmeira sobre as larvas de *R. microplus*, foi observada mortalidade acima de 80% em todas as concentrações testadas. Esses bioprodutos são uma alternativa promissora para o controle desse carrapato e podem ser adaptados aos atuais métodos integrados de controle para a pecuária.

Palavras-chave: Alternative control; Óleos fixos; Arecaceae; Carrapatos; Resistência acaricida.

#### Resumen

*Rhipicephalus microplus* es responsable de considerables pérdidas económicas en las regiones tropicales y subtropicales. Se han utilizado compuestos vegetales como alternativa a los acaricidas convencionales. El objetivo de este estudio fue evaluar los efectos de los aceites fijos extraídos de semillas de *Mauritia flexuosa* y *Mauritiella armata*, palmeras típicas del Cerrado brasileño sobre la garrapata *R. microplus*. Mortalidad por pruebas de paquetes larvarios (PPL). Las composiciones químicas de los aceites se evaluaron mediante cromatografía de gases utilizando un detector de ionización por impacto de electrones y mostraron la presencia de los compuestos: ácido palmitoleico, ácido linoleico, ácido palmítico y ácido mirístico. En el biocarrapaticidograma, el aceite de *M. flexuosa* y *M. armata* a concentraciones de 5% y 10% mostró eficacias > 80%. En cuanto al efecto de los aceites fijos de palmera sobre las larvas de *R. microplus*, se observó una mortalidad superior al 80% en todas las concentraciones ensayadas, estos bioproductos son una alternativa prometedora para el control de esta garrapata y pueden adaptarse a los métodos de control integrados actuales para la ganadería.

Palabras clave: Control alternativo; Aceites fijos; Arecaceae; Garrapatas; Resistencia acaricida.

## **1. Introduction**

*Rhipicephalus (Boophilus) microplus* is the most important ectoparasite of cattle that are widely distributed in tropical and subtropical regions (Canevari et al., 2017; Madder et al., 2012). The infestations of *R. microplus* cause considerably reducing the milk production and weight gain of cattle, compromising the viability of cattle ranching in regions with multi-resistant populations (Biegelmeyer et al., 2012; Roy et al., 2017).

Control with acaricides has been the method most used by producers. However, it has become less efficient, as resistant tick populations have been selected (Domingues et al., 2013). Additionally, intensive use of these chemicals has contributed to contamination and toxicity in the environment, with residues in milk and meat, representing a risk to public health (Waal & Danaher, 2014).

New proposals for minimizing the use of acaricides with plant metabolites have represented viable alternative in different bovine producing regions (Gonçalves et al., 2016). One of the main biomes present in South America is the Cerrado, which contains Veredas. This biome consists of soils with high water saturation that helps maintain water sources (Nunes et al., 2015). In these veredas, populations of the family Arecaceae, *Mauritia flexuosa* L.F. (Buriti) and *Mauritiella armata* Mart. (Burret) (Xiriri) are abundant. These palms are important for cycling nutrients and maintaining this ecosystem, as well as, contributing to food, medicine, and building supplies for houses and handicrafts (Martins et al., 2014; Souza & Lorenzi, 2008).

*Mauritia flexuosa* palm trees are particularly important because farmers and agribusinesses may produce oil from them (Sousa et al., 2012). "Buriti" oil has pharmacological interest since they contain a high concentration of tocopherols and carotenoids (Durães et al., 2006). However, very little is known about *M. armata* and its compounds, and the effects of fixed oils from these two palm trees against ectoparasites have not yet been investigated. Therefore, the objective in this study was to evaluate the effects of these palm oils against adult females and larvae of *R. microplus*.

## 2. Materials and Methods

#### 2.1 Evaluated plant materials

*Mauritia flexuosa* and *M. armata* fruits (mesocarps) from ten young specimens (from six to ten meters height) were collected in October (beginning of the rain season), in the most preserved area of the Água Doce vereda (15°13'30"S and 44°55'04"W) localized in the Pandeiros Environmental Protection Area (EPA), Pandeiros river, Januária, Minas Gerais, Brazil.

Plant identification was carried out by comparing samples to morphological characteristics and representative parts of species deposited in the collection of Montes Claros Herbarium, Minas Gerais (MCMG) of the Universidade Estadual de Montes Claros (UNIMONTES), under the voucher number 5777 and 5778 for *M. flexuosa* and *M. armata*, respectively.

#### 2.2 Fixed oil extraction from the palms

The mesocarp oils of the *M. flexuosa* and *M. armata* fruits were extracted with solvent using the *Goldfish* apparatus, according to Methodology of the National Institute of Science and Technology of Animal Science (INCT - CA 2012). Samples of 3 g of dehydrated and crushed mesocarps were placed in filter paper cartridges and 60 mL of petroleum ether were added. The oil extraction process was followed by continuous reflux of the solvent for 4 hours, with a condensation speed of 5 to 6 drops per second. After extraction, the ether was removed by the recycling scheme until a thin layer of the solvent remained on the bottom of the cups, which were returned to the oven at 105 °C for 30 minutes, for complete removal of the reagent. The yield calculation was obtained by the gravimetric relationship (Detmann et al., 2012).

In a round-bottomed flask (50 mL) were added 20 mg of *M. armata* or *M. flexuosa* oils. Then, 5 mL of potassium hydroxide (KOH) solution in methanol (0.5 mol/L) was added and heated at 100°C for 1 h under reflux. For esterification, 2 mL of HCl solution in methanol (4:1 v/v) were added to the mixture and heated again at 100°C, for 1 h. The methyl esters were extracted, and after cooling, 2 mL of distilled H<sub>2</sub>O was added. Then, the derivatives obtained were extracted with dichloromethane. After extraction, the organic phase was dried over anhydrous sodium sulfate, filtered and concentrated. The residue obtained, after complete removal of the solvent, was re-dissolved in 1 mL of dichloromethane and analyzed by gas chromatography with an electron impact ionization detector (GC-ME).

#### 2.3 Extract characterization (Gas Chromatography-Mass Spectrometry)

#### 2.3.1 Derivatization of fixed oils from Mauritia flexuosa and Mauritiella armata

For derivatization, aliquots of fixed oils (1mg) were dissolved in 60 µl of pyritine and 100 µl of BSTFA [(N, O-bis (trimetilsilil) trifluoroacetamida)] containing 1% of chlorotrimethylsilane. The reaction mixtures was heated at 60°C for 30 min (Jang & Kamens, 2001).

#### 2.3.2 Chromatographic analysis of fixed oils

The fixed oils were analyzed with an Agilent Technologies gas chromatograph (GC 7890A) equipped with electron impact ionization detector (GC-MS) and DB-5MS capillary column (Agilent Technologies, 30 m length  $\times$  0.25 mm internal diameter  $\times$  0.25 µm flm thickness). Helium (99.9999% purity) was used as carrier gas at the rate of 1 ml/min. Using a self-injector (CTC combiPaL), 1 µl of the solution was injected into the chromatograph at a 1:10 split ratio. The split/splitless injector was maintained at 220°C. The chromatographic column was heated initially to 160°C, maintained at 80°C for 5 min, and heated at a rate of 4°C/min to 240°C for 10 min. After compound separation, the temperature was raised to 300°C and maintained for 2 min (post run). The interface temperature was maintained at 240°C and the ionization was performed using 70 eV. The scanning range of m/z was from 30 to 600 Da and the all procedures were performed in triplicate. Individual components were

identified by comparing their mass spectra (MS) and retention indices with those reported in the previous studies and with the Wiley Registry of Mass Spectral Data, 6th edn (Wiley Interscience, New York) (Adams, 2001).

#### 2.4 Effect of fixed oils on reproductive parameters of Rhipicephalus microplus females

Engorged adult females of *R. microplus* were collected from Gyr X Hosten cattle, naturally infested, in Coração de Jesus – MG, Brazil at least 60 days after the most recent use of acaricide. The collected ticks were placed in plastic containers and ticks larger than 4-6 mm were selected, washed with distilled water, placed on paper towels, weighed and divided into groups of 10 females each based on the degree of engorgement and weight (Leite, 1995).

Fixed oils were prepared at concentration of 2.5%, 5%, and 10% in 5% Tween 80 (v/v) solution. Distilled water with 5% Tween 80 and distilled water served as negative controls. As positive controls, the following chemicals products were used: 0.025 mg/mL Cypermethrin (Barragem®, Zoestis, São Paulo, Brazil), 0.5 mg/mL Supona, 2-chloro-1-(2,4-dichlorophenyl)-vinyl diethyl phosphate (UCB, Uzinas Chimicas Brasileiras S/A, São Paulo, Brazil), and 0.25 mg/mL Amitraz (Triatox, MSD Animal Health, São Paulo, Brazil) all diluted as recommended by the manufacturers.

Acaricide efficacy was evaluated with an immersion test as described by Drummond et al. (1973). For each replicate, 10 ticks were immersed in 5 mL of the test solution for 5 min. Excess solution was removed with a paper towel, and ticks were placed in a Petri dish at 28°C with 70% relative humidity in a biological oxygen demand (BOD) incubator (Gallenkamp, United Kingdom, PSC 059). All procedures were performed in quadruplicate.

After 15 days of incubation, eggs were weighed for each group, transferred to 3 mL disposable syringes, sealed with hydrophilic cotton, and kept at 28°C with 70% relative humidity (Drummond et al., 1973). Thirty days after the start of hatching, syringe contents were transferred to Petri dishes, and 3 mL of a 50:50 solution of water and detergent was added. Then, three 200  $\mu$ L aliquots were pipetted onto glass slides to count unlaced eggs and larvae under a stereoscopic microscope to determine the hatching rate of each group (Vasconcelos et al., 2018; Figueiredo et al., 2019). All procedures were performed in triplicate. Each replicate was independently evaluated by five researchers.

To determine the oviposition capacity (OC), a modified version of the formula described by Bennett (1974) was used:

OC (oviposition capacity) = (weight of egg mass / initial weight of female)  $\times$  100

The efficacy of treatment was estimated using the equation from Drummond et al., (1973):

RE (reproductive efficacy) = (egg weight  $\times$ % hatching  $\times$  20000) / Initial weight of females PE (product efficacy) = (RE control group – RE treated group) / RE control group  $\times$  100.

Efficacy was calculated for each replicate using the mean value of reproductive efficiency (RE), and the value from the negative control was used as the ER control group. A randomized design was used to compare the four oil concentrations with the two negative control treatments and the three commercial acaricides (positive control) treatments, and mean values for each group were compared using analysis of variance (ANOVA) and the Scott–Knott test (p < 0.05). The concentration of oil that was sufficient to inhibit 90% of hatching (LC90) was estimated by probit analysis using the Sage 9.1 statistical package.

#### 2.5 Effects of fixed oils on mortality of Rhipicephalus microplus larvae

To test the effects of fixed oils, we adapted methods published by Stone & Haydock (1962). For the larval pack test (LPT), larvae of up to 15 days old post-hatching were used used. Efficacy was evaluated at concentration of 1.25%, 2.5%, 5%, and 10% and compared to the negative control consisting of distilled water with 5% Tween 80 and a positive control of 0.25 mg/mL amitraz (Triatox, MSD Animal Health).

Approximately 200 larvae were inserted into  $6 \times 6$  cm filter paper bags (Whatman #1) for each replicate. Bags were sealed with metal clips and impregnated with a given test solution, after which each group of replicates were deposited in Petri dishes and incubated under the same conditions as previously described for the engorged adult females for 24 h. The bags were opened on a white surface and the number of live and dead larvae were quantified. The relative numbers of dead larvae divided by total numbers of larvae were compared between treatments by ANOVA with a randomized design (p  $\leq$  0.05). The concentrations required to kill 90% of larvae (LC90) were estimated using the probit regression analysis function in Sage 9.1.

#### **3. Results**

#### 3.1 Chemical characterization of fixed oils

Twelve chemical structures that have been identified in the oil from *M. flexuosa* (OMF) were: myristic acid (tetradecanoic acid), palmitic acid (hexadecanoic acid), palmitoleic acid ((Z)-hexadec 9-enoic acid), linoleic acid (9.12 – octadecadienoic acid), oleic acid (octadic-9-enoic acid), stearic acid (octodenoic acid), arachinidic acid ((Z)-icos-9-enoic acid) and arachidic acid (eicosanoic acid) (Table 1 and Figure 1). The major compounds identified in oil from *M. armata* (OMA) were: suberic acid (octanedioic acid), myristic acid (tetradecanoic acid), palmitoleic acid ((Z)-hexadec-9-enoic acid), Palmitic acid (hexadecanoic acid), margaric acid (heptadecanoic acid), linoleic acid (9.12-octadecadienoic acid), oleic acid (octadec-9-enoic acid), stearic acid (octodecanoic acid), arachinidic acid ((Z) -icos-9-enoic acid), and arachidic acid (eicosanoic acid) (Table 1 and Figure 2).

Mauritia flexuosa					Mauritiella armata			
Ν	RT	Compounds	Area (%)	Ν	RT	Compounds	Area (%)	
1	10.20	Tetradecanoic acid	0.08	1	4.94	9-oxo nonanoic acid	0.17	
2	12.42	Pentadecanoic acid	0.07	2	5.05	Octanedioic acid	0.14	
3	14.09	(Z)-hexadec 9-enoic acid	0.08	3	6.64	Nonanodioic acid	0.24	
4	14.02	(E)-hexadec-9-enoic acid	0.36	4	10.20	Tetradecanoic acid	0.17	
5	14.74	Hexadecanoic acid	27.19	5	12.42	Pentadecanoic acid	0.10	
6	16.41	(Z)-heptadec-9-enoic acid	0.08	6	14.09	(Z)-hexadec-9-enoic acid	0.17	
7	16.96	Heptadecanoic acid	0.12	7	14.20	(E)-hexadec-9-enoic acid	0.23	
8	18.45	9.12 – octadecadienoic acid	1.63	7	14.75	Hexadecanoic acid	15.45	
9	18.67	Octadec-9-enoic acid	65.64	8	16.96	Heptadecanoic acid	0.07	
10	19.21	Octodenoic acid	3.86	9	18.45	9.12-octadecadienoic acid	1.10	
11	22.95	Cis-9-eicosenoico acid	0.81	10	18.67	Octadec-9-enoic acid	29.53	
12	23.59	Eicosanoic acid	0.12	11	19.21	Octodecanoic acid	31.23	
				12	20.56	Cis-9,12-octadecadienoic acid	3.66	
				13	22.96	Cis-9-eicosenoico acid	1.92	
				14	23.60	Eicosanoic acid	0.07	
				15	22.73	(Z)-octadec-10-enoic acid	0.28	

**Table 1.** Main compouds identified by *gas chromatography* in seed oils of the palms *Mauritia flexuosa* and *Mauritiella armata* its area (%) in chromatographic profile.

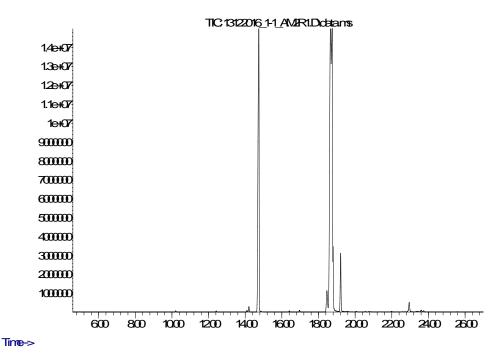


Figure 1. Chromatogram of the fixed oil of the *Mauritia flexuosa*.

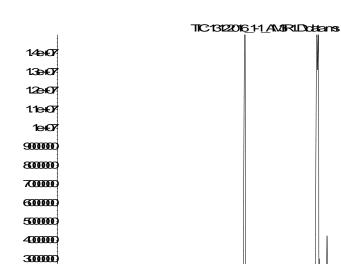
# Abundance

Source: Authors (2016).

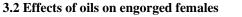








1000



Tine->

200000 100000

600

BOD

The OMF and OMA, at 2.5%, 5%, and 10% concentrations, significantly reduced the laying capacity of *R. microplus* (Table 2). There was a significant decrease in the average percentage of hatched larvae for all evaluated concentrations of OMF

1400

Source: Authors (2016).

1200

1600

1800

200

2200

2400

2600

and OMA. The LC90 was estimated at 6.45% ( $5.98\% \pm 7.03\%$ ) and 6.80% ( $6.30\% \pm 7.43\%$ ) for OMA and OMF, respectively. The efficacy of cypermethrin and supona acaricides were 5.87% and 61.20%, respectively, which were lower than the efficacy observed when using concentrations of fixed oils above 5% from both palm trees.

**Table 2.** Average oviposition capacity and hatchability of female of *Rhipicephalus microplus* treated with seed oils of the palms

 *Mauritia flexuosa* and *Mauritiella armata*.

Treatment (%)	Oviposition capacity (%)**	Hatchability (%)	Effectiveness (%)***
Mauritia flexuosa			
10.0	12.81a	10.64b	92.35a
5.0	19.02a	13.84b	82.92b
2.5	21.64a	27.93c	62.70c
1.25	28.88b	79.45e	0.00d
Mauritiella armata			
10.0	9.34a	5.18a	97.09a
5.0	26.00b	20.36b	67.15b
2.5	13.70a	40.37d	66.19b
1.25	31.63b	92.40f	0.00c
Distilled water*	40.28b	93.58f	0.00
Tween 80 (5% in water)*	17.48a	92.61f	-
Supona <sup>1</sup>	21.89a	31.82c	61.20c
Cypermetrina <sup>2</sup>	36.73b	54.83d	5.87d
Amitraz <sup>3</sup>	7.21a	0a	100a
Coefficient of variation (%)	43.98	8.82	18.92

Means followed by the same letter are statistically similar by the Scott-Knott test at 5% significance.

<sup>1</sup>Supona (2-chloro-1- (2.4-dichlorophenyl) vinyldiethylphosphate. UCB. UzinasChimicas Brasileiras S/A. São Paulo. Brazil).

<sup>2</sup>Cypermethrin (Butox MSD Animal Health. São Paulo Brazil).

<sup>3</sup>Amitraz (Triatox. MSD Animal Health. São Paulo Brazil).

\* Negative control.

\*\* OC (oviposition capacity) = (weight of egg mass/initial weight of female)  $\times$  100

\*\*\* Means obtained by the equation from Drummond et al. (1973) using the control with Tween 80 (5% in water.

Source: Authors (2017).

# 3.3 Mortality of Rhiphicepalus microplus larvae

Fixed oils of both palm trees induced a high mortality rate of *R. microplus* larvae. With all OMF concentrations, and with 2.5% and 5% concentrations of OMA, at least 85% mortality was observed at compared to the control group (Table 3). The LC90 of OMA was estimated at 1.56% (1.47%  $\pm$  1.72%). For OMF, the LC90 could not be estimated for the concentrations evaluated.

**Table 3.** Larval mortality of *Rhiphicepalus microplus* treated with seed oils of the palms *Mauritia flexuosa* and *Mauritiella armata*

Treatment (%)	Mauritia flexuosa (%)	Mauritiella armata (%)
5.0%	89.49c	100.00a
2.5%	85.27d	99.89a
1.25%	83.00b	66.72b
Distilled water*	0e	0c
Tween 80 (5% in water)*	0e	0c
Amitraz <sup>1</sup>	100a	100a
Coefficient of variation (%)	3.13	7.46

Means followed by the same letter are statistically similar by the Scott-Knott test at 5% significance.

\* Negative controls.

<sup>1</sup>Amitraz (Triatox, MSD Animal Health, São Paulo Brazil).

Source: Authors (2017).

# 4. Discussion

The intensive use of chemical acaricides has promoted rapid selection of ticks that are resistant of these products, which has been a challenge for cattle breeding in tropical and subtropical regions (Higa et al., 2016). In this study, cypermethrin (pyrethroid) was not effective against the evaluated tick strain detected in the northern region of Minas Gerais. However, in the same region, Carneiro et al. (2015) observed 100% efficacy against the strain they evaluated. *R. microplus* strains resistant to pyrethroids have been described (Mendes et al., 2019; Kumar et al., 2020), showing resistance in different geographical areas. Molecular studies have detected polymorphisms (Robbertse et al., 2016; Wyk et al., 2016; Bandara & Karunaratne, 2017; Sungirai et al., 2018) and changes in detoxification enzymes, such as esterases (Gaur et al., 2017) and monooxygenases (Graham et al. 2016).

The effects of essential oils extracted from plants to control populations of ticks are well described (Coelho et al., 2020; Santos et al., 2015; Medeiros et al., 2019), however, there are no reports using fixed oils. The use of fixed oils extracted from fruits or seeds has wider applicability, are a renewable and easily available source, and has a higher extraction yield compared to essential oils (Adekunle, 2015).

The palms *M. flexuosa* and *M. armata* belong to the Arecaceae family, which are known to contain compounds with antiparasitic activity (Batista et al., 2012; Metwaly et al., 2012; Tayler et al., 2019). However, there were not reports of using fixed oils from seeds extracted from these palm trees to control tick populations. In this study, fixed oils extracted from seeds of *M. flexuosa* and *M. armata* affected different stages of the life cycle of *R. microplus*, reducing the hatched larvae as well as inducing high larval mortality. Additionally, insecticidal effect of fixed oils, such as sunflower (*Helianthus annuus*), sesame (*Sesamum indicum*), cotton (*Gossypium hirsutum*), soybean (*Glycine max*), and pequi (*Caryocar brasiliense*), on *Callosobruchus maculatus* (woodworm) have been reported. These oils induced adult mortality (between 40% and 70%) and interfered with the laying of viable eggs (Pereira et al., 2008).

Saturated and unsaturated fatty acids with antioxidant activity can be identified in fixed oils extracted from the fruits of both palm trees, such as palmitoleic acid, linoleic acid, palmitic acid, and myristic acid. Chromatographic analyses have been revealed that vegetable oils are biologically active against arthropod pests (Sims et al., 2014). Although the effect of fatty acids against arthropods is not yet known, the toxicity of these compounds increases with the length of the carbon chain, as well as, the presence of certain chemical bonds (i.e. saturated or unsaturated) (Sims et al., 2014).

According to Ambrozin et al. (2006) and Silva and Nunomura (2012), oils that are rich in fatty acids, such as oleic, palmitic, stearic and linoleic acids, have biological activity against arthropods. Research has identified the presence of limonoids in oils extracted from andiroba seeds, which have proven insecticidal and repellent abilities (Ambrozin et al., 2006). According to Matos et al. (2010), several limonoids isolated from the *Cedrela* genus showed insecticidal activity belonging to the order Lepidoptera.

# 5. Conclusion

In this study, fixed oils from *Mauritia flexuosa* and *Mauritiella armata* compromised different stages of the life cycle of a resistant strain of *R. microplus*. These oils can be easily produced, representing a natural bioproduct for the control of this ectoparasite. Fixed oils of *M. flexuosa* and *M. armata* reduce the selection of resistant populations and this be used in integrated tick control. The extraction of species from the Cerrado is very common, so the sustainable management of *Mauritia flexuosa* and *Mauritiella armata* is necessary for their preservation, as well as concentrating the active principles of these species for the next biological tests.

# **Conflicts of interest statement**

The authors of this manuscript have no financial or personal relationship with individuals or organizations that may influence or impair the content of the document.

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