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Evaluation of phytotherapy alternatives for controlling *Rhipicephalus* (*Boophilus*) *microplus in vitro*

Avaliação de alternativas fitoterápicas no controle *in vitro* de *Rhipicephalus* (*Boophilus*) *microplus*

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Abstract

The objective of this study was to identify the main chemical components of the essential oil of *Cuminum cyminum* L. (cumin) and of the fixed oils of *Bertholletia excelsa* (Brazil nut) and of *Helianthus annuus* (sunflower seed). As well as testing the three oils and three different commercial synthetic acaricides against engorged females of *Rhipicephalus* (*Boophilus*) *microplus* in order to explore their acaricidal efficacy. Six different concentrations of the oils (200, 100, 50, 25, 12.5 and 6.25 mg/mL) and the active principles were evaluated with the Adult Immersion Test (AIT). The two main chemicals components of *C. cyminum* L. were the cuminaldehyde and the γ-terpinene. In both *B. excelsa* and *H. annuus* were the linoleic and oleic acid. *C. cyminum* L. showed high acaricidal activity (100%) over the engorged females and on their reproductive characteristat from the concentration of 100 mg/mL. *B. excelsa* and *H. annuus* had low acaricidal activity (39.39% and 58.75% in the concentration of 200 mg/mL respectively). The amidine and the pyrethroid (35.12% and 1.50% respectively). It can be concluded that the oil of *C. cyminum* L. may be a phytoterapic alternative for the cattle's tick control.

Keywords: *Cuminum cyminum* L., *Bertholletia excelsa*, *Helianthus annuus*, acaricides, tick.

Resumo

O objetivo do presente estudo foi identificar os componentes químicos majoritários do óleo essencial de *Cuminum cyminum* L. (comino) e dos óleos fixos de *Bertholletia excelsa* (castanha do Brasil) e de *Helianthus annuus* (semente de girassol). Assim como testar os três óleos e três diferentes acaricidas comerciais sintéticos contra fêmeas ingurgitadas de *Rhipicephalus* (*Boophilus*) *microplus*, para explorar sua eficácia acaricida. Seis concentrações dos óleos (200, 100, 50, 25, 12,5 and 6,25 mg/mL) e os princípios ativos foram avaliados por meio do Teste de Imersão de Adultas (AIT). Os dois componentes químicos majoritários de *C. cyminum* L. foram o cuminaldeído e o *y*-terpineno. Nos óleos de *B. excelsa* e *H. annuus* os componentes majoritários foram o ácido linoleico e oleico, respectivamente. *C. cyminum* L. mostrou alta atividade acaricida (100%) sobre as fêmeas ingurgitadas e suas caraterísticas reprodutivas, a partir da concentração 100 mg/mL., tornando-se uma fonte alternativa para controlar o carrapato do gado. No entanto sao necessários estudos adicionais, a serem conduzidos *in vivo*. *B. excelsa* e *H. annuus* tiveram baixa atividade acaricida (39,39% e 58,75% na concentração de 200 mg/mL respectivamente), não obstante apresentaram maior efeito que a amidina e o piretroide (35,12% e 1,50% respetivamente). Pode-se concluir que o óleo de *C. cyminum* pode ser uma alternativa fitoterápica para o controle do carrapato do gado.

Palavras-chave: *Cuminum cyminum* L., *Bertholletia excelsa*, *Helianthus annuus*, acaricidas, carrapato.

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Introduction

The direct effects caused by the tick species *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini, 1888) and the agents for bovine babesiosis and anaplasmosis that it transmits are extremely important and have high economic impact on livestock worldwide (JONGEJAN & UILENBERG, 2004; JONSSON, 2006; RODRIGUEZ-VIVAS et al., 2017). In Brazil the annual economic losses range from US$ 800 million to more than 3 billion (GRISI et al., 2014). This tick species is highly prevalent in tropical and subtropical regions, where high relative humidity soils and high temperature weather favor tick population survival (RODRIGUEZ-VIVAS et al., 2006; ESTRADA-PEÑA et al., 2006). In the southern region of Brazil, three to four tick generations are produced per year (PEREIRA et al., 2008b).

It is known that tick control is done mainly through synthetic acaricides worldwide. In Brazil the active agent amidine (Amitraz) and cypermethrin are the ones that have been used most over the last decade (SANTOS & VOGEL, 2012). The onset of resistance to synthetic acaricides exhibited by *R.* (*B.*) *microplus* has given rise to serious problems within cattle production worldwide, such as México, Africa, US and New Caledonia, between others countries (RODRÍGUEZ-VIVAS et al., 2017; VUDRIKO et al., 2016; CHEVILLON et al. 2007; ABBAS et al., 2014). This resistance has developed mainly because of intrinsic or biological factors related to the tick, such as production of genetic mutations in the dominant resistance allele and changes to enzyme metabolism in tick populations (GUERRERO et al., 2001; FOIL et al., 2004). These have occurred through operational factors related to human action aimed towards tick control, and this phenomenon has appeared because producers have used synthetic acaricides as the only tool for controlling this ectoparasite. Moreover, these control measures have commonly been used erroneously, such as excessive use of acaricides without knowledge of tick biology, ecology and prevalence, as well as failure to detect resistance (DENHOLM & ROWLAND, 1992).

Research efforts have been conducted towards the obtaintion of other therapeutic options in order to solve this problem. These investigations have focused on using botanical compounds and products for controlling *R.* (*B.*) *microplus*. In this context, studies have shown that plant-based oils and extracts have been gaining ground as control methods for *R. (B.) microplus* (SANTOS et al., 2013a; BARBOSA et al., 2013). The essential oil of *Cuminum cyminum* L. (Apiaceae) (cumin) shows antibacterial action (DERAKHSHAN et al., 2010), antifungic activity against fungus resistant to fluconazole (RABADIA et al., 2011) and insecticide activity (YEOM et al., 2012). It has been demonstrated its acaricidal activity against larvae of *R.* (*B.*) *microplus* (MARTINEZ-VELAZQUEZ et al., 2011), but its acaricidal activity against engorged female of *R. (B.) microplus*, as well as in their reproductive characteristics is unknown. Several studies using *Bertholletia excelsa* (Lecythidaceae) (Brazil nut) had demonstrated a tripanocidal activity and a bioactivity against *Plasmodium falciparum* (KLUCZKOVSKI et al., 2015; CAMPOS et al., 2005; SOUSA, 2013). Studies with the fixed oil of *Helianthus annuus* (Asteraceae) (sunflower seed) demonstrated an *in vitro* antimicrobial activity (ABOKI et al., 2012; TABASSUM & VIDYASAGAR, 2014) and insecticidal activity against *Callosobruchus maculatus,* reducing viable eggs and emerged insects (PEREIRA et al., 2008a). No reports were found in the literature about the fixed oil of *Bertholletia excelsa* (Brazil nut) and of *Helianthus annuus* (sunflower seed), or fixed oils in general, having any acaricide effect against *R.* (*B.*) *microplus*. The yield of these fixed oils is high, and they can be greatly produced in Brazil due to the high availability of these plants.

Given the above, the aim of the present work was: 1) to identify the main chemical components of the *Cuminum cyminum* L. (cumin) essential oil, *Bertholletia excelsa* (Brazil nut) and *Helianthus annuus* (sunflower seed) fixed oils; 2) evaluate oils and commercial synthetic acaricides against engorged females of *R.* (*B.*) *microplus* and their reproductive characteristics, in order to explore their acaricidal efficacy.

Materials and Methods

C. cyminum L.

Essential oil obtention

The essential oil was processed in the Laboratório de Pesquisa em Produtos Naturais (LPPN) of the Centro de Ciências Quimicas, Farmacêuticas e de Alimentos (CCQFA) of the Universidade Federal de Pelotas (UFPel), ubicated in the municipality of Capão do Leão in the state of Rio Grande do Sul, Brazil. The dry seeds of *Cuminum cyminum* L. used for the obtention of the oil were purchased from the producer Luar Sul® in Rio Grande do Sul, Brazil. The oil was obtained by means of hydrodistillation (1.5 L of distilled H_2O / 100 g of plant material) using a Clevenger apparatus. Once the volatile oil was obtained, it was separated from the water, dried with Na_2SO_4 sodium sulfate and stored in an amber bottle under refrigeration (RODRIGUES et al., 2004).

Chromatographic analyses

The analyses was made at the Laboratorio de Lipodômica e Bio-organicâ (LipBio) of the UFPel. The identification of the cumin essential oil was done using a gas chromatograph attached to a mass detector, model GC/MS-QP 2010SE (Shimadzu, Japan), equipped with an AOC-20i auto-injector. The separation occurred in a RTX-5MS (Restek, USA) capillary column; quantification was done by standard area and the compounds identification by the mass spectrometer, using the NIST 8 library of the definer GC/MS. The oil sample was diluted with hexane (analytic degree, ultra pure) (RODRIGUES et al., 2004).

B. excelsa and H. annuus

Fixed oils obtention

Both the Fixed oils of *B. excelsa* and *H. annuus* were processed in the LLipBio of the CCQFA of the UFPel. The nuts of *B. excelsa* used for the obtention of the oil were purchased from the producer

Castanhas of Rondônia® in Rondônia, Brazil and the dry seeds of *H. annuus* used for the obtention of the oil were purchased from the producer Argensun® in Buenos Aires, Argentina. Both fixed oils had the same process of obtention separately. The process consisted in the milling of the nuts and seeds samples in a Willey model B-602 cutting mill, further drying in an oven at 45ºC, followed by extraction where 50 g of the sample and 300 mL of hexane solvent were collocated in a Soxhlet apparatus at a temperature of 60ºC for a 6-hour period. At the end of the process, the solvent was removed through a route evaporator and the oil stored in an amber bottle under refrigeration.

Preparation of Fatty Acid Methyl Esters (FAMEs) of the fixed oils

The fixed oils of *B. excelsa* and *H. annuus* had the same preparation process of FAMEs separately. The fatty acids of each fixed oil were converted to their methyl esters using the boron trifluoride-methanol (BF_3) method, as shown in the literature (MOSS et al., 1974). The resulting mixture of fatty acid methyl esters (FAMEs) in hexane/chloroform (4:1, v/v) was subjected to gas chromatography-flame ionization detection (GC-FID).

Fixed oils (FAMEs) analysis by Gas-chromatography

Both *B. excelsa* and *H.annuus* fixed oils had the same FAMEs analyses separately, performed at the LLipBio of the UFPel. The quantitative GC analyses were performed according to the following conditions using a gas chromatograph GC/FID-2010 with an AOC-20i autosampler (Shimadzu Corporation, Kyoto, Japan) equipped with a fused-silica capillary column (Rtx-WAX, 30 m × 0.25 mm I.D. × 0.25 μm film thickness). Injections were performed with a 1:25 split ratio, and hydrogen was used as the carrier gas under constant flow mode at 1.2 mL/min. The injector was heated to 250 °C, and the flame-ionization detector operated at 250 °C. The initial programmed oven temperature was 100 °C, which was increased by 7 °C/min up to 200 °C, then increased by 5 °C/min to 202.6 °C and held isothermal for 2 min at this temperature. It was then increased by 5 °C/min to 222.9 °C and held isothermal for 2 min, and then increased by 5 °C/min to 230 °C and held isothermal for 10 min at 230 °C (TANG & ROW, 2013). The internal standard solution, containing nonadecanoate methyl ester (C19:0 ≥ 99.0%; Sigma-Aldrich, St. Louis, Missouri, USA), was prepared at a concentration of 2 mg/mL by dissolving 20 mg methyl nonadecanoate in 10 mL of n-hexane in a volumetric flask.

Acaricides

Three acaricides were selected based on the products most commonly used in the region. 1) Clipatic® (Batch number: 016/14, Expiration date. Ago/14-17), Active ingredient: Amitraz, Concentration: 0.125 mg/mL from Fagra Company. 2) Cyperbio® (Batch number: 002/15, Expiration date. Mar/15-Mar/17), Active ingredient: Cypermethrin, Concentration: 0.150 mg/mL from Biofarm Company. 3) Colosso FC30® (Batch number: 0001/14, Expiration date. Mai/14-16), Active ingredient: Chlorpyrifos + Cypermethrin + Fenthion, Concentration: 0.300 mg/mL, 0.150 mg/mL and 0.150 mg/mL respectively from Ourofino Company.

Tick collection

A population of around 600 engorged females of *R.* (*B.*) *microplus* was collected from a beef cattle ranch, situated in the municipality of Capão do Leão located on the southeastern slope of the state of Rio Grande do Sul in Brazil. The engorged females of *R.* (*B.*) *microplus* were collected directly from the cattle's bodies, naturally infested and with at least 40 days without acaricide treatment. The engorged females were subjected to further analysis to the Laboratório de Doenças Parasitarias (LADOPAR) in the Faculdade de Veterinaria of the UFPel.

In vitro assay (Adult Immersion Test)

The adult immersion test (AIT) described by Drummond et al. (1973) was performed. The population of engorged females of *R.* (*B.*) *microplus* were selected according to their state, discarding the dead, deformed, altered and hemorrhagic ones. Once the ticks were washed in distilled water and dried in filter paper, they were weighed and then divided into groups of ten females with homogeneous sizes and weights (weight difference of ± 0.2g). The commercial synthetic acaricides were diluted in water in accordance with the manufacturer's recommendations. For the three oils, six serial concentrations (200, 100, 50, 25, 12.5 and 6.25 mg/mL) were made using 75% ethyl alcohol as solvent. Water and 75% ethyl alcohol were used as controls groups; it has been previously assessed, these solvents do not interfere in the ticks' mortality (CHAGAS et al., 2003). Each test was performed in duplicate, and thus there were a total of 46 groups. Each test group of ten ticks was immersed for five minutes in each treatment, i.e. each diluted acaricide solution, each oil concentration and each control. After immersion, the groups were dried and dorsally fixed using a double-sided tape, on a previously identified Petri dish. The Petri dishes were taken for incubation in a BOD incubator, at a temperature of 27 °C (± 1 °C) and relative humidity higher than 80%, which are the ideal conditions for oviposition. After 14 days of incubation, the mortality rate of the adult females was evaluated and the fertile egg mass of each group was weighed and collocated in a glass vial for incubation in a BOD incubator, under the same conditions; after 30 day the egg hatching analysis was performed.

From these data, the estimated reproduction (ER) and the product effectiveness (PE%) of the treatments were determinate based on the following Formulas 1 and 2, described by Drummond et al. (1973):

RE = Reproductive efficiency:

$$RE = (\text{egg weight} \times \%\text{egg hatching} \times 20{,}000) / \text{engorged female weight} \quad (1)$$

PE = Product effectiveness %:

$$PE = (\text{RE of control group} - \text{RE of treated group} \times 100) / \text{RE of control group} \quad (2)$$

Statistical analysis

The mortality rate among engorged females (%), egg weight (g), hatching rate (%), as well as the mean efficacy of the products and the oils, were analyzed using the *IBM SPSS* software *V21.* Data was checked for normality and homogeneity. As these assumptions were not met, a non-parametric test (Kruskal-Wallis) was used to determine whether there were significant differences among the groups. Intergroup comparison was made using a Wilcoxon-Mann-Whitney test with Bonferroni correction, p values < 0.05 were considered statistically significant.

Results and Discussion

The main chemical components, with isolation proportions greater than 1% detected by gas chromatography-mass spectrometry of the three oils are shown in Table 1. The extraction yield for the essential oil of *C. cyminum* L. was of 2.5%.

The cuminaldehyde, γ-terpinene and β-pinene have shown insecticidal activity (PARK et al., 2008; YEOM et al., 2012), as well as a bactericidal activity against Gram-negative and Gram-positive bacteria (IACOBELLIS et al., 2005). *In vitro* studies have demonstrated the inhibitory and toxic effect of cuminaldehyde due to suffocation and inhibition of a variety of biosynthetic processes that are shown in the beetle's different development stages, such as an effect of inhibiting acetylcholinesterase enzymatic activity (CHAUBEY, 2008; ABDELGALEIL et al., 2009). The acaricidal activity shown in *R.* (*B.*) *microplus* tick larvae, of the essential oil of *C. cyminum* L., may be attributable to the high level of cuminaldehyde (22.03%), γ-terpinene (15.69%) and 2-caren-10-al (12.89%), as well as to minor components, such as the o-cymene and β-pinene (MARTINEZ-VELAZQUEZ et al., 2011); however, the proportions of cuminaldehyde, γ-terpinene, β-pinene and *o*-cymene obtained in this study were higher, which can explain the acaricidal activity reflected on the results shown in Table 2.

Based on the reproductive characteristics and product effectiveness with the *C. cyminum* L. (cumin) essential oil (Table 2), it is observed that in general, the acaricidal effect was directly proportional to the concentration. The highest mortalities of the engorged females were at concentrations of 200 mg/mL and 100 mg/mL (100%[a] in both), at concentration of 50 mg/mL, the mortality decreased to 85%[a], and the lowest mortalities obtained, were at concentrations of 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL with 45%[b], 20%[b] and 20%[b], respectively. All concentrations showed statistical difference with the control. In a study done in Mexico, five concentrations (20%, 10%, 5%, 2.5% and 1.25%) of cumin essential oil were tested in larvae of *R.* (*B.*) *microplus* and in all concentrations mortality of 100% was shown (MARTINEZ-VELAZQUEZ et al., 2011). However, it is known that larvae are more sensitive than engorged females, as it has been seen in studies with substances of plant origin (BORGES et al., 2011), because the cuticle of the tick is formed of an external layer called epicuticle (made externally of wax and internally of proteins). The layer of wax or lipids is seen only in *R.* (*B.*) *microplus* from the ecdise of the nymph and in higher amount in the adult (BALASHOV, 1972; ODHIAMBO, 1982).

Due to the high mortality in the engorged females shown by the two highest concentrations, no weights of the eggs and egg hatching results were obtained. The egg mass weights at concentrations of 50 mg/mL, 25 mg/mL and 12.5 mg/mL were of 184 mg, 464 mg and 1085 mg respectively, showing a negative effect ($p < 0.05$) on the egg posture, when compared to the control (1360 mg). Only the concentration of 6.25 mg/mL, did not show any negative effect on the egg posture regarding the control ($p < 0.05$), with weight of 1247 mg. It is worth mentioning that at concentrations of 50 mg/mL and 25 mg/mL, the egg hatching was below 10%,

Table 1. Main chemical components of *Cuminum cyminum* L. (Cumin), *Bertholletia excelsa* (Brazil nut) and *Helianthus annuus* (Sunflower seed) essential oils.

Chemical component	*Cuminum cyminum* L (%)	*Bertholletia excelsa* (%)	*Helianthus annus* (%)
Myristic Acid	-	0.044	0.071
Palmitic Acid	-	13.467	5.791
Palmitoleic Acid	-	0.232	0.073
Margaric Acid	-	0.129	0.039
Steric Acid	-	7.741	4.106
Oleic Acid	-	30.606	35.805
Linoleic Acid	-	47.256	53.638
Linolenic Acid	-	0.095	0.043
Arachidic Acid	-	0.194	0.244
Gadoleic Acid	-	0.07	0.096
Behenic acid	-	0.099	0.0866
Lignoceric Acid	-	0.065	0.228
Cuminaldehyde	32.66	-	-
γ-terpinene	19.87	-	-
β-pinene	15.22	-	-
O-cymene	14	-	-
2-caren-10-al	8.54	-	-
1-phenyl-1-butanol	8.01	-	-

Table 2. Evaluation of the commercial acaricides and of the six different concentrations of the Cumin essential oil, Brazilian nut and Sunflower seed fixed oils on reproductive indices of *R.* (*B.*) *microplus*, as well as their Product effectiveness (values are expressed in mean ±SD).

Commercial product	Female mortality %	Egg mass weight (mg)	Egg Hatching %	Product Effectivness (%)
Amitraz	25.00±21.23 [b]	1110±0.18 [a]	80.00±0.0 [b]	35.12±9.44
Pyrethtoid	0.00±0.0 [c]	1480± 0.06 [a]	92.50±3.54 [a]	1.50±2.12
Organophosphate-Pyrethroid Association	100.00±0.0 [a]	0.00±0.0 [b]	0.00±0.0 [c]	100.00±0.0
Water	5.00±0.0 [b]	1360±0.03 [a]	88.75±1.77 [a]	-
Cuminum cyminum L				
6.25mg/mL	20.00±0.0 [b]	1247±0.18 [a]	88.75±1.77 [a]	18.58±9.69
12.5mg/mL	20.00± 14.14 [b]	1085±0.32 [b]	27.50±24.75 [b]	74.51±27.37
25mg/mL	45.00±7.07 [b]	464±0.02 [c]	7.75±3.18 [b]	97.34±1.12
50mg/mL	85.00±21.21 [a]	184±0.26 [c]	1.50±2.12 [b]	99.58±0.59
100mg/mL	100.00±0.0 [a]	0.00±0.0 [c]	0.00±0.0 [c]	100.00±0.0
200mg/mL	100.00±0.0 [a]	0.00±0.0 [c]	0.00±0.0 [c]	100.00±0.0
Alcohol	5.00±0.0 [c]	1360±0.06 [a]	96.00±0.0 [a]	-
Bertholletia excelsa				
6.25mg/mL	0.00±0.0 [a]	1437±0.08 [a]	91.50±9.19 [a]	8.16±11.54
12.5mg/mL	10.00±14.14 [a]	1263±0.25 [a]	93.00±0.0 [a]	13.96±19.74
25mg/mL	15.00±7.07 [a]	1194±0.19 [a]	92.00±2.83 [a]	19.69±14.86
50mg/mL	25.00±35.36 [a]	1049±0.46 [a]	90.00±7.07 [a]	29.96±32.92
100mg/mL	10.00±0.0 [a]	1228±0.0 [a]	87.50±10.61 [a]	20.91±6.41
200mg/mL	25.00±35.36 [a]	937±0.43 [a]	90.00±7.07 [a]	39.39±20.70
Alcohol	5.00±0.0 [a]	1360±0.06 [a]	96.00±0.0 [a]	-
Helianthus annuus				
6.25mg/mL	5.00±7.07 [a]	1425±0.15 [a]	96.00±0.0 [a]	4.66±6.60
12.5mg/mL	0.00±0.0 [a]	1501±0.0 [a]	92.00±2.83 [a]	1.51±2.14
25mg/mL	5.00±7.07 [a]	1380±0.15 [a]	88.50±4.95 [a]	11.31±16.00
50mg/mL	10.00±0.0 [a]	1298±0.05 [a]	92.50±3.54 [a]	11.48±3.15
100mg/mL	5.00±7.07 [a]	1230±0.09 [a]	92.50±3.549 [a]	16.24±0.95
200mg/mL	25.00±7.07 [a]	781±0.14 [b]	72.50±10.61 [a]	58.75±3.29
Alcohol	5.00±0.0 [a]	1360±0.06 [a]	96.00±0.0 [a]	-

Common corresponding letters a-c in a given column indicates no significant differences (p<0,05).

having high negative effect. At concentration of 12.5 mg/mL, the egg hatching went up to 27.50%, but still indicating a negative effect ($p < 0.05$). Only the concentration of 6.25 mg/mL (88.75% of egg hatching) did not presented statistical significance toward the control (96%), showing a low effect. The cumin essential oil presented high Product Effectiveness (PE) at concentrations of 200 mg/mL, 100 mg/mL, 50 mg/mL and 25 mg/mL, with 100%, 100%, 99.58% and 97.34%, respectively, being above the level stipulated in Decree no. 48 of year 1997 from the Ministerio da Agricultura, Pecuaria e Abastecimento (BRASIL, 1997), which states that a product is considered effective as an antiparasitic agent, when the PE is ≥95%, having similar results with the Organophosphate-Pyrethroid Association (Table 2), at concentration of 12.5 mg/mL. The PE decreased to 74.51% and at concentration 6.25 mg/mL decreased to 18.58%. The effective results obtained in the engorged female mortality and in the reproductive characteristics, are due to the bioactivity acaricidal effect of the main multiple chemical compounds and by their high percentage amount showed by the gas chromatography-mass spectrometry (Table 1).

The obtained results support that this essential oil as an alternative source to control the cattle tick, delaying the development of resistance; on the other hand, this is only the acaricidal effect *in vitro*; additional studies conduct *in vivo* are necessary, applied as pour-on, on the animal's back and/or with aspersion (MARTINS & GONZÁLEZ, 2007), in order to see if the lowest acaricidal concentration tested in this study, can cause the same activity, due to the difficulties related to external environmental conditions (MULLA & SU, 1999). Regarding the time of storage and conservation of the oil and the seeds, as seen in others plant extracts (BORGES et al., 2011), as well as to the low yield of the oil, we believe that further studies testingt other types of extracts of the cumin seeds and other parts of the plants should be done, identifing the mode of action of the main bioactive compounds and those minor compounds with synergism, to evaluate their toxicity.

The chromatographic profile of the fixed oil of *B. excelsa* (Brazil nut), showed as the major compounds the unsaturated fatty acids. In the polyunsaturated fatty acids, the linoleic acid (C18:2n6c) was the main component with 47.25% and in the monounsaturated fatty acids was the oleic acid (C18:1n9c) with 30.60%. The extraction yield of the oil was of 69%, which is high. This results were close to those obtained by Ryan et al. (2006), where they obtained 42.80% of linoleic acid and 29.09% of oleic

acid, as well as those obtained by Venkatachalam & Sathe (2006), with 45.43% of linoleic acid and 28.75% of oleic acid.

The six different concentrations of the fixed oil of *B. excelsa* (Brazil nut), did not show statistical difference with the control ($p > 0.05$) in the mortalities of the engorged females and on each of the reproductive characteristics, with some of the results not being directly proportional with the concentrations and with variations on the results between them. In the mortality of the engorged females, both concentrations of 200 mg/mL and 50 mg/mL showed 25%, the concentration of 12.5 mg/mL had 15%, at concentrations of 100 mg/mL and 12.5 mg/mL the mortality was of 10% in each, the concentration of 6.25 mg/mL, did not present any mortality in the engorged females. The eggs mass weights were proportional to the mortality of the engorged females at each concentration. The lower egg mass weight was at concentration of 200 mg/mL with 937 mg, the concentration of 100 mg/mL showed the lowest egg hatching (87.50%); however, no statistical difference with the control was seen, having a low effect. The fixed oil presented the higher PE (39.39%) at the concentration of 200 mg/mL, below the average established by the MAPA.

The results in the chromatographic profile of the fixed oil of *H. annuus* (Sunflower seed), also showed that the unsaturated fatty acids were the main components, with 53.63% of linoleic acid (C18:2n6c) and 35.80% of oleic acid (C18:1n9c). The extraction yield for the oil was of 61%. These results were close to those reported by Mandarino (1992), with 50% to 70% of linoleic acid and 26% to 40% of oleic acid. In general, the differences in the quantities of the unsaturated fatty acids components of the two fixed oils in this study with those mentioned in the other studies can be related to the origin and genotype of the nuts and seeds.

The fixed oil of *H. annuus* (sunflower seed), like the fixed oil of *B. excelsa* (Brazil nut), obtained results not directly proportional to the concentrations, none of the concentrations showed a statistical difference with the control. The highest mortality (25%) was at the concentration of 200 mg/mL. At the concentration of 50 mg/mL, the mortality was of 10%, in the concentrations of 100, 25 and 6.25 mg/mL the mortalities were of 5% and the concentration of 12.5 mg/mL not showed mortalilty. In the fixed oil of *H. annuus*, the concentration of 200 mg/mL presented the lowest egg mass weight (781 mg) ($p < 0.05$); also, this concentration showed the lowest hatching rate with 72.50% (no statistical difference with the control). Between the fixed oils, this oil presented the higher PE (58.75%) at the concentration of 200 mg/mL, but below the average established by the MAPA. It is important to establish that in both fixed oils the PEs were higher than those obtained by the pyrethroid and the amidine tested in this study.

In general, both fixed oils showed low mortalities, attributed to the fact that the main components are unsaturated fatty acids (Table 1). Also, they did not have a high acaricidal activity, like the high mortality effect shown in the essential oil. In addition, it can be deduced that the low mortality was because the fixed oils never evaporate or volatilize completely, remaining as viscous liquids. Once in contact for a period of time with the tick's spiracles, where the normal route for gas exchanges through holes or aeropyles and the ambient air is made (HINTON, 1967), the demand for oxygen and release of carbon dioxide is not balanced and excessive water loss occurs, causing suffocation of the tick and consequent dehydratation. Therefore, the variations of the results between the six diferent concentrations may be due to several factors such as the time of contact of the oil after the immersion test in the cuticle in each tick and the misture at dilutions of the oils with the solvent. It is important to menthion that irregularities in mortality were shown in a study with essential oil in the study by Olivo et al. (2008), in which citronella oil (0.5% and 1%) was used against engorged adult *R. (B.) microplus* females. Thus, the phenomenon of passivation was observed, where the concentrated product started to be absorbed, but a passivator film began to form, thereby inhibiting passage of the oil. In such situations, the oil therefore penetrates better when it is more diluted, because no protective film has formed, causing higher absorption (CHAGAS et al., 2002).

Plants of the same species can vary in the quantity of chemical compounds due to their interspecific variation and other factors such as seasonality, circadian rhythm, development, temperature, ultraviolet radiation, water availability, altitude and atmospheric pollution, among others; this can coordinate or change the rate of production of the secondary metabolites (GOBBO-NETO & LOPES, 2007). Generally, the acaricidal effect of an essential oil is attributed to the components isolated in higher quantity; however, the activity of the main compound can be regulated by other components present in minor quantity (CAMPOS et al., 2012).

The reproductive indices and PEs obtained from the three commercial acaricides (Table 2), showed that between the three acaricides, only the organophosphate-pyrethroid association had a product effectiveness of 100%, being above the established by the MAPA. The amidine presented low effectiveness (35.12%), and the pyrethroid presented the lowest PE (1.50%) among the acaricides.

Conclusions

The essential oil of *C. cyminum* L., demonstrate high acaricidal activity (100%), *in vitro* from the concentration of 100 mg/ml, against engorged females of *R.* (*B.*) *microplus.* The oil's activity was attributed to the high content of bioactive biodegradables compounds. There is a need for additional studies, to be conduct *in vivo*. On the other hand, the fixed oils of *B. excelsa* and *H. annuus* had low acaricidal effect (39.39% and 58.75% in the concentration of 200 mg/ml respectively). The amitraz and pyrethroid. Had low acaricidal effect (35.12% and 1.50% respectively), only the association showed high acaricidal effect (100%).

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