CHEMICAL COMPOSITION OF *Melinis minutiflora* EXTRACT AND ITS REPELLENT AND IXODICIDE EFFECT ON *Amblyomma cajennense* LARVAE

Composición química del extracto de *Melinis minutiflora* y su efecto repelente e ixodicida sobre larvas de *Amblyomma cajennense*

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ABSTRACT

The objective of this study was to identify the chemical compounds of extracts from the stem, leaves and stem/leaves of *Melinis minutiflora* to evaluate the repellent and ixodicide activity against *Amblyomma cajennense* larvae. The extracts from the stem (S), leaves (L) and stem/leaves (SL) of *M. minutiflora* were processed on Soxhlet with ethanol at 96%. The components identification was carried out by gas chromatograph-mass spectrometer (GC-MS). The repellent effect and ixodicide action were evaluated with the olfactometer test and immersion of larvae test, respectively. In S, L and SL were identified 11, 6 and 8 compounds, respectively. The percentages of repellency were: 6, 12, 25, 32, 53 and 71 with S; 6, 15, 23, 32, 57, 79 with L and 6, 12, 32, 48, 64, 90 with SL, while, the percentages of mortality were: 8, 10, 19, 32, 49 and 75 with S; 5, 9, 20, 38, 56, 82 with L; and 10, 10, 25, 42, 64 y 93 with SL at for the 5, 10, 15, 20, 25 y 30 mg mL^{-1} concentrations, respectively, in both tests. The repellent concentration in stem/leaves was RC_{50} 9.94(16.52-23.18) mg mL^{-1} and RC_{95} 42.25(32.47-90.18) mg mL^{-1} (R^2 = 0.934); subsequently leaves RC_{50} 22.25(18.49-28.49) mg mL^{-1} and RC_{95} 60.11(40.04-239.39) mg mL^{-1} (R^2=0.972), while, stem requires more repellent concentration with RC_{50} 23.55(21.13-27.20) mg mL^{-1} and RC_{95} 67.01(48.57-130.25) mg mL^{-1} (R^2=0.938). The lethal concentration (LC) with SL for LC_{50} 19.73(14.29-23.79) mg mL^{-1} and LC_{95} 38.22(29.20-126.27) mg mL^{-1} (R^2=0.974), lower compared to L, LC_{50} 21.78(19.28-24.56) mg mL^{-1} and LC_{95} 45.94(36.31-78.54) mg mL^{-1} (R^2=0.974), and S LC_{50} 24.00(22.30-25.57) y LC_{95} 40.19(36.92-45.33) mg mL^{-1} (R^2=0.938). The results showed that extract from stem/leaves of *Melinis minutiflora* have high repellent effect and ixodicide activity on *A. cajennense* larvae.

Key words: Natural products; *Amblyomma cajennense*; secondary metabolites; ixodicide.

RESUMEN

El objetivo del estudio fue identificar los compuestos químicos de los extractos de tallo, hojas y tallo/hojas de *Melinis minutiflora* y evaluar su actividad repelente e ixodicida contra larvas de *Amblyomma cajennense*. Los extractos tallo (S), hojas (L) y tallo/hojas (SL) de *M. minutiflora* fueron procesados en Soxhlet con etanol (96 %). La identificación de los componentes fue mediante cromatografía de gases-espectrometría de masas (CG-EM). El efecto repelente e ixodicida se determinó en pruebas de olfactómetro y de inmersión de larvas, respectivamente. En S, L y SL fueron identificados 11; 6 y 8 compuestos, respectivamente. Los porcentajes de repelencia fueron 6; 12, 25, 32; 53 y 71 con S; 6; 15; 23; 32; 57; 79 y 6 con L; y 12; 32; 48; 64; 90 con SL, mientras que, los porcentajes de mortalidad fueron 8; 10; 19; 32; 49 y 75 con S, 5; 9; 20; 38; 56; 82 con L y 10; 10; 25; 42; 64 y 93 con SL, a concentraciones de 5; 10; 15; 20; 25 y 30 mg mL^{-1}, respectivamente, en ambas pruebas. La concentración repelente (CR) en tallo/hojas la CR_{50} 9,94(16,52-23,18) mg mL^{-1} y CR_{95} 42,25(32,47-90,18) mg mL^{-1} (R^2= 0,934); en hojas CR_{50} 22,25(18,49-28,49) mg mL^{-1} y CR_{95} 60,11(40,04-239,39) mg mL^{-1} (R^2= 0,972), mientras que, tallo requiere más concentración repelente CR_{50} 23,55(21,13-27,20) mg mL^{-1} y CR_{95} 67,01(48,57-130,25) mg mL^{-1} (R^2= 0,975). La concentración letal (LC) en tallo/hojas fue para LC_{50} 19,73(14,29-23,79) mg mL^{-1} y LC_{95} 38,22(29,20-126,27) mg mL^{-1} (R^2=0,974) más baja respecto a extracto hojas LC_{50} 21,78(19,28-24,56) mg mL^{-1} y LC_{95} 45,94(36,31-78,54) mg mL^{-1} (R^2=0,974), y que tallo LC_{50} 24,00(22,30-25,57) y LC_{95} 40,19(36,92-45,33) mg mL^{-1} (R^2=0,938). Los resultados muestran que el extracto de tallo/hojas de *M. minutiflora* tienen un elevado efecto repelente e ixodicida sobre larvas *A. cajennense*.

Palabras clave: Productos naturales; *Amblyomma cajennense*; metabolitos secundarios; ixodicida.

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INTRODUCTION

In tropical and subtropical regions of the world, the diseases transmitted by ticks are one of the main zoonotic problems. Ticks affect 80% of the world´s cattle [31, 46] and are ranked in second place (after mosquitoes) as a main vector of diseases in humans, wild and domesticated animals [14, 17, 40]. In North America, Central and South America, the tick (*Amblyomma cajennense)* is considered to be very aggressive, especially in the stadiums of nymphs and adult. The ticks also transmit bacterium *Rickettsia rickettsii* to humans cause of Rocky Mountain spotted fever and Tick Bite Fever in Africa disease [6, 14, 33,]. The genus *Amblyomma* involves nearly 130 species of ticks distributed worldwide [4]. Among these, *Amblyomma cajennense* (Fabricius, 1787) (Acari: ixodidae) cause skin detriment, loss of body weight, low productive performance and cattle (*Bos taurus* y *Bos indicus*) death [19, 47]. Ticks have caused economics losses of 13-18 billion dollars around the world [9, 15, 32], one billion dollars of Latin America and 48 million dollars in Mexico [11, 26]. *A. cajennense* is the second place in importance in México and it is found on 609,857 km^2 (31% of total area) of tropical, arid and temperate zones [25].

Among control methods against these ticks, the utilization of synthetic chemical compounds is the most used [21]. Often use of pesticide has promoted the development of resistant strains of ticks. Moreover, chemical compounds can be accumulated in food of animal origin and consequently affect the environment [29, 38].

Natural pesticides derived from plants are an alternative to reduce the use of synthetic products [10, 12]. These are natural products generated from secondary metabolism of plants [5, 34]. They may help in the development of an integrated program for tick control. These programs include: management of resistant breeds of cattle, nutritional programs, plot rotation, fire control, entomopathogenic fungi, vaccines and natural pesticides against ticks [1, 31].

M. minutiflora, which belongs to Poaceae family, secretes an oleoresin from its thrichomes located in stem and leaves. This oily resin negatively affects ticks [28, 36]. Nevertheless, the chemical compounds producing these beneficial actions have not been identified.

The aim of the present research was to identify chemical compounds of extracts from stem leaves and stem/leaves of *M. minutiflora* and, evaluate the repellence and ixodicide effect on *Amblyomma cajennense* tick larvae.

MATERIALS AND METHODS

M. minutiflora (Mm) seeds were obtained from Centro Nacional de Investigación Disciplinaria en Parasitología Veterinaria, Jiutepec, Morelos, Mexico [National Research Center of Veterinary Parasitology]. It was seeded by hand in Tepic County located at 21°, 31' LN y 104°, 54' LO, 920 m meters over sea level (m.o.s.l), 1121 milimeter (mm) of precipitation, temperature of 15-25 °C, dry tropical to subhumid weather and, most of the precipitation during summer [27]. Plots were established on surfaces of 5 x 7 meters (m), areas with 25 grooves. Seed density of 12 kilogram/hectare (kg/ha) was used. Fertilization (100-50-50 kg de NPK/ha) was carried out 45 and 70 d after they have been planted. Later on, only Nitrogen (50%) was applied.

Extracts of stem, leaves and stem/leaves of *M. minutiflora* by Soxhlet

It took place at the Universidad Autónoma de Nayarit [Autonomous University of Nayarit], when the plant was 90 d of age. For the extraction process, plant samples were cut to obtain a particle size of 0.3-0.5 centimeters (cm). The plant material 50 grames (g) was weighted and extracted by Soxhlet (Kimax® of 250 mL). Filter paper (Whatman No. 1) with a grass sample was placed on the extractor equipment (Kimax® of 250 mL, model: 24005, México). One hundred fifty milliliters (mL) of the ethanol 96 % solvent was poured into the flask and placed at by constant reflux until plant exhaustion; this procedure was at 50 60°C.
Once the extraction was done, extracts were concentrated in a roto-evaporator with reduced pressure (ACME®) at 40 - 50 °C. Yield of the crude extract was obtained. One part of crude extract was resuspended in ethanol 99.5% for phytochemistry analysis by Gas Chromatograph-Mass Spectrometer (CG-MS; Hp Agilent 5973, Selective mass detector 6890, USA), the rest of the extract was resuspended at concentrations 5, 10, 15, 20, 25 and 30 mg mL^{-1} on ethanol 96% for *in vitro* assays.

Culture *A. cajennense* larvae

A. cajennense larvae were obtained by hand from beef cattle in Jesús María Corte, Country Tepic, Nayarit, México located at 21°, 43' LN and 104°, 52' LO. They were placed in portable coolers at 4 - 6°C for transportation to the laboratorio de Resistencia y Taxonomía de Garrapata del Comité para el Fomento y Protección Pecuaria de Nayarit [Ticks Resistance and Taxonomy Laboratory of Nayarit Cattle Development and Protection Committee], to be identified under stereoscopic microscope (ZEISS®). To obtain the eggs, 10 ticks were placed in petri dishes for incubation (Precision Incubator®, model 6) at 27 ± 2°C, with a relative humidity (RH) of 80 - 90%. The eggs were placed in 15 mL vials and then were placed 19 d in the incubator at the same conditions until hatched. The larvae were used at 14 d post-hatch, in the Olfactometer test (OT) and larval immersion test (LIT), described below [18, 36].

Chemical compound identification by CG-MS

The identification of chemical compounds from stem, leaves and stem/leaves extract was carried out at Phytochemical Laboratory, CINVESTAV Irapuato, México. Gas Chromatograph-

Mass Spectrometer (CG-MS) Agilent Technologies 7890ª (origin United States of North America), with a capillary column (J&W No. 122-0162DB-1ms), of 60 m, with 0.25 mm of external diameter and 0.25 µm of internal diameter was used. Helium was used as a carrier gas, with a flow rate of 1mL/min. Injector temperature was 250°C. The injection volume was 1 µL. The initial temperature was 150°C for three minutes (min), subsequently increasing the temperature at a rate of 4ºC per min up to 280°C, for a total runtime of 60.5 min; a pressure of 24.942 pounds force per square inch (psi) was used. Flame ionization detector (FID) was operating at 70 eV. Mass range was from m/z 50 to 550 amu.

Olfactometer Test

Repellence was determined by Olfactometer Test (OT). Olfactometer is a glass tube, "Y" shaped, 9 centimeters (cm) long with 0.5 cm of internal diameter and 0.8 cm of external diameter (Pírex®). A cotton ball was soaked with 1 mL either crude extract of stem, crude extract of leaves and crude extract of stem/leaves, concentrations (5, 10, 15, 20, 25 and 30 miligrams (mg) mL^{-1}) or ethanol 96% (negative control) with ten repetitions for each treatment. They were dried at laboratory temperature for 24 hours (h) and then the cotton ball (200 mg) was placed in an arm of the olfactometer. In the other arm, another cotton ball (200 mg) was placed soaked with ethanol 96 %, and at the bottom of the olfactometer a batch of 1,000 approx. *A. cajennense* larvae was placed. It was covered with a cotton ball to prevent larvae escape. Larvae were allowed to ascend through the walls for 20 min, and then they were quantified in each arm. A new olfactometer was used for each trial. The percentage of repellency was calculated, with the formula of Muro [36]:

$$\text{Repellence} = \frac{\text{Larvae in the arm with cotton impregmated only with the solvent corresponding to the evaluated extract}}{\text{Larvae that climbed both arms of the olfactometer}} \text{ X 100}$$

Larval immersion test

This test consisted in measuring the larvicide effect of the natural extract from either stem, leaves or stem/leaves of *M. minutiflora* or ethanol 96 % (control). First, a sheet of filter paper (Whatman No. 1 of 8.5 x 7.5 cm) was placed in Petri dishes and, above them a 14 d old batch of 100 ± 10 larvae was placed. Then they were treated homogeneously with different concentrations (5, 10, 15, 20, 25 and 30 mg mL^{-1}) from evaluated *M. minutiflora* extract; these dilute concentration on ethanol 96% with 5 mL for each concentration. Another sheet of Whatman 1 paper was placed and it was immersed for 5 min for each treatment respectively, (with modifications at Drummond *et al*. [18] and Soberanes *et al*. [44] tests. Then the filtre papers was pulled and folded with snaps to allow solvent to evaporate for 40 min at room temperature. The respective control was conducted using ethanol 96 % [41] instead of the extract. For each treatment, ten repetitions were performed. After each Whatman 1 paper was identified, according to treatment. Larvae were placed in the incubator (Precisión Incubator®, model 6, USA) for 72 h at 27 ± 2°C and 80 - 90 relative humidity (RH). Afterwards, the larvae were counted, considering live larvae those with movement and dead larvae those without movement. Mortality rate of larvae was calculated following the next formula [2]:

$$\text{Mortality} = \frac{\text{\% Mortality treated - \% Control Mortality}}{\text{100 - \% Control Mortality}} \text{ X 100}$$

Statistical analysis

The data obtained in mortality percentage were analyzed through one-way ANOVA test and then compared by Tukey test ($P<0.05$) when it was necessary according to SAS [45]. The lethal concentrations (LC_{50} and LC_{95}) and their confidence limits were obtained following the Probit method with Polo Plus Software Version 2.0 [39].

RESULTS AND DISCUSSION

Phytochemical characterization

The composition of the *M. minutiflora* extract analyzed on GC–MS is shown in TABLE I. The identified components for stem, leaves or stem/leaves from the extract of *M. minutiflora*, revealed on stem 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester (33.42%) as best component, following hexadecanoic acid (6.96%), while for leaves, the best component was 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester (59.44%) similar to stem, and for last heneicosane (3.19%), in stem/leaves extract from *M. minutiflora* were observed 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester (60.07 %), the majority of the extract, on the other hand, n-hexadecanoic acid (12.81 %), octadecanoic acid (10.74 %) and eicosanoic acid (9.28%), were found in different concentrations. It shows that the components with the major concentration were to stem/leaves, regarding other extracts.

Meanwhile, Magano *et al*. [30] it was found that the components 2- benzenedicarboxylic acid, dibutyl ester (2.32 %), 1,2 -benzenedicarboxylic acid, bis (2-ethylhexyl)-ester (20.19 %), hexadecanoic acid (51.55 %), 9-hexadecanoic acid (11.84 %) from *Senna itálica* extract (Arachoides subspecie) present ixodicide effect in *Hyalomma marginatum* [30]. Two of these compounds were found in *M. minutiflora*. Previous studies,

TABLE I
CHEMICAL COMPOUNDS IDENTIFIED FROM EXTRACTS OF *Melinis minutiflora* BY GC-MS

Compound Plant extract	TR*	Peakarea %
	Stem	
Propanoic acid,2-methyl-,(1,1-dimethylethyl-1,3-propanediyl ester	7.13	4.33
Isopropyl Myristate	11.91	1.06
Phthalic acid, bytilundecyl ester	15.50	5.62
Hexadecanoicacid, ethylester	15.72	6.96
Isopropyl Palmitate	16.40	0.81
9,12-Octadecadienoic acid (Z,Z)-	19.29	1.49
7-Methyl-Z-tetradecene-1-ol- acetate	24.93	1.00
1,2-Bencenedicarboxylic acid, mono(2-ethylhexyl)ester	27.34	33.42
Squalene	32.24	1.34
α-Amyrin	42.36	2.88
Lanosterol	46.03	5.51
	Leaves	
Propanoic acid,2-methyl-,(1,1-dimethylethyl-1,3-propanediyl ester	7.01	6.67
Heneicosane	18.00	3.19
17-Octadecynoic acid	19.58	1.19
7-Metyl-Z-tetradecen-1 olacetate	20.14	1.09
1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	27.37	59.44
Heptacosane	29.96	0.44
	Stem/Leaves	
Propanoic acid,2-methyl-,(1,1-dimethylethyl-1,3-propanediyl ester	7.11	8.32
n-Hexadecanoic acid	18.89	12.81
9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	22.68	4.35
Octadecanoicacid	23.35	10.74
Eicosanoicacid	27.54	9.28
7-Pentadecyne	30.55	5.34
1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	30.70	60.07
2,3-Dicyano-4-[4-(1-imidazoyl)-butoxy]-phenol	34.48	1.32

*TR= Retention time (minutes).

reported some derived sterols from plants with toxic activity against arthropod pests (ß-sitosterol, stigmasterol, ß –sitosterol -3-O- ß-D-glucoside, 3 ß –sitosterol, 7α -hydroxy- ß –sitosterol and ecdysterone, cyasterone) [24] these components are similar to the present study.

Bioassays repellent and acaricide effect

The repellent and acaricide effects of the extracts from stem, leaves or stem/leaves against *A. cajennense* larvae were variable. These effects depended on the concentration applied in each bioassay. It has been found that the effect is dependent on the species, phytochemical composition and applied concentration [3].

Repellent effect

The repellency of extract from *M. minutiflora*, was denominated repellent concentration (RC_{50} and RC_{95} mg mL^{-1}). The results are shown on TABLE II. The percentage repellence for the extract from stem, leaves or stem/leaves at concentration 5 mg mL^{-1} did not show significant difference compared to the control ($P < 0.05$). The minimum and maximum percentages of repellence were: 12 and 71 with stem extract; 15 and 79 with leaves extract and stem/leaves 12 and 90 with the stem/leaves extract, at 10 and 30 mg mL^{-1} concentrations, respectively (TABLE II), showing significant difference among the concentrations, for each extracts ($P < 0.05$). Bissinger *et al*. [8, 30] studied repellent effect of synthetic compounds and terpenoids, the latter are derived from various plants,

TABLE II
REPELLENCY EFFICIENCY FROM THE EXTRACTS OF *Melinis minutiflora* AGAINST *Amblyomma cajennense* LARVAE

Plant extract	Concentration (mg mL^{-1})	Percent[a] repellent ± SD	RC_{50} (mg mL^{-1}) (LCL-UCL)	RC_{95} (mg mL^{-1}) (LCL-UCL)	R^2
Stem	30	71.77Ca ± 4.31	23.55(21.13 - 27.20)	67.01(48.57-130.25)	0.975
	25	53.01DEb ± 4.15			
	20	32.03EFGc ± 3.84			
	15	25.01FGHc ± 6.13			
	10	12.62GHd ± 4.84			
	5	6.20Hd ± 1.98			
	Control	6.09Hd ± 1.91			
Leaves	30	79.42Ba ± 8.42	22.25(18.49- 28.49)	60.11(40.04-239.39)	0.972
	25	57.87Db ± 4.20			
	20	32.87EFGc ± 4.44			
	15	23.84GHcd ± 4.50			
	10	15.53GHde ± 5.73			
	5	6.60He ± 1.71			
	Control	5.54He ± 0.76			
Stem/ Leaves	30	90.83Aa ± 5.83	19.94 (16.52-23.18)	42.25 (32.47-90.18)	0.934
	25	64.31BCDb ± 13.46			
	20	48.13DEFc ± 14.21			
	15	32.15EFGcd ± 8.99			
	10	12.03GHde ± 6.59			
	5	6.55He ± 1.42			
	Control	7.29He ± 2.13			

RC_{50} repellent concentration 50% of the exposed parasite, RD_{95} repellent concentration 95% of the exposed parasite, UCL upper confidence limit, LCL lower confidence limit, Determination coefficient *(R^2)*, significant at P<0.05 level. [a]Mean value of ten replicates. Averages followed by equal letters in the column of plant extract (capital letter) or row for in every plant extract (small letter) do not differ statistically at significance level of 5 %.

and are effective on different species of ticks. De Santana *et al*. [16], report the use of acaricides and repellents derived from plants, as an alternative tick control [22, 43]. Bioassay in *Amblyomma americanum* with vertical paper filter technique [35] exhibited the effective concentration of N, N-diethyl-m-toluamide (DEET) that repels 50 % of tick (EC_{50}) of 0.02 mg/cm^2, while EC_{50} from essential oils of commercial origin is between 0.113 and 0.297 mg/cm^2. Based on estimations of EC_{50}, oregano (*Origanum vulgare*) essential oils was the most effective between all the oil tested, followed by clove (*Eugenia caryophyllta)*, thyme (*Thymus saturelloides*), vetiver (*Vetiveria zizanioides*), sandalwood (*Sanalum album*), cinnamon (*Cinnamomum zelanicum*), cedarwood (*Cedrus atlantica*) and peppermint (*Mentha piperita*) oils, whereas in this research the best repellency was 30 mg mL^{-1}. The compounds callicarpenal, intermedeol, in extract from *Mentha pulegium* and leaves from *Memora nodosa* showed a high repellency percentage against *A. cajennense* nymphs of 77 and 87 % [43] similar to *M. minutiflora*. Instead, Fernandes *et al*. [20], found repellence effect against *A. cajennense* nimphs with extracts of *Melia azedarach, Cymbopogon nardus, Spiranthera odoratissima, Chenopodium ambrosioides, Ageratum conyzoides, Mentha pulegium, Ruta graveolens and Memora nodosa*, the best repellence was obtained with *C. nardus* (90 %) compared to other extracts tested (66 %) which presented lower effect than *M. minutiflora*.

The repellent concentration was determined by Probit Analysis (RC_{50} and RC_{95}), for extracts from stem, leaves or stem/leaves of *M. minutiflora*. The extracts from stem/leaves exhibited the best repellency on RC_{50} 19.94(16.52-23.18) mg mL^{-1} and RC_{95} 42.25(32.47-90.18) mg mL^{-1} (R^2 = 0.934), this repellency is comparable to that observed by Ashintani *et al*. [7] on *Ixodes ricinus* nymphs.

TABLE III
ACARICIDE EFFICACY OF *M. minutiflora* EXTRACT AGAINST LARVAE OF *Amblyomma cajennense*

Plant extract	Concentration (mg mL^{-1})	Percent[a]mortality ± SD	LC_{50} (mg mL^{-1}) (LCL-UCL)	LC_{95} (mg mL^{-1}) (LCL-UCL)	R^2
Stem	30	75.28Ca± 2.01	24.00(22.30-25.57)	40.19(36.92-45.33)	0.938
	25	49.50DEFb±11.09			
	20	32.21FGHc±10.21			
	15	19.10HIJdc±5.88			
	10	10.58IJd±5.54			
	5	8.62IJd±3.49			
	Control	7.43IJd±2.55			
Leaves	30	82.52Ba±3.04	21.78(19.28-24.56)	45.94(36.31-78.54)	0.986
	25	56.36CDEb±3.41			
	20	38.40EFGHc±6.73			
	15	20.51HIJd±4.23			
	10	9.62IJe±0.93			
	5	5.53IJe±1.06			
	Control	4.99Je±1.94			
Stem/ Leaves	30	93.34Aa±3.60	19.73(14.29-23.79)	38.22(29.20-126.27)	0.974
	25	64.42CDb±4.03			
	20	42.60EFGc±11.08			
	15	25.01GHId±6.89			
	10	10.36IJe±2.77			
	5	10.44IJe±2.54			
	Control	5.45IJe±1.58			

LC_{50} lethal concentration that kills 50% of the exposed parasite, LC_{95} lethal concentration that kills 95% of the exposed parasite, UCL upper confidence limit, LCL lower confidence limit, Determination coefficient *(R^2)*, significant at P<0.05 level. [a]Mean value of ten replicates. Averages followed by equal letters in the column of plant extract (capital letter) or row for in every plant extract (small letter) do not differ statistically at significance level of 5 %.

Ixodicide effect

The percentages of mortality with the stem, leaves or stem/ leaves extracts at 5, 10 and 15 mg mL^{-1} concentrations were to the control (P > 0.05), TABLE III. The mortality increased (P< 0.05) at greater concentrations (25 and 30 mg mL^{-1}) with the three extracts. Da Silva *et al*. [13] observed the best acaricide activity at 20 mg mL^{-1} concentration when used thymol at increasing concentrations against *A*. *cajennense* larvae unfed and fed by larval packet and larval immersion tests (94.5 % and 100% of mortality, respectively). In this experiment, the mortality percentages observed in the larval immersion tests were lower compared to those reported by Da Silva *et al*. [13, 42].

The effect of extract of leaves at 25 and 30 mg mL^{-1} concentrations showed difference among them (P < 0.05) and were greater than that at 5, 10 and 15 mg mL^{-1} concentrations (P <0.05). The mortality percentage in larvae with 30 mg mL^{-1} concentration from stem extract was of 75.28 %. For leaves extract was observed an intermediate effect in 30 mg mL^{-1} concentrations, reaching 82.52 % of dead larvae. The higher effect at 30 mg mL^{-1} concentration was observed with the extracts from stem/leaves with larvae mortality of 90.83 % (P < 0.05). De Santana *et al.* [16] assayed, two variations of essential oil from the Poacea *Chrysopogon zizanioides*, one of these variations had a high acid value (lower quality) and the other lower acid value (high quality). The quality is related with the concentration of fatty

acids and sesquiterpene zizanoic and khusimol (alcoholic portion) acid. The acid value of essential oils of *C. zizanioides* was 0.45 mg/g potassium hydroxide for that with acid high value (HAV) and 7.5 mg/g of oil low acid (LAV), reporting that in HAV the CL_{50} for *Amblyomma cajennense* larvae is 2.076 (1.853–2.311) (µL ml^{-1}) and the CL_{99} of 13.030 (9.596–20.390) (µL ml^{-1}). For LAV, the CL_{50} was 1.17(1.117–1.226) (µL ml^{-1}) and the CL_{99} 2.190 (2.002–2.490) (µL ml^{-1}). The CL_{50} and CL_{99} obtained from *C. zizanioides* were lower than *M. minutiflora* in the the present study.

The LC_{50} and LC_{95}, were determined with the extracts from stem, leaves or stem/leaves of *M. minutiflora*. The stem/leaves extract was the one that show best effect for LC_{50} 19.73 (14.29-23.79) mg mL^{-1} and LC_{95} 38.22 (29.20-126.27) mg mL^{-1} (R^2 = 0.974, TABLE III). According to Gomes *et al*. [23], they identified monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpene and caryophyllene oxide from essential oil of *Lippia sidoides*, in concentrations of 2.35 and 14.10 mg mL^{-1}. They caused a mortality of 41 and 82 % on unfed larvae of *A. cajennense* and 20.6 and 99. 5 % *R. sanguineus*, because the penetration of an insecticide varies with the thickness of the layer of lipids cuticle, and may vary according to species and stage of development, of the tick [23].

The repellent activity and ixodicide from the extracts of *M. minutiflora* to the concentrations 5, 10, 15, 20, 25 and 30 mg mL^{-1} against *A. cajennense* larvae was depending on the dose (FIG. 1).

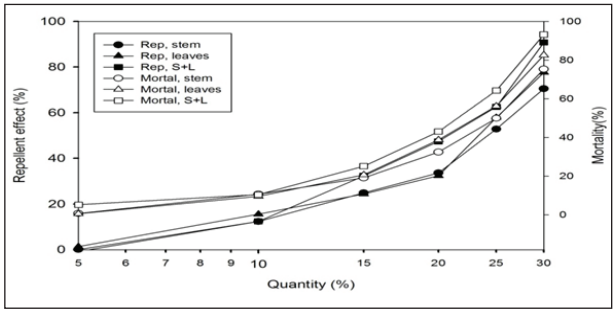

FIGURE 1. THE REPELLENT AND ACARICIDE ACTIVITY FROM EXTRACTS *M. minutiflora* AGAINST *A. cajennense* LARVAE.

CONCLUSIONS

In summary, extract from *M. minutiflora* has a strong activity against *A. cajennense*, based on the test of repellency and ixodicide; this may be due the additive effect of components from extracts. Further research is recommendable with the purified and isolated active components from extracts is necessary, and its assessment on bovine host.

BIBLIOGRAPHIC REFERENCES

[1] ABBAS, R.Z.; ZAMAN, M.A.; COLWELL, D.D.; IQBAL, Z. Acaricide resistance in cattle ticks and approaches to its management: The state of play. **Vet**. **Parasitol**. 203(1): 6-20. 2014.

[2] ABBOTT, W.S. A method of computing the effectiveness of an insecticide. **J**. **Econ**. **Entomol**. 18: 265-267. 1925.

[3] ADENUBI, O.T.; FASINA, F.O.; MCGAW, L.J.; ELOFF, J.N.; NAIDOO, V. Plant extracts to control ticks of veterinary and medical importance: A review. **S. African J. Bot.** 105: 178-193. 2016.

[4] ALONSO, D.M.; FERNÁNDEZ, S.A.; MARTÍNEZ I.F.; OSORIO, M.J. *Amblyomma cajennense* (Acari: Ixodidae) tick populations susceptible or resistant to acaricides in the Mexican Tropics. **Vet**. **Parasitol**. 197(1): 326-331. 2013.

[5] APARECIDO, C.M.; OLIVEIRA, M.C.; GOLDNER, S.M.; TEIXEIRA, G.F.; AZEVEDO, P.M.; DAEMON, E. Acaricidal activity of the essential oils from *Eucalyptus citriodora* and *Cymbopogon nardus* on larvae of *Amblyomma cajennense (*Acari: Ixodidae) and *Anocentor nitens* (Acari: Ixodidae). **Parasitol**. **Res**. 107(4): 987-992. 2010.

[6] ARRIETA, F.H. Garrapatas (Acarina: Ixodoidea) del ganado bovino y controles utilizados en el Municipio Jesús Enrique Lossada, Estado Zulia, Venezuela. **Rev. Cientif. FCV-LUZ.** IX(1): 47-51. 1999.

[7] ASHITANI, T.; GARBOUI, S.S.; SCHUBERT, F.; VONGSOMBATH, C.; LIBLIKAS, I.; PÅLSSON, K.; BORG, K.A. Activity studies of sesquiterpene oxides and sulfides from the plant *Hyptis suaveolens* (Lamiaceae) and its repellency on *Ixodes ricinus* (Acari: Ixodidae). **Exp. Appl. Acarol**. 67(4): 595-606. 2015.

[8] BISSINGER, B.W.; ROE, R.M. Tick repellents: past, present, and future. **Pest**. **Biochem**. **Physiol**. 96(2): 63-79. 2010.

[9] BURGER, T.D.; SHAO, R.; BARKER, S.C. Phylogenetic analysis of mitochondrial genome sequences indicates that the cattle tick, *Rhipicephalus* (*Boophilus*) *microplus*, contains a cryptic species. **Mol**. **Phylogenet**. **Evol**. 76: 241-253. 2014.

[10] CAMPOS, R.N.; BACCI, L.; ARAÚJO, A.P.; BLANK, A.F.; ARRIGONI, B.M.; SANTOS, G.R.; RONER, M.N. Óleos essenciais de plantas medicinais e aromáticas no controle do garrapato *Rhipicephalus microplus*. **Arch**. **Zoot**. 61: 67-78. 2012.

[11] CORTES, V.J. Garrapatas: estado actual y perspectivas. **Biomed**. 31: 268-283. 2011.

[12] DA SILVA, L.A.; SOUSA, F.J.; GARCÍA, P.S.; PINHEIRO, G.G.; DA SILVA, S.L.; COSTA, J.L. Acaricide activity of different extracts from *Piper tuberculatum* fruits against *Rhipicephalus microplus*. **Parasitol**. **Res**. 113(1): 107-112. 2014.

[13] DA SILVA, M.A.; DAEMON, E.; DE OLIVEIRA, M.C.; MATURANO, R.; BRITO, F.C.; MASSONI, T. Acaricidal activity of thymol on larvae and nymphs of *Amblyomma cajennense* (Acari: Ixodidae). **Vet**. **Parasitol**. 183(1): 136-139. 2011.

[14] DANTAS, T.F.; CHOMEL, B.B.; OTRANTO, D. Ticks and tick-borne diseases: a One Health perspective. **Trends Parasitol**. 28(10): 437-446. 2012.

[15] DE CASTRO, J.J. Sustainable tick and tick-borne disease control in livestock improvement in developing countries. **Vet. Parasitol**. 71(2): 77-97. 1997.

[16] DE SANTANA, C.R.N.; LIMA, C.B.N.; OLIVEIRA, A.P.; ARAÚJO, A.P.A.; BLANK, A.F.; ALVES, P.B.; BACCI, L. Acaricidal properties of vetiver essential oil from *Chrysopogon zizanioides* (Poaceae) against the tick species *Amblyomma cajennense* and *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae). **Vet**. **Parasitol**. 212(3): 324-330. 2015.

[17] DOMÍNGUEZ, G.D.; ROSARIO, C.R.; ALMAZÁN, G.C.; SALTIJERAL, O.J.; DE LA FUENTE, J. *Boophilus microplus*: Aspectos biológicos y moleculares de la resistencia a los acaricidas y su impacto en la salud animal. **Trop. Subtrop. Agroecosyst**. 12: 181-192. 2010.

[18] DRUMMOND, R.O.; ERNST, S.E.; TREVINO, J.L.; GLADNEY, W.J.; GRAHAM, O.H. *Boophilus annulatus* and *B. microplus*: laboratory tests of insecticides. **J. Econ. Entomol.** 66(1):130-133. 1973.

[19] ESTRADA, P.A.; TARRAGONA, E.L.; VESCO, U.; DE MENEGHI, D.; MASTROPAOLO, M.; MANGOLD, A.J.; NAVA, S. Divergent environmental preferences and areas of sympatry of tick species in the *Amblyomma cajennense* complex (Ixodidae). **Int. J. Parasitol**. 44(14): 1081-1089. 2014.

[20] FERNANDES, S.S.; FERREIRA, B.L.; SOUSA, B.R.; LOPES, F.R.; BRAZ, L.C.; FAUSTINO, T.L.; REALINO, P.J.; HENRIQUE, F.P. Repellent activity of plant-derived compounds against *Amblyomma cajennense* (Acari: Ixodidae) nymphs. **Vet. Parasitol**. 167(1): 67-73. 2010.

[21] FERNÁNDEZ, E.; BETTENCOURT, V. Entomopatogenic fungi against South American tick species. **Exp. Appl. Acarol**. 46(1-4): 71 - 93. 2008.

[22] FERREIRA, M.M.; MORE, S.J. Plant-based insect repellents: a review of their efficacy, development and testing. **Malaria J**. 10(1): S11. 2011.

[23] GOMES, G.A.; MONTEIRO, C.M.; JULIAO, L.S.; MATURANO, R.; SENRA, T.O.; ZERINGÓTA, V.; CALMON, F.; MATOS, R.S.; DAEMON, E.; CARVALHO, M.G. Acaricidal activity of assential oil from *Lippia sidoides* on unengorged larvae and nymphs of *Rhipicephalus sanguineus* (Acari: Ixodidae) and *Amblyomma cajennense* (Acari: Ixodidae). **Exp. Parasitol**. 137: 41-45. 2014.

[24] GONZÁLEZ, C.A.; REINA, M.; DIAZ, C.E.; FRAGA, B.M.; SANTANA, M.O. Natural Product-Based Biopesticides for Insect Control. Reference Module in Chemistry. **Mol. Sci. Chem. Eng**. 3: 237-268. 2013.

[25] GONZÁLEZ, C.F.; BECERRIL, P.C.; TORRES, H.G.; DÍAZ, R.P.; SANTELLANO, E.E.; ROSENDO, P.A. Infestación natural por *Amblyomma cajennense* y *Boophilus microplus* en bovinos criollo lechero tropical durante la época de lluvias. **Agrocien.** 43: 577-584. 2009.

[26] GUERRERO, F.F.; LOVIS, L.; MARTINS, J.R. Acaricide resistance mechanisms in *Rhipicephalus* (*Boophilus*) *microplus.* **Rev. Bras. Parasitol. Vet.** 21(1): 1-6. 2012.

[27] INSTITUTO NACIONAL DE ESTADÍSTICA Y GEOGRAFÍA (INEGI). México. On Line: http://www.inegi.org.mx/geo/contenidos/topografía.html. 06/10/2013.

[28] IRIARTE, H.P.; AGUIRRE, O.J.; MARTÍNEZ, G.S.; GÓMEZ, D.A.; LOYA, O.L.; FERNÁNDEZ, R.M.; ULLOA, C.R. Repelencia de los pastos *Melinis minutiflora, Andropogon gayanus, Brachiaria brizantha y Cenchrus ciliaris* sobre larvas de garrapata *Amblyomma cajennense* F. (Acari:Ixodidae). **Biocien.** 2(3): 140-147. 2013.

[29] LINARES, V.S. Manejo integral de las garrapatas una propuesta eficiente y sostenible con el medio ambiente. **Agron.** 16(2): 13-21. 2008.

[30] MAGANO, S.R.; THEMBO, K.M.; NDLOVU, S.M.; MAKHUBELA, N.F. The anti-tick properties of the root extracts of *Senna italic* subsp. *Arachoides.* **Afr. J. Biotechnol**. 7(4): 476-481. 2008.

[31] MANJUNATHACHAR, H.V.; SARAVANAN, B.C.; KESAVAN, M.; KARTHIK, K,; RATHOD, P.; GOPI, M.; BALARAJU, B.L. Economic importance of ticks and their effective control strategies. **Asian Pac. J. Trop. Dis**. 4: S770-S779. 2014.

[32] MAPHOLI, N.O.; MARUFU, M.C.; MAIWASHE, A.; BANGA, C.B.; MUCHENJE, V.; MACNEIL, M.D.; DZAMA, K. Towards a genomics approach to tick (Acari: Ixodidae) control in cattle: a review. **Ticks Tick Borne Dis**. 5(5): 475-483. 2014.

[33] MCGARRY, J.W. Travel and disease vector ticks. **Travel Med. Infec. D**. 9(2): 49-59. 2011.

[34] MENDOZA, M.; DARY, L.; TABORDA, M. Composición química y actividad acaricida del aceite esencial de *Cymbopongon citratus* Stapf contra el ácaro intradomiciliario *Dermatophagoides farinae* (Acari: Pyroglyphidae). **Biosalud.** 9: 21-31. 2010.

[35] MENG, H.; LI, A.Y.; JUNIOR, L.M.C.; CASTRO, A.I.; LIU, J. Evaluation of DEET and eight essential oils for repellency against nymphs of the lone star tick, *Amblyomma americanum* (Acari: Ixodidae). **Exp. Appl. Acarol**. 68(2): 241-249. 2016.

[36] MURO, F.J.; CRUZ, V.C.; FERNÁNDEZ, R.M.; MOLINA, T.J. Efecto repelente de extractos de *Melinis minutiflora* sobre larvas de la *Boophilus microplus*. **Vet**. **Méx**. 35(2): 153-159. 2004.

[37] NERIO, L.S.; OLIVERO, V.J.; STASHENKO, E. Repellent activity of essential oils: a review. **Biores**. **Tech**. 101(1): 372-378. 2010.

[38] PÉREZ, S.; PATIÑO, T. Validación en Campo del Efecto de *Beauveria bassiana* (Balsamo) Vuillelim y *Metarhizium anisopliae* (Metsch.) Sorokin como Agentes Reguladores Biológicos sobre *Boophilus microplus* Canestreni y *Amblyomma cajennense* Fabricius (Arachnida: Ixodidae). In: Murgueitio, RE: Cuartas, CC: Naranjo, RJ. (Eds). **Ganadería del futuro, Investigación para el desarrollo.** 2da. Ed. Fundación CIPAV. Cali, Colombia. Pp 351-465. 2009.

[39] POLO PLUS PROBIT AND LOGIT ANALYSIS. User´s Guide. Pacific Southwest Forest and Range Experiment Station, Forest Service, U.S. Department of Agriculture, Berkeley. California. Version 2.0. 2002.

[40] RAVINDRAN, R.; RAMANKUTTI, S.A.; JULIET, S.; THUMADATHP, A.; GOPI, J.; KARAPPARAMBU, G.A.; NARAYANA, N.S.; GHOSH, S. Comparison of *in vitro* acaricidal effects of commercial preparations of cypermethrin and fenvalerate against *Rhipicephalus* (*Boophilus*) *annulatus.* **Springer Plus**. 3(1): 90. 2014.

[41] RESENDE, J.D.; DAEMON, E.; DE OLIVEIRA, M.C.; MATURANO, R.; DE AZEVEDO, P.M.; RODRIGUES, A.F. Toxicity of solvents and surfactants to *Amblyomma cajennense* (Fabricius, 1787) (Acari: Ixodidae) and *Dermacentor rnitens* (Neumann, 1897) (Acari: Ixodidae) larvae. **Exp. Parasitol**. 131(2): 139-142. 2012.

[42] SENRA, T.O.S.; CALMON, F.; ZERINGÓTA, V.; MONTEIRO, C.M.; MATURANO, R.; DA SILVA, M.R.; DAEMON, E. Investigation of activity of monoterpenes and phenylpropanoids against immature stages of *Amblyomma cajennense* and *Rhipicephalus sanguineus* (Acari: Ixodidae). **Parasit. Res**. 112(10): 3471-3476. 2013.

[43] SOARES, S.F.; BORGES, L.M.; DE SOUSA, B.R; FERREIRA, L.L.; LOULY, C.C.; TRESVENZOL, L.M.; FERRI, P.H. Repellent activity of plant-derived compounds against *Amblyomma cajennense* (Acari: Ixodidae) nymphs. **Vet. Parasitol**. 167(1): 67-73. 2010

[44] SOBERANES, C.N.; SANTAMARÍA, V.M.; FRAGOSO, S.H.; GARCÍA, V.Z. Primer caso de resistencia al amitraz en la garrapata del ganado *Boophilus microplus* en México. **Téc. Pec. Méx**. 40(1): 81-92. 2002.

[45] STATISTICAL ANALYSIS SYSTEM INSTITUTE (SAS). Version 9.0. 2002.

[46] TARRAGONA, E.L.; CICUTTIN, G.L.; MANGOLD, A.J.; MASTROPAOLO, M.; DE SALVO, M.N.; NAVA, S. Rickettsia infection in *Amblyomma tonelliae*, a tick species from the *Amblyomma cajennense* complex. **Ticks Tick Borne Dis.** 6(2): 173-177. 2015.

[47] VILLAR, M.; POPARA, M.; MANGOLD, A.J.; DE LA FUENTE, J. Comparative proteomics for the characterization of the most relevant *Amblyomma* tick species as vectors of zoonotic pathogens worldwide. **J. Proteom**. 105: 204-216. 2014.