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Acaricidal activity of cashew nut shell liquid associated with essential oils from *Cordia verbenacea* and *Psidium guajava* on *Rhipicephalus microplus*

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ABSTRACT

The aim of this study was to assess the *effectiveness of cashew nut shell liquid (CNSL),* Cordia verbenacea and *Psidium guajava*, both alone and in association against *Rhipicephalus microplus*. Larval packet and adult immersion tests were conducted in concentrations ranging from 3.1 at 100.0mg mL^{-1}. CNSL was effective against engorged females (99.6%) at 100.0mg mL^{-1} and against larvae (99.2 %) at 50.0mg mL^{-1}. The highest efficacy on engorged females was achieved by the essential oils of *P. guajava* at 12.5mg mL^{-1} (99.9%), followed by those of *C. verbenacea* at 25.0mg mL^{-1} (96.9%), while a low larvicidal activity of 5.8 and 59.0% was, respectively, obtained. The association at 50.0mg mL^{-1} had asignificant effect on both life stages of *R. microplus*. This association caused considerable larval mortality (95.3%) and high efficacy on engorged females (93.9%).

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1. Introduction

Rhipicephalus microplus stands out as one of the parasites that most impair scattle productivity, mainly due to reduction in meat and milk production. Annual losses due to this type of parasitism are estimated to be US$ 3.24 billion in Brazil (1). Control of this parasite has been achieved by intensive use of synthetic acaricides, which cause serious problems, such as selection of resistant ticks and soil and water pollution, as well as bioaccumulation of these chemicals in meat and milk (2,3). There is increasing resistance to available acaricides worldwide, which makes it urgent to develop new acaricidal products (2). An alternative is the development of formulations based on natural products, which reduce the risk of food and environment contamination. Natural products can be effective and to control resistant strains of ticks (4). One advantage of the use of plant oils is that the development of resistance can be slower than normally occurs when single compounds are used. This is because of the inherent complexity of plant-derived oils (5). The richness of native species and traditional knowledge of rural populations makes Brazil an important source for the discovery of new molecules with pharmacological potential (6).

Anacardium occidentale L. (Anacardiaceae), the cashew tree, is a tropical species native to Northeast Brazil where its cultivation is one of the most important agricultural activities (7). The main product from this plant is the fruit, known as the cashew nut, which is marketed and consumed all over the world. The processing of the cashew nut also produces a dark resin, known technically as cashew nut shell liquid (CNSL), a by-product of cashew agribusiness. CNSL is a potential natural insecticide (8), but can cause injury when in contact with the skin (9).

Aromatic plants produce secondary metabolites such as essential oils, complex mixtures of volatile and lipohilic compounds characterized by a strong aroma and biologically active properties (10). *Cordia verbenacea* DC. (Boraginaceae) is a shrubby species found widely in the coastal regions of Brazil (11). In folk medicine, its aerial parts are used mainly as antimicrobial, anti-inflammatory and analgesic agents (12), and insecticidal potential (13).

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Psidium guajava L. (Myrtaceae) is another aromatic plant native to tropical America (14). The leaves of *P. guajava* are traditionally used for treatment of diarrhea (6). The leaf extract and essential oil of *P. guajava* showed strong activity against rat cestodes (15).

The low toxicity of the essential oils from *C. verbenacea* and *P. guajava* in mammals (12,15) and the possibility of synergistic effects with CNSL have the potential to make a mixture of these oils an efficient and low-cost approach for control of *R. microplus*.

The aim of this study was therefore to investigate the acaricidal activity of an association of CNSL and essential oils isolated from *C. verbenacea* and *P. guajava* on engorged females and larvae of *R. microplus*, and correlate the size of the effect with the chemical composition of the mixture.

2. Material and methods

2.1. Plant material

Cordia verbenacea was collected at Fazenda Tabuleiros II, a property belonging to the company Anidro do Brasil Extrações S.A. (03°01′41″ S, 41°44′56″ W) and *Psidium guajava* was harvested from local farmers at Tabuleiros Litorâneos (03°01′18″ S, 41°46′92″ W), in the municipality of Parnaíba, Piauí state, Brazil. Voucher specimens were deposited in the Herbarium (CEN) of the *Embrapa Recursos Genéticos e Biotecnologia*, under registration numbers 81.102 and 81.100, respectively. CNSL was purchased from Eurocaju Company (Altos, Piauí, Brazil). The essential oils were obtained by applying the technique of distillation by water vapor drag in a Linax® essential oil extract model D20 (16). Nearly 20 kg of fresh leaves of *C. verbenacea* and *P. guajava* were extracted during 3 h and the yields obtained were 0.12% and 0.15%, respectively. The essential oils were stored at 4°C prior to testing and analysis.

2.2. CNSL analysis

CNSL was quantified by high performance liquid chromatography (HPLC) using a recently developed method (17). The samples were solubilized in methanol at concentration 1 mg mL^{-1}, filtered in filter disc PTFE 45 µm and injected on HPLC. The analyses were performed on a LC-20AB pump system coupled to a diode array detector SPD-M20A (Shimadzu) and equipped with a reverse-phase column CLC–ODS(M) C_{18} (150 × 4.6 mm × 5 µm). The mobile phase used consisted of acetonitrile, water and acetic acid in the proportions 80:20:1, respectively. The separations were carried out isocratically at a flow of 1.5 mL min^{-1} in 30 min, 30°C and an injection volume of 20 µL. Chromatograms were recorded over a range from 200 to 400 nm and monitored at a UV wavelength of 280 nm. Anacardic acids and cardanols were determined from the calibration curve of an external standard.

2.3. Essential oil analysis

The essential oils were analyzed by Gas Chromatography Mass Spectroscopy (GC-MS) and Gas Chromatography with a Flame Ionization Detector (GC-FID) to determine their chemical composition according to chromatographic method described earlier (18). GC–MS analysis was performed on a Varian 450-GC/240-MS instrument equipped with a non-polar VF-5MS fused silica capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness), using helium as carrier gas and a flow rate of 1.5 mL/min, with a split ratio of 1:30. The injector temperature and detector temperature were set at 250°C. The oven temperature was programmed to increase from 70 to 180°C at 4°C/min, and afterwards to 250°C at 10°C/min. Mass spectra was recorded in a range of mass-to-charge ratio (*m/z*) between 30 and 450. GC-FID analysis was carried out on a Shimadzu GC-2010 Plus chromatograph under the same chromatographic conditions employed for the GC–MS analysis, except for the carrier gas (hydrogen). The retention indices were determined by the injection of a mixture of C7–C30 homologous *n*-alkanes (Sigma–Aldrich, St. Louis, Missouri, USA). The volatile compounds were identified by comparison of the mass spectra recorded with those provided by the spectrometer database (NIST – 147,198 compounds), as well as matching the retention indices and mass spectra with data from literature (19). The relative content of oil constituents was determined by the peak area normalization method and expressed as percentages.

2.4. Preparation of the dilutions

For each essential oil and CNSL, solutions were prepared at concentrations of 6.2, 12.5, 25.0, 50.0 and 100.0 mg mL^{-1}. The essential oils are freely soluble in CNSL due to the nonpolar chemical characteristics of both. For the association, solutions were prepared with 25.0 mg mL^{-1} of CNSL and *C. verbenacea* and *P. guajava* essential oils at concentrations of 3.1, 6.2, 12.5, 25.0 and 50.0 mg mL^{-1}. The negative control consisted of ultrapure water and 50% ethanol +1% DMSO. A mixture of cypermetrin (0.18 mg mL^{-1}), chlorpyrifos (0.30 mg mL^{-1}) and citronellal (0.012 mg mL^{-1}) diluted to a 0.125% concentration in ultrapure water was used as positive control. Each treatment contained three replicates.

2.5. Preparation of ticks

Engorged *R. microplus* females were collected in February 2013 from naturally infested cattle. The ticks were washed with water and dried with paper towels. These females were incubated at 27°C and 80% relative humidity (RH) until oviposition was finished. Previously, acaricide tests had demonstrated resistance of this tick population to amidinic and pyrethroid compounds. This study was approved by the Ethics Committee for Animal Experimentation of the Universidade Federal do Maranhão (23115018061).

2.6. Adult immersion test

The adult immersion test was performed as described by literature (20). After selection of the ticks based on their mobility, body integrity and size (≥4.5 mm), engorged females were weighed to obtain groups with weights ranging from 170 to 210 mg. Each tick group was dipped for five minutes in solutions containing the various treatments, and then dried on a paper towel and stored in a biochemical oxygen demand (BOD) incubator at 27°C and RH > 80% for 18 days. After that period, the egg mass was weighed, transferred to adapted syringes, and incubated for 20 days (27°C and RH > 80%). Hatchability was estimated from the average numbers of eggs and larvae. The egg production index (EPI), oviposition reduction (OR), hatchability and efficiency were calculated according to literature (20,21).

2.7. Larval packet test

The larval packet test was performed according to literature (22) and modified by Food and Agriculture Organization of the United Nations (23). Approximately 100 larvae, 14–21 days old, were placed between two filter papers (2 × 2 cm) impregnated with the appropriate essential oil and concentration, to form a sandwich. Each 'sandwich' was placed in a filter paper envelope and then sealed, identified and incubated at 27° C with RH ≥ 80% for 24 h (24). Living and dead larvae were counted 24 h later, and mortality was calculated from the arithmetic average of three replicates.

2.8. Statistical analysis

The lethal concentration of CNSL and essential oils for 50% of the population (LC50) of larvae and engorged females was calculated by Probit analysis with GraphPad Prism 6.0 software. Formulations were considered significantly different when the 95% confidence intervals of LC50 did not overlap (25). The differences among the concentrations of mortality against larvae, EPI, OR, hatchability and efficiency were analyzed by the *F* test of ANOVA followed by Tukey test ($p < 0.05$).

3. Results and discussion

The main components of CNSL used in this study were Cardanol (73.7%) and Cardol (9.0%), in addition to other non-identified compounds (17.3%). This is a typical composition from technical CNSL. Cardanol is produced by the decarboxylation of anacardic acid during CNSL heating (26). Cardanol and Cardol has known larvicidal activity against *Aedes aegypti* (27), but there are no studies show inactivity of these compounds on ticks.

The chemical composition of the essential oils from *C. verbenacea* and *P. guajava* leaves, along with the retention indices and percentages are shown in Table 1 and the chromatographic profile are shown in Figure 1. Thirty-three and 30 components were identified, respectively, in the *C. verbenacea* and *P. guajava* essential oils. The most abundant compounds in *C. verbenacea* essential oils were the monoterpene α-pinene (49.0%), and the sesquierpenes β-caryophyllene (12.4%) and alloaromadendrene (5.3%), while in *P. guajava* essential oils the three most abundant constituents were sesquiterpenes: β-caryophyllene (39.0%), β-selinene (9.7%) and α-selinene (9.7%). α-Pinene has been reported in other oils and plant species effective against *R. microplus* (28). It is also the major compound from *Tithonia diversifolia* oil (63.6%), which has been used traditionally for the control of *R. appendiculatus* by the Bukusu community (29). β-caryophyllene, which was present in both essential oils studied, was found earlier in the essential oil of *Lippia sidoides*, with high larvicidal activity on *R. microplus* (30) and β-selinene, one of the compounds of *P. guajava*, have larvicidal effect against *A. aegypti* (31).

CNSL was effective against engorged females (99.6%) at 100 mg $mL^{-1}$and led to larvae mortality (99.2%) at 50 mg mL^{-1}. The highest efficacy on females was achieved by the essential oils of *P. guajava* at 12.5 mg mL^{-1} (99.9%), followed by *C. verbenacea* at 25.0 mg mL^{-1} (96.9%). The effect of *P. guajava* essential oils on the reproduction of the engorged females, even at concentrations as low as 12.5 mg mL^{-1}, resulted from significant ($p < 0.05$) egg production (1.5%), reduction of oviposition (97.2%) and reduced hatchability (0.6%) of eggs (Table 2). In turn, *C. verbenacea* essential oils at 25 mg mL^{-1} caused significant ($p < 0.01$) egg production (15.9%), reduction of oviposition (71.8%) and lowered hatchability (12.3%) (Table 2). On the other hand, a low larvicidal activity was observed for *P. guajava* (11.8%), and *C. verbenacea*

Table 1. Chemical composition of the essential oils from *Cordia verbenacea* and *Psidium guajava.*

Class	Peak	Compounds	RI[a]	RI_{lit}[b]	Essential oils	
					Cordia verbenacea (%)[c]	*Psidium guajava* (%)[c]
Monoterpenes Hydrocarbons	1	α-thujene	934	928	1.7	-
	2	α-pinene	942	936	49.0	0.4
	3	Sabinene	982	973	1.0	-
	4	β-pinene	989	978	1.4	-
	5	β-myrcene	996	989	0.6	0.2
	6	Limonene	1035	1029	0.6	1.0
	8	*(E)*-β-ocimene	1050	1048	-	0.3
	9	γ-terpinene	1064	1060	-	0.2
Monoterpenes Oxygenated	10	α-terpineol	1199	1190	-	0.5
	7	Eucalyptol	1040	1032	0.9	6.9
	11	Bornyl acetate	1294	1283	0.5	-
	13	Citronellyl acetate	1356	1352	0.4	-
Sesquiterpenes Hydrocarbons	12	δ-elemene	1346	1337	2.0	-
	14	α-copaene	1386	1376	0.4	3.9
	15	β-bourbonene	1394	1384	0.1	-
	16	β-elemene	1401	1390	2.3	-
	18	Sesquithujene	1412	1405	0.8	-
	17	α-gurgujene	1422	1409	-	0.2
	19	α-*cis*-bergamotene	1424	1414	0.8	-
	20	β-caryophyllene	1433	1420	12.4	39.0
	21	β-gurgujene	1445	1431	0.7	-
	22	α-*trans*-bergamotene	1448	1434	0.1	-
	23	Aromadendrene	1452	1441	-	6.3
	24	*cis*-β-farnesene	1460	1446	0.1	
	25	α- caryophyllene	1464	1453	1.2	4.0
	26	*trans*-β-farnesene	1467	1456	2.7	-
	27	Allo-aromadendrene	1474	1460	5.3	1.0
	28	*trans*-cadina-1 (6),4-diene	1487	1476	-	1.6
	29	γ-muuroleno	1487	1476	0.3	-
	31	Germacrene D	1494	1481	1.8	-
	30	β-selinene	1499	1486	-	9.7
	32	α-selinene	1506	1493	-	9.7
	33	Bicyclogermacrene	1508	1494	1.6	-
	34	α-muurolene	1512	1498	-	0.0
	35	Germacrene A	1516	1502	1.7	-
	36	γ-cadinene	1526	1513	-	0.5
	37	δ-cadinene	1534	1523	1.0	2.0
	42	*trans*-γ-bisabolene	1541	1533	0.4	-
	38	α-calacorene	1553	1540	-	0.1
	39	Germacrene B	1564	1551	-	1.5
Sesquiterpens Oxygenated	41	Cubebol	1529	1515	0.2	-
	40	*trans*-nerolidol	1574	1561	-	0.7
	44	Espatulenol	1589	1576	0.3	-
	50	β- caryophyllene oxide	1594	1581	1.1	-
	43	Globulol	1595	1582	-	4.3
	45	Viridiflorol	1604	1591	-	0.3
	47	Guaiol	1610	1597	-	0.3
	46	Epoxi-allo-aromadendrene	1653	1641	-	1.9
	48	α-muurolol	1656	1643	-	0.5
	49	Pogostol	1687	1653	-	2.2
	51	*cis*-α-santalol			1.1	
		Total			94.5	99.2

[a]RI: Kovats index displayed by compounds in a RTX column; [b]RI_{lit}: Literature data (Adams, 2007; [c]Peak relative areas determined in the GC-FID chromatogram.

(83.3%) essential oils, even in concentrations of 100.0 mg mL^{-1} (Table 3). Although *P. guajava* and *C. verbenacea* essential oils provided the best results on engorged females, their larvicidal actions were the worst (Table 4), compromising the effectivity of these oils in the control of *R. microplus* in cattle. As the cattle are infested by ticks at different life stages, the best compound would be that which is effective against all of them (30). The activity of the essential oils increased remarkably, when the target was engorged females. This may suggest that the effect of these oils is greater on the reproductive system. Extracts from plants can harm germ cells by decreasing their capacity to produce viable eggs and form new individuals (32). With the available methods for evaluation of larvae, the effect on the reproductive system is not verified, only the mortality. Greater activity on engorged females, when compared with those on larvae, was also verified by the action of the extract of *Pilocarpus microphyllus* and (33) of the essential oil of *Maesosphaerum suaveolens* (34).

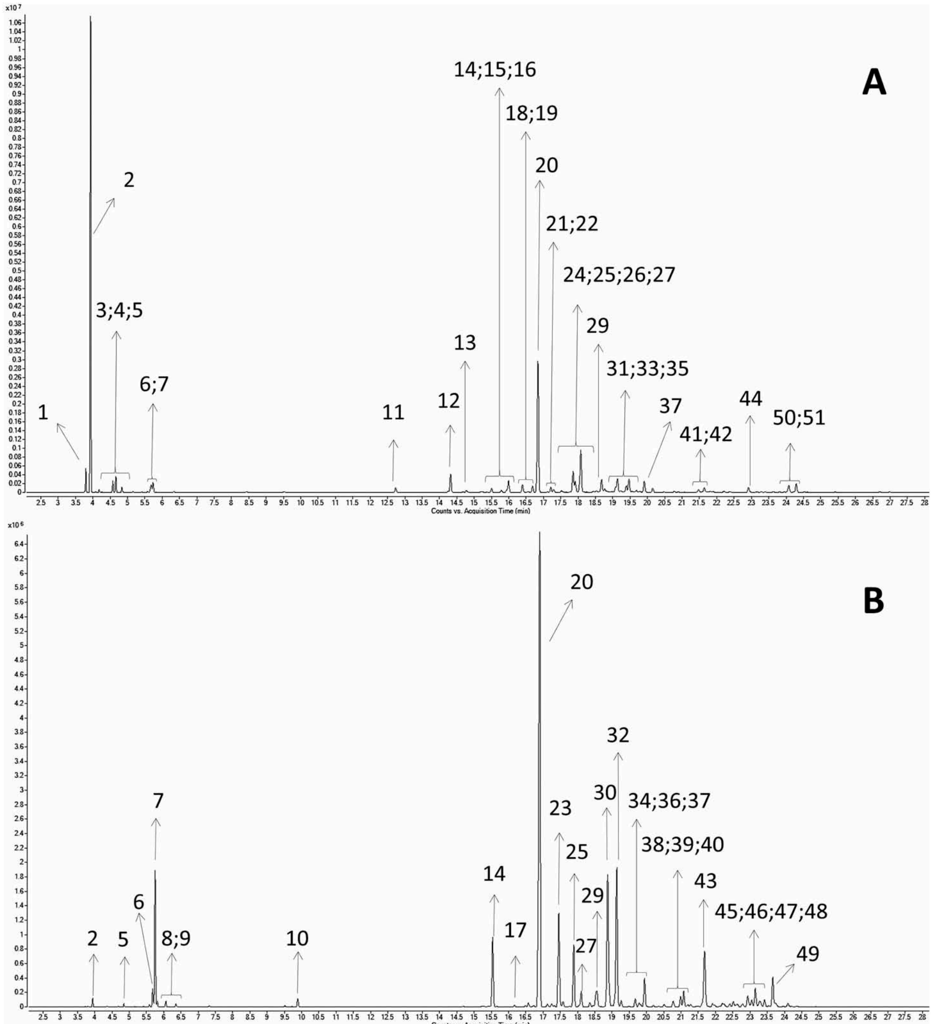

Figure 1. Chromatograms (TIC) of essential oils of *Cordia verbenaceae* (A) and *Psidium guajava* (B).

CNSL showed efficacy on both engorged female and larvae, but it has the practical disadvantage in causing contact dermatitis if skin is directly exposed to it (9). To avoid this problem an association between CNSL and the two essentials oils was prepared. This may reduce the CNSL concentration and consequently its caustic action when in contact with skin. We found that CNSL at a concentration below 25.0 mg mL^{-1} associated with *C. verbenacea* and *P. guajava* essential oils at 50.0 mg mL^{-1}had a significant effect ($p < 0.05$) on both life stages of *R. microplus*. In this concentration, the association caused egg production of 23.7%, reduction of oviposition (58.1%), low hatchability of eggs (13.2%) and consequently high efficacy on adults (93.9%), besides high larval mortality (95.3%) (Tables 2 and 3). The synergistic effect was demonstrated only on *R. microplus* larvae, since the efficiency on engorged females was high even at concentrations as low as 12.5 and 25.0 mg mL^{-1} for the respective essential oils of *P. guajava* and *C. verbenacea*. This synergism can be observed by comparing the results of high larval mortality caused by association at 50.0 mg mL^{-1} (95.3%) with those determined by CNSL at 25.0 mg mL^{-1} (66.9%) and essential oils of *C. verbenacea* (75.8%) and *P. guajava* (6.6%) at 50.0 mg mL^{-1} (Table 3).

The associations are a useful alternative in the control of bovine ticks. Santos et al. (35) associated *Cymbopogon nardus* (citronella), *Chenopodium ambrosioides* (Santa Maria herb) with *Quassia amara* (quassia) tincture and obtained high *in vitro* efficiency on engorged females of *R. microplus*, however, this formulation was not evaluated against larvae.

Low toxicity of *C. verbenacea* leaf extract was demonstrated when administered topically and orally in rats (12). In addition, an acute toxicity study in rats concluded that leaf extracts of *P. guajava* are non-toxic (15). Thus, the association of the essential oils of this species with lower concentrations of CNSL facilitates the development of formulations against *R. microplus*, which can be used for topical application in cattle. Nevertheless, it is still

Table 2. Activity of cashew nut shell liquid (CNSL) and essentials oils from *Cordia verbenacea* and *Psidium guajava* isolated and in association* on engorged female (egg production index, reduction of oviposition, hatchability and efficiency) of *Rhipicephalus microplus.*

Treatment	Concentration (mg mL^{-1})	Egg Production Index (%)	Reduction of oviposition (%)	Hatchability (%)	Efficiency (%)
CNSL	6.2	52.7 ± 2.9a	8.8 ± 5.0a	92.0 ± 5.3a	13.2 ± 6.4a
	12.5	48.3 ± 3.0a	16.5 ± 5.2b	76.7 ± 1.8b	33.8 ± 4.1a,b
	25.0	39.5 ± 1.1bA	31.6 ± 1.8cA	66.8 ± 13.0bA	52.6 ± 10.4bA
	50.0	24.1 ± 3.6b	58.2 ± 6.2d	19.8 ± 8.0c	91.4 ± 3.8c
	100.0	4.0 ± 2.1b	93.1 ± 3.6d	3.8 ± 6.5c	99.6 ± 0.7c
C. verbenacea	6.2	42.6 ± 5.9a	24.5 ± 10.5a	79.9 ± 9.4a	36.9 ± 11.1a
	12.5	21.8 ± 15.1a,b	61.3 ± 26.8b	44.5 ± 14.5b	79.5 ± 16.0b
	25.0	15.9 ± 18.5a,b	71.8 ± 32.8c	12.3 ± 7.6b	96.9 ± 2.9b
	50.0	0.2 ± 0.4b	99.6 ± 0.7c	1.5 ± 2.6b	100.0 ± 0.0b
	100.0	0.3 ± 0.5b	99.5 ± 0.9c	0.0 ± 0.0b	100.0 ± 0.0b
P. guajava	6.2	46.7 ± 5.4a	13.2 ± 10.0a	85.1 ± 2.8a	12.4 ± 7.4a
	12.5	1.5 ± 2.6b	97.2 ± 4.8b	0.6 ± 1.0b	99.9 ± 0.1b
	25.0	0.0 ± 0.0b	100.0 ± 0.0b	0.0 ± 0.0b	100.0 ± 0.0b
	50.0	1.8 ± 3.1b	96.6 ± 5.8b	3.1 ± 5.4b	99.6 ± 0.6b
	100.0	0.4 ± 0.7b	99.2 ± 1.4b	1.9 ± 3.3b	99.9 ± 0.1b
Association*	3.1	31.8 ± 3.3a,bA	29.3 ± 5.7aA	50.2 ± 5.7aA	70.4 ± 5.9aA
	6.2	25.3 ± 3.6a,bB	55.3 ± 6.4bB	22.9 ± 9.6bB	89.6 ± 3.9aA
	12.5	23.5 ± 2.8aB	58.5 ± 5.0bB	20.5 ± 2.4bB	91.2 ± 0.5bB
	25.0	35.6 ± 5.0bA	37.0 ± 8.9bA	30.4 ± 10.9bA	80.4 ± 5.4aA
	50.0	23.7 ± 6.3aB	58.1 ± 11.2bB	13.2 ± 7.3bB	93.9 ± 3.9bB
Positive control	-	5.3 ± 2.0	99.2 ± 0.4	0.0 ± 0.0	100.0 ± 0.0

Positive control = 0.18 mg mL^{-1} cypermetrin, 0.30 mg mL^{-1} chlorpyrifos, and 0.012 mg mL^{-1} citronellal. *Association of CNSL at 25 mg mL^{-1} + different concentrations of *C. verbenacea* and *P. guajava* essential oils. Different lower case letters showed statistically significant differences ($p < 0.05$) among concentrations of the same essential oil. Different upper case letters showed statistically significant difference ($p < 0.05$) between each concentration of associations with 25 mg mL^{-1}CNSL.

Table 3. Activity of cashew nut shell liquid (CNSL) and essentials oils from*Cordia verbenacea* and *Psidium guajava*isolated and in association* on larvae mortality of *Rhipicephalus microplus.*

Concentration (mg mL^{-1})	CNSL (%)	*C. verbenacea* (%)	*P. guajava* (%)	Association* (%)
3.1	-	-	-	67.7 ± 6.4aA
6.2	5.2 ± 4.7a	0.0 ± 0.0a	4.9 ± 4.6a	68.7 ± 9.9aA
12.5	50.2 ± 9.8b	16.7 ± 10.1a	5.8 ± 5.0a	99.5 ± 0.9bB
25.0	66.9 ± 15.0bA	59.0 ± 8.7b	9.8 ± 4.2a	80.4 ± 8.1aA
50.0	99.2 ± 1.4c	75.8 ± 14.2c	6.6 ± 1.9a	95.3 ± 0.9bB
100.0	100.0 ± 0.0c	83.3 ± 11.9d	11.8 ± 4.8a	-

*Association of CNSL at 25 mg mL^{-1} + different concentrations of *C. verbenacea* and *P. guajava* essential oils. Different lower case letters showed statistically significant differences ($p < 0.05$) among concentrations of the same essential oil. Different upper case letters showed statistically significant difference ($p < 0.05$) between each concentration of associations with 25 mg mL^{-1} CNSL.

Table 4. Lethal concentration of the essential oils from *Cordia verbenacea* and *Psidium guajava* and of the cashew nut shell liquid (CNSL) and Association* against *Rhipicephalus microplus* engorged females and larvae.

Treatment	LC_{50} (mg mL^{-1})	CL 95%	R^2
Engorged female			
(CNSL)	19.6b	16.8–22.8	0.95
Cordia verbenacea	7.5a	6.6–8.5	0.91
Psidium guajava	12.3b	10.7–18.2	0.97
Association*	ND	ND	ND
Larvae			
(CNSL)	14.7b	12.2–17.8	0.92
Cordia verbenacea	25.1c	20.1–31.8	0.89
Psidium guajava	>100.0d	-	-
Association*	1.00a	0.07–2.30	0.74

LC_{50} = Lethal concentration 50% (mg mL^{-1}) *R. microplus* larvae or engorged females; CL = 95% confidence limits; R^2 = Regression coefficient.*Association of the cashew nut shell liquid (CNSL) at 25 mg mL^{-1} withessentials oils of *C. verbenacea* and *P. guajava*. Values with different letters in a column are significantly different. ND – Not determined.

necessary to evaluate the toxicity of CNSL and these essential oils on the skin of the cattle.

The results obtained by association on females (93.9%) and larvae (95.3%) are comparable with those obtained by positive control also on female and larvae (data not shown), where both reached 100%. It can be inferred that the association between CNSL, *C. verbenacea* and *P. guajava* essential oils is a promising acaricidal formulation for control of *R. microplus*.

Brazilian companies generate 45 thousand tons of CNSL per year as a byproduct of the processing of 360 thousand tons per year of cashew nuts (7). The use of residues generated industrially from abundant and renewable raw material is viewed very favorably nowadays, both because of the benefits to the environment and because of the reduction obtained in processing costs. Furthermore, *C. verbenacea* is a native species with an extensive distribution in Brazil, while *P. guajava* is a naturalized species, widely cultivated throughout the country (11,14). These features add to the potential for the use of these species in the development of an acaricide product.

This is the first report evaluating the effects on *R. microplus* of an association containing CNSL, and the essential oils of *C. verbenacea* and *P. guajava*.

4. Conclusion

CNSL showed high efficacy against engorged females and larvae of *R. microplus*. The highest efficacy on engorged female was obtained with the essential oils of *P. guajava*, followed by those of *C. verbenacea*, while the lowest larvicide activity was recorded using essential oils of *P. guajava*, followed by those of *C. verbenacea*. However, the association of CNSL and the essential oils of *C. verbenacea* and *P. guajava* obtained high efficiency *in vitro* on larvae, as well as on engorged females of *R. microplus*. It is necessary evaluated the toxicity of this association on cattle skin in order to ensure the practical effectiveness of this treatment.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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