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ACTION OF BOTANICAL AQUEOUS EXTRACT OF *Cymbopongon nardus* ON THE BIOLOGY OF *Rhipicephalus (Boophilus) microplus*

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ABSTRACT

The aim of this study was to evaluate the *in vitro* action of the botanical aqueous extract of *Cymbopogon nardus* (Citronella) on *Rhipicephalus* (*Boophilus*) *microplus* biology. Engorged female ticks were collected from bovines in the West's Mesoregion of Maranhão, Brazil. In the laboratory, the engorged female ticks passed through a trial procedure to be distributed in five groups (G) of ten, in triplicate, and immersed in a 50% citronella aqueous extract for 10 (G1), 20 (G2) and 40 (G3) minutes. Control groups were immersed in 15% Cypermethrin (G4) and distilled water (G5) for five minutes. They were placed in Petri dishes, kept in a moisture chamber (27±2ºC and 90±5%RH). Statistical analysis of biological parameters from non-parasitic stages followed the non-parametric method of KrusKall–Walls test and the median comparison by Dunn's test using 5% of probability level. Biological parameters such as female after oviposition mortality period (G3 4.86), larval longevity period (G1 96.03; G3 96.2), larval mortality period (G1 75.73; G3 75.56) and female posture rhythm were significant lower when compared to the control group G5. Citronella activity reduced the biological parameters of *Rhipicephalus* (*B.*) *microplus* non-parasitic stages.
KEYWORDS: acaricide, citronella, phytotherapic

AÇÃO DO EXTRATO BOTÂNICO AQUOSO DE *Cymbopongon nardus* NA BIOLOGIA DE *Rhipicephalus* (*Boophilus*) *microplus*

RESUMO

Objetivou-se avaliar a ação *in vitro* do extrato botânico aquoso (EBA) de *Cymbopogon nardus* Rendle (Citronela) sobre a biologia de *Rhipicephalus* (*Boophilus*) *microplus*. Fêmeas ingurgitadas foram colhidas em bovinos oriundos da Mesorregião Oeste do Maranhão, Brasil. No laboratório as fêmeas ingurgitadas passaram por uma triagem, distribuídas em cinco grupos (G) de 10, em triplicata, imersas em 10 (G1), 20 (G2) e 40 (G3) minutos no extrato de citronela a 50% e os controles em Cipermetrina 15% (G4) e água destilada (G5) por cinco minutos, acondicionadas em placas de Petri, mantidas em câmara úmida (27±2ºC e

90±5%UR). A análise estatística dos parâmetros da fase não-parasitária foi pelo método não paramétrico do teste de KrusKall-Walls e a comparação entre as médias pelo teste de Dunn ao nível de probabilidade de 5%. Os parâmetros biológicos como o Período de Mortalidade das fêmeas após postura (G3 4,86), Período de Sobrevivência das Larvas (G1 96,03; G3 96,2), Período de Mortalidade das Larvas (G1 75,73; G3 75,56) e Ritmo de postura das fêmeas dos grupos tratados com citronela foram significativamente inferiores quando comparados ao grupo controle G5. A citronela apresentou atividade de redução nos parâmetros biológicos da fase não parasitária de *Rhipicephalus* (*B*.) *microplus.*
PALAVRAS-CHAVE: acaricida, citronela, fitoterápico

INTRODUÇÃO

Rhipicephalus (Boophilus) microplus is an ectoparasite distributed between the parallels 30º S and 40º N, except in the United States of America, where it was eradicated, and in very high or arid areas (CORDOVÉS, 1996). Although is a typical bovine tick, other animals can be their hosts such as equines, ovines, caprines, canines and cervines (GONZÁLES, 1974) and also buffalo, cats, pigs, jaguars, sloths, kangaroos and rabbits (PEREIRA, 1980).

Brazil is a notable tropical country, with climate characteristics that are favorable to *R.* (*B.*) *microplus* development and survival on most months of the year (EVANS, 1992). Ticks are hard to control, and lead to a vast economic lose on milk exploitation, low feed conversion and weight gain. Tick infestation increases direct and indirect costs with treatment and preventive care on infectious parasitic diseases (VIDOTTO, 2002).

Indiscriminate and incorrect use of acaricides results in genetic information for future generations on how to survive to that product (FURLONG & PRATA, 2006). NOLAN (1990) said that the acaricide resistance observed in arthropods comes from genetic and depending on the involved species, can be characterized in many ways, such as a life cycle modification.

Plants have been an important source of substances with different chemical structures and a vast activity against arthropods (VIVAN, 2005). Thereby it is believed that the use of herbal extracts in an isolated or associated form can cause a slower development of the resistance. Another important factor is the reduction on the problem of residues, as well as its biodegradable characteristic (MORALES & GARCÍA, 2000, ROEL, 2001; IANNACONE & LAMAS, 2002).

Cymbogonon nardus has essential oil as its composition, high in geraniol and citronellal. Citronellal is used as a base material for the synthesis of important chemical compounds, called ionones and for vitamin A synthesis (CRAVEIRO et al., 1981). The essential oil of *C. nardus* is also used in perfumery and cosmetic industry, as insects repellent, fungicide and bactericide (BILLERBECK et al., 2001; TRONGTOKIT et al., 2005; WONG et al., 2005).

The aim of this study was to evaluate the *in vitro* action of botanical aqueous extract (BAE) of dehydrated citronella leaves (*C. nardus*) on the biological parameters of the *Rhipicephalus* (*B.*) *microplus* females and non-parasitic stages.

MATERIAL AND METHODS

Extraction of the vegetal material

The citronella leaves were harvested at the University's farm, located at Universidade Estadual do Maranhão – UEMA campus, in São Luís, Maranhão, Brazil

(02º 31’ 47” S 044º 18’ 10” W), the voucher specimen was registered under the number 1796 in the Herbarium Rosa Mochel/UEMA.

The leaves were dried in the shadow, in room temperature (27±2ºC and 70±10%RH), dehydrated in a dry heater at 60ºC and crushed in a mechanical crusher until powder was obtained. The preparation of the botanical aqueous extract (BEA) in a 50% concentration (g/mL), was trough hot infusion in distilled water (1:2), homogenized three times a day and a 72 hours rest, filtered in a nylon fabric at the moment of the *in vitro* test.

In vitro test

Tick engorged females were manually collected in naturally infected crossbreed cattle, from the West mesoregion of Maranhão (4º 43’ 15,44” S and 48º 28’ 36,11” W). The animals chosen for tick collection were the ones that were not submitted for chemical acaricides in the past three months. The collected engorged females of *Rhipicephalus* (*B.*) *microplus* were sorted, observing mobility, agility and/or trauma existence. After individually cleaning and weighing, in an analytical scale (precision of 0,0001g), they were distributed in five groups (G), in triplicates. Each replica contained ten females, according to the treatment used as described below.

The groups were submitted to the following treatments: immersion in 50% of citronella aqueous extract for 10, 20 and 40 minutes (G1, G2 and G3). Positive control was immersed in 15% Cypermethrin (G4) and the negative control was immersed in sterilized distilled water (G5), for 5 minutes both.

After the immersions, the females were dried with filter paper, placed in sterile Petri dishes, fixed in dorsal decubitus, numbered and placed in a moisture chamber (length x width x height = 35,0 x 28,0 x 7,0cm, respectively), containing 2000 mL of distilled water, intern temperature and relative humidity kept at 27±2ºC and 90±5%RH, respectively, daily measured using a hygrometer, over the laboratory counter.

After the eggs were laid, each female eggs mass (EM) was weighted, every three days, the EM were placed in an identified 15 mL test tube, closed with hydrofile cotton and replaced in its place of origin. The biological parameters evaluated were pre-oviposition period (POP), oviposition period (OP), incubation period (IP), hatching period (HP), percentage hatching larvae (%H-LL), engorged female initial weight (EFIW), eggs mass weight (EMW), reproduction efficiency index (IER%), nutritional efficiency index (IEN%), weight of female at end of oviposition (WFO) larval longevity period (PL-LL), female survival period (PS-F), female after oviposition mortality period (PM-F), larval mortality period (PM-LL) and posture regularity (PR).

Statistical analysis of parameters from non-parasitic stage followed the non-parametric method of KrusKall – Walls test and the median comparison by Dunn’s test using 5% of probability level.

RESULTS AND DISCUSSION

The mean period, in days, of POP and OP did not show a significant statistical difference between the groups treated with citronella and the control group (P>0,05) (Table 1). The mean variation for POP and OP results in this study, are in accordance with most of the authors who observed variations from 2 to 4 and 9 to 12 days, respectively, in studies with the species *Riphicephalus* (*B.*) *microplus* and *Dermacentor nitens* in different relative humidity and fixed temperature (95; 70; 50;

30% RH and 25 ± 2ºC, respectively) (HITCHCOCK, 1955; DESPINS, 1992; GUIMARÃES DA SILVA et al., 1997).

No alteration in the morphology of the eggs was observed during IP, except for G4, where there was a dehydrated parcel, also occurring a decrease in embryogenesis days (17.36 ± 10.73) when compared to the other groups, which had a statistical significance difference (P<0,05) (Table 1).

Larvae emergence in the citronella treated groups and G5 didn't show variation in the hatching period, with a mean above 6.16 ± 3.02 (P>0,05), which demonstrated no interference of the extract in that biological parameter. However; G4 showed a considerable decrease in the period, in days, of hatching period (3.76 ± 4.09) when compared to the other groups, probably because of the drug aggression suffered by the engorged female, during immersion, which differed from the other groups (P<0,05) (Table 1). Differently than the results found from the BAE – citronella treated groups, MARTINS (2006) confirmed larvicide and acaricide action when testing Java's citronella (*Cymbopogon winterianus*) oil, controlling the engorged females with posture and eggs hatching inhibition.

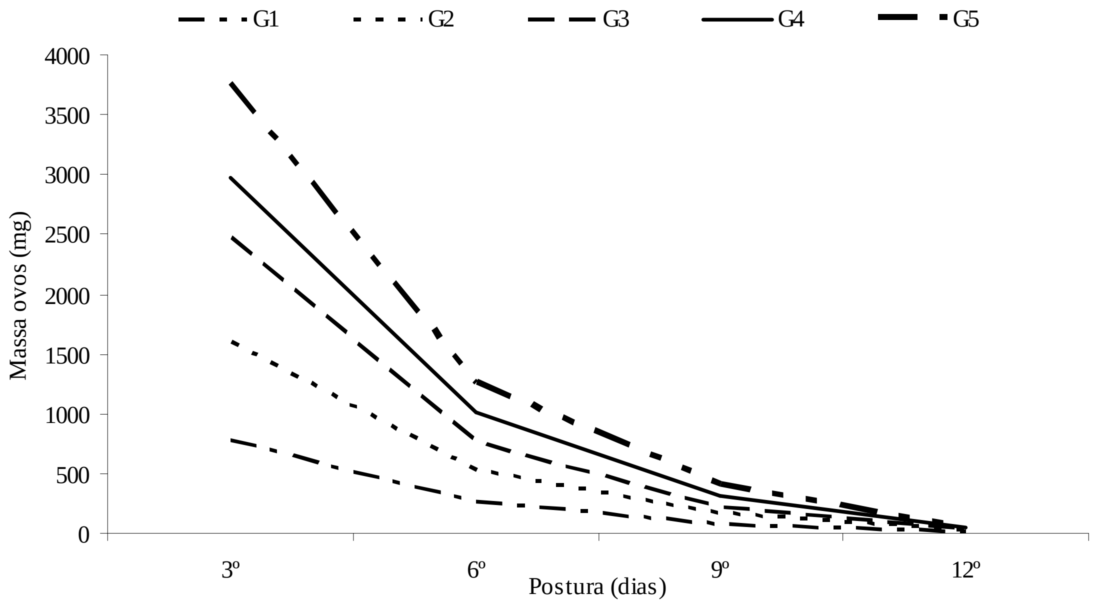
The extract didn't show a negative action on percentage hatching larvae in the different groups tested, when compared to G5; however G4 showed a 46% of mortality by the eggs dehydration, without embryo formation. Furthermore, when the results were statistically analyzed, a difference between G4 and the other groups was verified (P<0,05) (Table 1). This biological event always occur when ixodides had a previous contact with the active ingredient of the chemical drug, leading to generations of resistant strains of *Rhipicephalus* (*B.*) *microplus* (HITCHCOCK, 1955; FURLONG, 1996). As well as citronella, neem's emulsifiable oil used by BROGLIO-MICHELETTI et al. (2010) showed larvae hatch up to a 100%. OLIVO et al. (2013) evaluated the effect *in vitro* of the essential oil of *Crymbia citriodora* im engorged females of *Rhipicephalus* (*Boophilus*) *microplus* and observed the effect on hatching larvae at of 2% and there was no hatching at 5%.

TABLE 1. – Mean periods of the non–parasitic stages and hatching percentage of *Rhipicephalus* (*Boophilus*) *microplus* in the different immersion times in 50% of *Cymbopogon nardus* (Citronella) botanical aqueous extract under laboratorial conditions (27 ± 2ºC, 90 ± 5% RH)

Grupos	POP (dias) X ± DP (LI-LS)	OP (dias) X ± DP (LI-LS)	IP (dias) X ± DP (LI-LS)	HP (dias) X ± DP (LI-LS)	%H-LL X ± DP (LI-LS)
G1	2,8 ± 0,76a (9 - 3)	10,1 ± 3,29a (0 - 12)	21,63 ± 6,05a (0 - 25)	7,36 ± 1,82a (4 - 10)	92,53 ±25,23a (0 - 100)
G2	2,96 ± 0,18a (2 - 3)	10,8 ± 2,31a (3 - 12)	22,2 ± 0,40a (22 - 23)	6,16 ± 3,02a (0 - 11)	98,03 ± 9,09a (50 - 100)
G3	2,6 ± 1,03a (0 - 3)	9,7 ± 3,58a (0 - 12)	21,76 ± 4,28a (0 - 25)	7,66 ± 1,64a (4 - 9)	95,36 ± 18,21a (0 - 100)
G4	2,96 ± 0,18a (0 - 3)	9,3 ± 4,19a (0 - 12)	17,36 ± 10,73b (0 - 28)	3,76 ± 4,09b (0 - 9)	54,56 ± 42,20b (0 - 100)
G5	2,96 ± 0,18a (2 - 3)	10,3 ± 2,91a (3 - 12)	22,6 ± 0,89a (22 - 24)	7,46 ± 2,64a (0 - 10)	91,59 ± 17,59a (50 - 100)

Means with the same letters on the vertical didn't differ by KrusKall – Walls' test, followed by Dunn's test at 5%.

Females laid their eggs until the 21st day, with a peak on the third day, in all groups studied, with a decrease until the end of the posture (Graphic 1). This data was compatible to the registered in studies about the biological parameters of *Rhipicephalus* (*B.*) *microplus* non–parasitic stage by SNOWBALL (1957), BENNETT (1974) and different from HITCHCOCK (1955), that verified the peak on the fourth day.



GRAPH 1. – *In vitro* posture regularity of engorged females of *Rhipicephalus* (*Boophilus*) *microplus* treated with Citronella aqueous extract at 50% and the positive and negative control, under laboratorial conditions (27 ± 2ºC, 90 ± 5% RH)

The eggs mass weight of *Rhipicephalus* (*B.*) *microplus* is directly related to female nutrition during parasitic stage, which sometimes compromises eggs posture over 120mg (BENNETT, 1974). In this study the females had its medium weight within the parameters recommended by BENNETT (1974), therefore, the EMW between the citronella treated groups and G5, didn't show a statistical significance difference ($P>0,05$), only when compared to G4 ($P<0,05$), showing that G4 females, didn't have nutritional conversion mean above 85mg and/or the drug was deleterious to the egg production ($P<0,05$) (Table 2). BORGES et al. (2003) studying *in vitro Melia azedarach* (Meliaceae) in the concentration of 0,25% observed total inhibition of posture in engorged females when immersed in a crude extract of ripe fruits extract with different solvents. Besides that, high mortality rate of the larvae and on engorged females were verified.

The regularity of the posture of the engorged females immersed in citronella's BAE had lower mean on the eggs mass weight, when compared to the other groups, however the posture peak of the posture happened on the third day in all groups, being compatible with other studies done by HITCHCOCK (1915), SNOWBALL et al. (1957), LONDT (1997) and BENNETT et al. (1974). The decrease on the eggs mass weight on the citronella treated groups could probably characterize sensibility to the product on strain of *Rhipicephalus* (*B.*) *microplus* females. These data are in agreement with BROGLIO–MICHELETTI et al. (2010) which found out that the use of plants such as Neem's extract and oil related to the posture, showed a great potential to reduce the eggs' mass weight.

The rates of IER (%) and IEN (%) didn't show statistical difference within the citronella treated groups and G5 (P>0,05), but differed from G4 that had low rates (P<0,05), demonstrating the acaricide action of the chemical drug. Even though, the citronella treated groups showed a sensibility to the plant, because of the high weight of female at end of oviposition (mg), which didn't show statistical difference within themselves (P>0.05) (Table 2). COSTA et al. (2008) when testing the dehydrated hydroalcoholic botanical extract of 20% concentrated Neem (*A. indica*), Citronella's (*C. nardus*) 20% and Eucalyptus' (*Eucalyptus* spp.) 10%, respectively showed the efficiency of the product (EP%) of 32%, 17% and 96%.

TABLE 2. – Mean periods of female, eggs mass and reproductive and nutritional efficiency rate and final weight of females of *Rhipicephalus* (*Boophilus*) *microplus* in the different immersion times in 50% of *Cymbopogon nardus* (Citronella) botanical aqueous extract under laboratorial conditions (27 ± 2ºC, 90 ± 5% RH)

Grupos	EFIW (mg) X ± DP (LI-LS)	EMW (mg) X ± DP (LI-LS)	IER (%) X ± DP (LI-LS)	IEN (%) X ± DP (LI-LS)	WFO (dias) X ± DP (LI-LS)
G1	208,76 ± 48,65a (143 -328)	113,24 ± 38,33a (0 - 178)	54,42 ± 16,13ª (0 - 68,68)	68,73 ± 20,57a (0 - 94,78)	41,73 ± 16,99a (19 - 81)
G2	209,2 ± 29,23a (159 - 276)	119,38 ± 31,88a (5 - 167,10)	56,73 ± 12,03ª (2,5 - 66,84)	70,58 ± 12,02a (16,66 - 82,73)	43,66 ± 26,54a (23 - 170)
G3	210,86 ± 29,16a (163 - 271)	115,83 ± 37,83a (0 - 178)	54,69 ± 16,80ª (0 - 80,64)	68,52 ± 19,89a (0 - 95,25)	47,93 ± 25,33a (21 - 120)
G4	194,1 ± 33,32b (151 -303)	84,24 ± 42,97b (0 - 144)	43,75 ± 22,24b (0 - 76,19)	54,90 ± 29,29b (0 - 100)	51,53 ± 41,61a (19 - 198)
G5	219,5 ± 31,71a (165 - 294)	118,21 ± 40,42a (30 - 181,25)	53,26 ± 16,90ª (17,03 - 80,19)	70,82 ± 14,35a (37,80 - 95,39)	53,86 ± 25,10a (26 - 115)

Means with the same letters on the vertical didn't differ by KrusKall – Walls' test, followed by Dunn's test at 5%.

The mean of the larval longevity period within the citronella treated groups showed significant statistical difference, when compared to the control groups, even though the survival period was over 96 days, the same event didn't occur with the G4 group, which showed only 82.5 days of life (P<0.05) (Table 3). The longevity period of the larvae of the citronella treated groups weren't compatible with the ones found by NDUMU et al. (1999) which demonstrated an *in vitro* toxicity of the Neem's seeds oil to the larvae of *Amblyomma variegatum*, observing that in the pure product there was a 100% of larvae mortality after 48 hours and that the toxicity was related to the concentration and the exposure time. The results found are probably related to the concentration, exposure time and the active ingredient (citronellal) of the extract.

The mean of the females survival periods didn't show a significant statistical difference within the citronella treated groups and the control groups (P>0.05), therefore occurring a stretching on the days on those citronella treated groups, in G1 and G2 over 20 days, while the others were under this (Table 3). The mean of the mortality period of the female at end of oviposition showed a significant difference for G3 and G4 when compared to other groups, occurring a decrease on days (P<0.05). CHUNGSAMARNYART & JIWAJINDA (1992) when studying distilled from leaves of *C. citratus* or lemon grass (citral) and of *C. nardus* or citronella grass (citronella) found that both caused the *in vivo* death of the engorged female of *Rhipicephalus* (*B.*) *microplus*. CASTRO et al. (2009) evaluated the effect *in vitro* of the extract of *Araucaria angustifolia* in *Rhipicephalus* (*Boophilus*) *microplus* females and observed 50% of efficacy in the concentration of 30%.

Related to larvae mortality in the different treated groups, it was verified that some larvae didn't survive for a long time, when compared to PL-LL, in which the days present themselves really close, probably if those larvae came after the 12th day of posture, when there is a decrease on the female's energy and posture, besides dehydration suffered because of the atmospheric pressure and hemolymph decrease, therefore those larvae showed low motility, energy and time of life decrease (Table 3). These prerogatives were observed by BENNNETT (1974), LEES (1946) and LONDT (1997). CLEMENTE et al. (2010) obtained satisfactory results when evaluated the acaricidal activity of *Cymbopogon nardus* in non engorged larvae of *Anocentos nitens*, the percentage of efficacy was of 90,8% and 100% for the concentrations of 25 and 50%, respectively. The repellent action of this plant was proved by SOARES et al (2010) against nymphs of *Amblyomma cajennense*.

TABLE 3. – Mean periods of larvae and engorged female survival and mortality of of *Rhipicephalus* (*Boophilus*) *microplus* in the different immersion times in 50% of *Cymbopogon nardus* (Citronella) botanical aqueous extract under laboratorial conditions (27 ± 2ºC, 90 ± 5% RH)

Grupos	**PL-LL (dias)** **X ± DP** **(LI-LS)**	**PS-F (dias)** **X ± DP** **(LI-LS)**	**PM-F (dias)** **X ± DP** **(LI-LS)**	**PM-LL (dias)** **X ± DP** **(LI-LS)**
G1	96,03 ± 27,23b (0 -122)	22,53 ± 6,03a (10 - 37)	7,4 ± 5,34a (0 - 21)	75,73 ± 21,92b (0 - 99)
G2	107,80 ± 55,9a (100 - 121)	22,33 ± 6,37a (9 - 33)	7,83 ± 5,31a (0 - 16)	85 ± 8 ,42a (78 - 99)
G3	96,2 ± 18,74b (0 -107)	19,0 ± 5,88a (0 - 28)	4,86 ± 3,67b (0 - 13)	76,56 ± 14,92b (0 - 92)
G4	82,5 ± 46,69c (0 -120)	18,9 ± 4,87a (3 - 26)	4,36 ± 2,73b (0 - 10)	66,2 ± 37,71c (0 - 99)
G5	106,73 ± 6,46a (92 - 121)	19,6 ± 5,18a (7 - 34)	6,16 ± 4,15a (0 - 18)	85.9 ± 6,4a (78 - 99)

Means with the same letters on the vertical didn't differ by KrusKall – Walls' test, followed by Dunn's test at 5%.

CONCLUSION

It was concluded that the botanical aqueous extract of citronella has deleterious action on *Rhipicephalus* (*B.*) *microplus* life parameters larval longevity period, larval mortality period and female posture rhythm. The ixodide shows resistance to cypermethrin 15%.

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