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Acaricidal activity of extracts from the leaves and aerial parts of *Neoglaziovia variegata* (Bromeliaceae) on the cattle tick *Rhipicephalus (Boophilus) microplus*

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ABSTRACT

This experiment was carried out to study the bioacaricidal activity of *Neoglaziovia variegata* against engorged females of *Rhipicephalus (Boophilus) microplus*. The mortality and fecundity of groups of engorged adult females exposed to different concentrations of ethanol, hexane, chloroform, and ethyl acetate extracts obtained from the leaves and aerial parts of *N. variegata* were evaluated, using three treatments with concentrations of 5, 10 e 25 mg/ml; two controls (distilled water and distilled water with drops of cremophor); with three replicates. The hexane extract of the leaves demonstrated significant results, presenting 94.1% inhibition of oviposition; 0.33% the average percentage of eclosion of eggs; and 99.8% of effectiveness. These results indicate *N. variegata*, particularly the hexane extract of leaves, as potential alternative control agents of *R. (B.) microplus*. Pharmacological and chemical studies are continuing in order to characterize the mechanism responsible for this effect.

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1. Introduction

The tick *Rhipicephalus (Boophilus) microplus* (Canestrini, 1887) (Acari: Ixodidae) is the most important ectoparasite in areas of livestock productivity, both in tropical and subtropical regions, causing severe economic losses (Athayde et al., 2001). Several methods of control of this arthropod are employed based on the use of chemical acaricides. The intense and indiscriminate use of these products along the years has caused the problem of resistant populations; in addition, they lead to waste in products of animal origin and the environment (Bittencourt et al., 1999; Farias et al., 2008). There is a global trend to reduce, as far as possible, the use of chemical insecticides and miticides, mainly due to the aggressions caused to the environment and the food chain. The use of plants with pesticide properties may be an alternative, with less environmental impact and which might present a decrease in the problematic occurrence of resistant strains (Fernandes et al., 2007; Hernandez et al., 1987; Valente et al., 2007).

Neoglaziovia variegata, family Bromeliaceae, characterized by having flowers protected by bracts with bright coloring, striped leaves and succulent fruits (Silveira et al., 2009), is an endemic species of the Caatinga biome (Pereira and Quirino, 2008). The leaves are made up of highly resistant fibers, generating commercial value. Handmade hats, bags, and fishing nets are some examples of products from these leaves (Pereira and Quirino, 2008). Several studies have demonstrated that *N. variegata* have biological properties such as gastroprotective (Machado et al., 2013), photoprotective (Oliveira-Júnior et al., 2013), antibacterial (Oliveira-Júnior et al., 2012), antinociceptive (Lima-Saraiva et al., 2012a) and antioxidant (Lima-Saraiva et al., 2012b). However there is no report of its activity against tick.

This work aimed the extraction, analysis and evaluation of the acaricidal activity of the leaves and aerial parts of *Neoglaziovia variegata* on the cattle tick *Rhipicephalus (Boophilus) microplus* using adult immersion tests.

2. Materials and methods

2.1. Plant material

The leaves and aerial parts of *Neoglaziovia variegata* (Arruda) Mez were collected in the municipality of Petrolina (S 08°59′16″; W 40°35′20″), State of Pernambuco, Brazil, in January of 2011. The samples were identified by André Paviotti Fontana, a botanist from Centro de Recuperação de Áreas Degradadas da Caatinga (CRAD/Univasf). A voucher specimen (6441) was deposited at the Herbarium

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Vale do São Francisco (HVASF) of the Federal University of San Francisco Valley.

2.2. Extract preparation

The leaves and aerial parts were dried and pulverized, then macerated with 95% ethanol at room temperature for 72 hours. The solution was filtered and concentrated under reduced pressure in a rotatory evaporator at 50 °C; producing 30 g of crude ethanol extract (Nv–EtOH). The fractions were obtained using a sequence of solvents increasing polarity (hexane, chloroform and ethyl acetate), after extraction the solutions were decanted, filtered and the solvents were removed by evaporation under reduced pressure. Distilled water was used for the preparation of working solutions of each extract.

2.3. Analysis of by high performance liquid chromatography (HPLC)

The polyphenol profile analyses were carried out using a liquid chromatograph (Prominence LC-20AT, Shimadzu®, Japan) with a diode array detector (DAD, SPD-M20A) coupled to an LC Solution ChemStation data-processing station. The column used was a C18 Betasil (250 mm × 4.6 mm; 5 µm; Thermo Fisher Scientific, Runcorn, UK), operated at 27 °C. The mobile phase consisted of solvent A (ultrapure water and acetic acid, 99:1) and solvent B (methanol). The gradient (v/v) began with 38% of solvent B, rising to 70% at 20 min, 85% at 25 min, and 100% at 30 min, and remaining at this concentration for another 5 min. The injection volume was 20 µl, and the flow rate was 1 ml/min. The identification of phenolic compounds was based on a comparison of chromatographic data (retention times and UV spectra) with authentic chemical markers. Authentic markers available at the market were used for chromatographic comparison of data. Isoquercetin, gallic acid, protocatechuic acid, kaempferol-3-*O*-rhamnoside, vanillic acid and *p*-coumaric acid were supplied by Sigma-Aldrich® Chemie (Steinheim, Germany) for HPLC analysis with purity ≥99%. The HPLC analysis was performed with all the extracts and fractions of *N. variegata* (leaves and aerial parts).

2.4. Preparation of ticks

The engorged female ticks were collected from a naturally infested herd, kept on farms, localized in the municipality of Petrolina, State of Pernambuco. These females were incubated at 27–28 °C and 70–80% relative humidity for 2 weeks until the eggs were laid. After hatching the eggs, two cows were artificially infested with the larvae, to ensure that these ticks were free of chemicals. *Rhipicephalus (Boophilus) microplus* females in the later stages of engorgement, measuring more than 4.5 mm, were collected from infested animals, washed with water and dried in paper toweling.

2.5. Adult immersion test (AIT)

These females were used in the Adult Immersion Test (Drummond et al., 1973) to measure the acaricidal activity of extracts (leaves and aerial parts) from *N. variegata* against *R. (B.) microplus* adult engorged females. Three hundred eighty (380) engorged females separated into groups of ten (10), approximately 0.2 mg, were immersed in each solutions for 5 minutes. Six groups were made: four treated (hexane, ethyl acetate, chloroform and ethanol), at concentrations of 5 mg/ml, 10 mg/ml and 25 mg/ml, and two controls were also prepared, one containing only distilled water and other containing cremophor surfactant with distilled water, once cremophor was used at preparation of all extracts at high concentrations. Each extract concentration has been tested three times. After the immersion, the ticks were dried on paper towel, placed in sterile Petri dishes and incubated at 27–28 °C, 70–80% relative humidity. After 14 days the eggs were collected, weighed and observed. The eggs were placed in glass tubes, incubated at the same conditions and after 14 days the tubes were observed and the hatching rates of the different treatments were estimated with the aid of magnifying glass and compared to the controls.

The parameters were calculated as follows: Inhibition of oviposition (%IO) = [IO (control) – IO (treated)/IO (control)] × 100; Percentage estimated reproduction (%RE) = [(EW) × (%E × 0.01)/(IFW)] × 20,000; Reproduction control (%CR) = [CR(control) – CR(treated)/CR(control)] × 100; where (%IO) = percentage inhibition of oviposition; (%RE) = percentage reproductive efficiency; IFW = initial female weight; EW = egg weight; (%E) = percentage eclosion, and (%CR) = percentage reproduction control.

2.6. Statistical analysis

The data obtained were analyzed using the GraphPad Prism program version 5.0 and expressed as mean ± S.E.M. Statistically significant differences between groups were calculated by the application of analysis of variance (ANOVA) followed by Tukey test and confirming with Student-*t* test.

3. Results

The profile of phenolic compounds of *N. variegata* was analyzed by HPLC–DAD. Based in the chromatograms (data not shown), it was possible to identify the presence of flavonoid isoquercetin in ethanolic; *p*-coumaric acid in chloroform; and vanillic, *p*-coumaric and protocatechuic acids in ethyl acetate extracts from the leaves. From the aerial parts were identified flavonoid isoquercetin in ethanolic extract; vanillic acid in chloroform; and flavonoid isoquercetin, *p*-coumaric acid, vanillic acid, protocatechuic acid, caffeic acid and kaempferol-3-*O*-rhamnoside in ethyl acetate extracts from the aerial parts. However, the content of individual compounds was different between the extracts. These compounds were identified by comparison with the parameters retention time and absorption bands in the ultraviolet spectra of the standards that were used.

The results for initial weight of the female (IFW), egg weight (EW), percentage inhibition oviposition (%IO), hatching rate (%E), percentage estimated reproduction (% RE) and percentage control of reproduction (%CR) of the extracts of leaves and aerial parts are shown in Tables 1 and 2, respectively.

All concentrations of ethanol and hexane extracts of *N. variegata* leaves produce a significant effects on the adult female *R. (B.) microplus* ticks. At the highest concentration (25 mg/ml) the %CR was verified extremely toxic for ethanolic (92.55%) and hexanic (99.81%) extract, obtaining nearly 100% efficiency (Table 1). However, the results of aerial parts (Table 2) were different from those observed in the leaves; for the highest concentration (25 mg/ml) the %CR was 57.25% for ethanolic extract and 61.92% for hexanic extract. All fractions have shown dose dependent response on the reproductive parameters of adult ticks. The weight of engorged female maturation showed no statistical difference. There was no statistical difference between control 1 (distilled water) and control 2 (distilled water with cremophor), showing that the cremophor surfactant did not demonstrate any toxicity against the ticks.

4. Discussion

The Bromeliaceae is a botanical family characterized by the presence of steroids, terpenoids and phenolic compounds, mainly flavonoids. The phytochemical characterization of leaves and aerial parts of *N. variegata* demonstrated in EtOH and hexane extracts the presence of anthracene derivatives, flavonoids, tannins, mono and diterpenes. Hexane extract also showed positive reaction for the

Table 1
Engorged female *Rhipicephalus (Boophilus) microplus* ticks were exposed to different concentrations of extracts from the leaves of *N. variegata*.

Extract (mg/ml)	IFW	EW	%IO	%E	%RE	%CR
Hexane						
5	0.20 ± 0.02	0.08 ± 0.01	18.59 ± 8.95[ab]	70.17 ± 10.61[ab]	6139 ± 975.6[ab]	37.63 ± 11.84[ab]
10	0.18 ± 0.03	0.03 ± 0.01	61.22 ± 16.28[a]	26.67 ± 18.24[ac]	2135 ± 1,603[ac]	78.07 ± 16.33[ac]
25	0.18 ± 0.03	0.004 ± 0.006	94.14 ± 8.49[b]	0.33 ± 0.57[bc]	19.83 ± 34.35[bc]	99.81 ± 0.32[bc]
Chloroform						
5	0.19 ± 0.03	0.08 ± 0.02	13.04 ± 11.26	66.50 ± 22.10	6157 ± 2,450	38.42 ± 26.47
10	0.20 ± 0.03	0.06 ± 0.02	31.04 ± 9.94	50.00 ± 22.75	4008 ± 2,190	61.07 ± 20.35
25	0.18 ± 0.03	0.07 ± 0.03	33.22 ± 21.73	45.67 ± 37.65	3933 ± 4,025	61.80 ± 37.99
Ethyl acetate						
5	0.18 ± 0.03	0.08 ± 0.01	18.8 ± 3.25	68.67 ± 16.50	6335 ± 1,909	40.30 ± 18.33
10	0.19 ± 0.03	0.08 ± 0.01	22.52 ± 18.76	72.33 ± 29.48	6552 ± 3,171	38.38 ± 29.80
25	0.20 ± 0.03	0.07 ± 0.02	28.56 ± 13.89	74.00 ± 17.35	6333 ± 2,103	40.38 ± 19.89
Ethanol						
5	0.22 ± 0.06	0.08 ± 0.01	21.65 ± 12.52[a]	78.83 ± 6.890[a]	7023 ± 903.1[a]	33.91 ± 8.040[a]
10	0.18 ± 0.04	0.06 ± 0.04	41.3 ± 22.34[b]	58.83 ± 23.53[b]	4749 ± 2,672[b]	54.83 ± 23.76[b]
25	0.20 ± 0.03	0.01 ± 0.05	84.56 ± 12.48[ab]	10.00 ± 3.460[ab]	768.5 ± 193.6[ab]	92.55 ± 1.742[ab]
Control 1: distilled water	0.19 ± 0.01	0.10 ± 0.01		99.83 ± 0.16	10,236 ± 75,488	
Control 2: distilled water with cremophor	0.18 ± 0.02	0.09 ± 0.01	0.91 ± 2.33	99.67 ± 0.33	10,116 ± 586.7	3.88 ± 4.32

IFW: initial female weight; EW: egg weight; %IO: percentage inhibition oviposition; %E: percentage hatching; %RE: percentage estimated reproduction; %CR: control of reproduction. Means in the same column followed by the same letters were significantly different under the Tukey test.

Table 2
Engorged female *Rhipicephalus (Boophilus) microplus* ticks were exposed to different concentrations of extracts from the aerial parts of *N. variegata*.

Extract (mg/ml)	IFW	EW	%IO	%E	%RE	%CR
Hexane						
5	0.19 ± 0.03	0.07 ± 0.01	20.76 ± 13.90	56.17 ± 20.65	4829 ± 1890	49.88 ± 21.09
10	0.18 ± 0.02	0.06 ± 0.00	26.50 ± 5.62	52.83 ± 27.77	4140 ± 1942	56.37 ± 22.09
25	0.17 ± 0.02	0.04 ± 0.01	46.57 ± 35.09	42.50 ± 21.27	3604 ± 1914	61.92 ± 21.43
Chloroform						
5	0.18 ± 0.03	0.08 ± 0.02	8.82 ± 11.80	69.67 ± 19.22	6315 ± 2249	34.84 ± 24.07
10	0.19 ± 0.03	0.07 ± 0.03	22.46 ± 30.16	58.50 ± 34.77	5461 ± 3254	42.15 ± 36.03
25	0.20 ± 0.03	0.07 ± 0.03	25.84 ± 30.15	51.67 ± 29.16	4656 ± 2955	50.66 ± 32.43
Ethyl acetate						
5	0.21 ± 0.01	0.08 ± 0.02	13.64 ± 26.40	85.17 ± 13.25	6963 ± 1069	27.86 ± 14.93
10	0.22 ± 0.03	0.09 ± 0.03	16.30 ± 26.41	74.00 ± 14.00	6162 ± 365.7	36.19 ± 7.62
25	0.19 ± 0.04	0.08 ± 0.03	17.13 ± 26.62	70.00 ± 16.46	6276 ± 2571	34.42 ± 29.28
Ethanol						
5	0.19 ± 0.02	0.08 ± 0.01	18.73 ± 8.75[a]	84.33 ± 15.18[a]	7109 ± 722.2[a]	30.76 ± 9.10[a]
10	0.20 ± 0.04	0.08 ± 0.03	30.24 ± 13.58	65.57 ± 13.51	5412 ± 1593	38.04 ± 14.92
25	0.18 ± 0.05	0.05 ± 0.02	40.34 ± 7.50[a]	57.17 ± 9.87[a]	4219 ± 1356[a]	57.25 ± 13.03[a]
Control 1: distilled water	0.20 ± 0.02	0.10 ± 0.01		99.99 ± 0.001	52.21 ± 1.61	
Control 2: distilled water with cremophor	0.18 ± 0.03	0.09 ± 0.02		99.99 ± 0.001	48.55 ± 3.25	7.07 ± 3.47

IFW: initial female weight; EW: egg weight; %IO: percentage inhibition oviposition; %E: percentage hatching; %RE: percentage estimated reproduction; %CR: control of reproduction. Means in the same column followed by the same letters were significantly different under the Tukey test.

presence of triterpenes and steroids; $CHCl_3$ extract for the presence of anthracene derivatives, coumarins, flavonoids, tannins, monoterpenes, diterpenes and naphthoquinones; and ethyl acetate extract for the presence of coumarins, flavonoids and tannins, lignans, naphthoquinones (Oliveira-Júnior et al., 2012).

Lima-Saraiva et al. (2012a) demonstrated that the chromatogram of ethanolic extract of leaves showed the presence of eight peaks with different retention times. Based on their retention time, the compounds have UV band characteristic for cinnamic acid, coumarin, and flavonoid derivatives. These results are in accordance with the compounds identified in this study, whose main constituents were flavonoids and cinnamic acid derivatives.

In this study, we evaluated the acaricidal activity of *N. variegata* leaves and aerial part extracts for the control of adults *R. (B.) microplus*. Hexane extract showed high effectiveness with high mortality reducing the weight of eggs and egg hatch. This effect is probably because large quantities of terpene compounds that were identified at this stage and this class of secondary metabolites are known for insecticidal activity. The plants with pesticidal activity may cause different effects on arthropods such as repellency, inhibition oviposition and feeding, cause developmental disorders, deformation, infertility, and death in various phases. The extent of the effect and the time actions are dependent on the dosage used (Roel, 2001). According to Romero (2008), terpenes and its derivatives such as monoterpenes and sesquiterpenes are substances found commonly in the essential oils and form together with the phenylpropanoid potent natural insecticides like pyrethroids.

Hexane extract of the leaves of *N. variegata* in low concentrations showed noticeable toxicity levels similar to other natural products and extracts, like azadiractin, thymol, carvacrol, citronella oil of well-known insecticidal activity. Singh et al. (2014) reported the efficacy of 93.7% at the 5.0% concentration of *Cymbopogon winterianus* against *Hyalomma anatolicum* ticks. Olivo et al. (2008) reported testing various concentrations of citronella oil (*Cymbopogon nardus*) to perceive an efficacy of 89.5% for the highest concentration (100%). This action is due to the active principal citronellal, proven insecticide and acaricide action (Chagas et al., 2002). Similar results were obtained by Monteiro et al. (2010), who evaluated the effect of monoterpene thymol against *R. (B.) microplus*, obtaining an efficacy of 99%–2% concentration, concluding that the isolated substance

has an *in vitro* effect against the tick. Another metabolite widely studied is azadirachtin, present in neem (*Azadiracta indica*), since it has strong insecticidal action; however, a study by Broglio-Micheletti et al. (2010) detected only 73.2% of success for the hexane extract of the seeds. Crude extracts of *Allium sativum* and *Carica papaya* at lower concentrations demonstrated complete failure of eclosion of eggs of *R. (B.) microplus* (Shyma et al., 2014).

The experiments reported here are preliminary, but they provide clear evidence that the ethanolic and hexane extracts of the leaves from *N. variegata* have potential acaricidal property and appear auspicious for tick control.

5. Conclusions

The ethanolic and hexane extracts of leaves from *N. variegata* produced a high acaricidal activity and reduced the egg-laying ability of *Rhipicephalus (B.) microplus* suggesting that they are good candidates for the development of new acaricidal agents. Additional studies will be conducted in order to identify the active compounds and their effects on other life stages of ticks.

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