Research Article

Bioactivity of ethanolic and hexanic extracts of *Annona montana* Macfad. in the control of *Aphis craccivora* Koch (Hemiptera: Aphididae)[1]

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ABSTRACT

The use of synthetic insecticides to control pests in agriculture may negatively affect the environment, human health, and contribute to the emergence of resistant pest populations. This study aimed to investigate the chemical composition and evaluate the bioactivity of ethanolic leaf extracts and hexanic seed extracts of *Annona montana* Macfad. against *Aphis craccivora* Koch. Bioassays were conducted to assess toxicity, feeding repellency and translaminar activity. For the chemical characterization of the hexanic seed extract, 97.2 % of the constituents were identified, with the linoleic (37 %), oleic (36.7 %), palmitic (16.9 %) and stearic (5.2 %) acids as the major compounds. For the ethanolic leaf extract, 83.31 % of the constituents were identified, mainly α-tocopherol (19.97 %), β-sitosterol (10.16 %), cyclohexanecarboxylic acid, heptadecyl ester (6.26 %), methyl linolenate (5.35 %) and phytol (4.43 %). Both extracts showed toxic effects on *A. craccivora*, with the ethanolic extract exhibiting a higher toxicity against nymphs and adults of the cowpea aphid. Repellent and translaminar effects were also observed, with the hexanic seed extract showing a more pronounced activity in both cases.

KEYWORDS: Bioinsecticide, cowpea aphid, insecticide plant.

RESUMO

Bioatividade de extratos etanólicos e hexânicos de *Annona montana* Macfad. no controle de *Aphis craccivora* Koch (Hemiptera: Aphididae)

O uso de inseticidas sintéticos para o controle de pragas na agricultura pode afetar negativamente o meio ambiente, a saúde humana e contribuir para o surgimento de populações de pragas resistentes. Objetivou-se investigar a composição química e avaliar a bioatividade de extratos etanólicos de folhas e extratos hexânicos de sementes de *Annona montana* Macfad. sobre *Aphis craccivora* Koch. Bioensaios foram realizados para avaliar a toxicidade, repelência alimentar e atividade translaminar. Na caracterização química do extrato hexânico de sementes, 97,2 % dos constituintes foram identificados, com os ácidos linoleico (37 %), oleico (36,7 %), palmítico (16,9 %) e esteárico (5,2 %) sendo os principais compostos. No extrato etanólico de folhas, 83,31 % dos constituintes foram identificados, principalmente α-tocoferol (19,97 %), β-sitosterol (10,16 %), ácido ciclohexanocarboxílico, éster heptadecílico (6,26 %), linolenato de metila (5,35 %) e fitol (4,43 %). Ambos os extratos mostraram efeitos tóxicos sobre *A. craccivora*, com o extrato etanólico exibindo maior toxicidade contra ninfas e adultos do pulgão do feijão-caupi. Efeitos repelentes e translaminares também foram observados, sendo que o extrato hexânico de sementes demonstrou atividade mais pronunciada em ambos os casos.

PALAVRAS-CHAVE: Bioinseticida, pulgão do feijão-caupi, planta inseticida.

INTRODUCTION

Pest control remains one of the major challenges in crop production. Aphids, in particular, cause both direct and indirect damage at various phenological stages of agricultural crops and act as vectors of plant diseases.

Aphis craccivora (Hemiptera: Aphididae), commonly known as cowpea aphid, is a key pest of several economically important crops worldwide (Mitra et al. 2021), with a marked preference for legumes (Thakshila et al. 2022).

Chemical control remains the most widely used strategy for managing this hemipteran, as with

[1] Received: May 25, 2025. Accepted: Sep. 05, 2025. Published: Sep. 29, 2025. DOI: 10.1590/1983-40632025v5578060.
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Editor: Luis Carlos Cunha Junior/Data Availability Statement: Research data are only made available by authors upon request.

most pests of economic importance (Doracenzi et al. 2021). However, the excessive and indiscriminate application of synthetic insecticides has led to adverse effects on the environment and human health, as well as the emergence of resistant pest populations (Giunti et al. 2022).

Within the framework of integrated pest management, plant-based insecticidal formulations have gained increasing attention as alternatives for agricultural pest control. Botanical insecticides are often reported to exhibit a greater selectivity (Santana et al. 2025).

Among tropical species of the *Annona* genus with insecticidal potential, *Annona montana* stands out. Extracts of this species have shown detrimental effects on a range of arthropod pests of agricultural relevance, including *Sitophilus zeamais* Motchulsky (Coleoptera: Curculionidae) (Ribeiro et al. 2014b), *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) (Blessing et al. 2010) and, most notably, *A. craccivora* (Bandeira et al. 2017).

Secondary metabolites commonly found in Annonaceae species include acetogenins, compounds unique to this family, which have demonstrated insecticidal activity against several crop pests and disease vectors (Durán-Ruiz et al. 2024). In addition to acetogenins, *A. montana* contains alkaloids, glycosides, flavonoids, sesquiterpenes, phenolic compounds, saponins and terpenoids (Guerrero-Álvarez & Giraldo-Rivera 2023), all of which are associated with insecticidal action (Giraldo-Rivera & Guerrero-Álvarez 2021).

Despite this potential, studies assessing the insecticidal activity of *A. montana* extracts against *A. craccivora* remain scarce. From this perspective, the present study aimed to investigate the chemical composition and evaluate the bioactivity of ethanolic leaf extracts and hexanic seed extracts of *A. montana* on *A. craccivora*.

MATERIAL AND METHODS

The botanical material was collected in March 2018, in the municipality of Boa Vista, Roraima state, Brazilian Amazon. The collection site was georeferenced (2º49′10″N; 60º40′22″W), the plant identified and a voucher specimen deposited in an official indexed herbarium under the registration nº 9045. The access was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen), in compliance with the Brazilian legislation, under the number AE6C7D9.

After collection, *Annona montana* leaves and seeds were sorted, weighed, washed with running water and dried in a forced-air oven at 40 ºC, until a constant mass was achieved. The dried plant structures were separately ground in a knife mill to obtain powders with granulometry of 20-40 mesh. Leaf and seed powders were stored in hermetically sealed glass containers until extraction.

The ethanolic leaf extract was prepared following the methodology of Carvalho et al. (2017), and the hexanic seed extract according to Melo Filho et al. (2018). To identify the chemical constituents of the hexanic seed extract, gas chromatography coupled with mass spectrometry was performed after two preparatory procedures: microwave hydrolysis and methylation of free fatty acids, as described by Sande et al. (2018) and Melo Filho et al. (2018). The chemical composition of the ethanolic leaf extract was determined by gas chromatography with flame ionization detection (Carvalho et al. 2013).

Liquid emulsions were prepared for aphid bioassays using reverse osmosis water, Tween 80® and the active botanical ingredients (ethanolic and hexanic extracts of *A. montana*). To minimize experimental error, a stock (standard) emulsion was first prepared, from which aliquots were taken and diluted to obtain the working solutions used in the bioassays against *Aphis craccivora*.

For rearing and maintenance of *A. craccivora*, nymphs and adult females were collected from an ecological cowpea field. Cowpea plants (‘BRS Aracê’ cultivar) were grown in pots and maintained under ambient conditions in 2.0 × 2.0 m cages covered with anti-aphid screen.

Bioassays were performed to estimate the median lethal concentration (LC_{50}) and 90 % lethal concentration (LC_{90}) of the extracts. Two preliminary tests were conducted with concentrations of ethanolic and hexanic extracts to establish ranges resulting in mortality close to 0 % (lower limit) and 100 % (upper limit). The concentrations were calculated using the Bliss’ formula. For ethanolic extracts (bioassay 1), the treatments were: reverse osmosis water + Tween 80® (0.06 %); 0.10, 0.21, 0.46, 0.98, 2.09, 4.47 and 9.55 mg mL^{-1}. For hexanic extracts (bioassay 2), the treatments were: reverse osmosis water + Tween 80® (0.06 %); 0.10, 0.17, 0.29, 0.49, 0.83, 1.41 and

2.40 mg mL^{-1}. A total of 1,715 nymphs were used in the ethanolic extract bioassays, and 1,888 nymphs in the hexanic extract bioassays.

To establish the bioassays, three adult aphids from the stock colony were placed on host plants grown in 500-g pots, with one leaf per plot, and maintained for 48 h in individualized mini-cages (Ø of 4 cm; 2 cm high) to allow colony formation and nymph production. After this period, adults were removed and only nymphs (2-3 days old) were retained on the leaves. The treatments were applied by spraying with a professional double-action airbrush (0.5-mm nozzle) coupled to a pneumatic pump operating at 5 psi. Preliminary tests were conducted to ensure adequate coverage, and a 30-µL aliquot of the working solution (prepared from the m/v ratio) was standardized for each treatment. All bioassays were conducted under controlled conditions (25 ± 2 ºC; 60 ± 10 % of RH; 12-h photoperiod).

The experimental design was completely randomized, with 8 treatments and 10 replicates, each replicate consisting of one leaf containing a variable number of nymphs (average: 22). To avoid damage to the insects' mouthparts, nymphs were not manipulated at this stage. Lethal concentrations (LC_{50} and LC_{90}) were estimated using the Probit analysis in the R Studio. Nymph mortality was assessed at 24 h after spraying, with individuals unable to move a distance equivalent to their body length considered dead.

The LC_{50} values of the ethanolic and hexanic extracts of *A. montana* were subsequently used to assess the extract toxicity in adult aphids. The same methodology and spray application were employed, except that 10 adult aphids (8-10 days old) were confined in mini-cages attached to host leaves. Treatments included: reverse osmosis water + Tween 80® (0.06 %) as control, LC_{50} of ethanolic leaf extract and LC_{50} of hexanic seed extract. The design was completely randomized, with 3 treatments and 10 replicates, each replicate consisting of one leaf with 10 adults. Mortality was assessed at 24 h after application. Homoscedasticity and normality were verified using the Levene's and Shapiro-Wilk's tests, respectively. Data were subjected to analysis of variance (Anova) and means compared using the Tukey test at 5 % of probability.

For repellency assays, two approaches were used: a) choice test: 10 adults (8-10 days old) were released in the center of a Petri dish arena (Ø of 7 cm; 5 cm high) containing four leaf discs (Ø of 4 cm), placed equidistantly. Two discs were treated with *A. montana* extracts and two with water + Tween 80® (control). Before testing, the aphids were starved for 2 h. Counts of aphids on treated and untreated discs were made at 30, 60, 90 and 120 min. The design was completely randomized, with subdivided plots over time. Each treatment had 10 replicates, each consisting of one arena with 10 adults. Data were analyzed by Anova and means compared with the Student's t-test at 5 % of probability; b) no-choice test: the same procedures were followed, except that treated and control discs were offered individually, preventing aphids from choosing between the treatments. The experiment was conducted in a completely randomized design, with subdivided plots over time, comprising 12 treatments (extract × time) and 10 replicates. Data were analyzed by Anova and means compared by the Tukey test at 5 % of probability.

To evaluate the translaminar effect of *A. montana* extracts, the methodology used for LC_{50} and LC_{90} determination was adopted, except that the treatments (control, LC_{50} ethanolic leaf extract and LC_{50} hexanic seed extract) were applied to the abaxial leaf surface, whereas insects were confined to the opposite side. The design was completely randomized, with three treatments and 15 replicates, each replicate consisting of one leaf with approximately 20 nymphs (2-3 days old). After the drying treatment, nymphs were placed on the leaf surface and covered with mini-cages. Handling was minimized to avoid injury to the insects' mouthparts. Mortality was recorded at 72 h after application. Data were analyzed by Anova and means compared using the Tukey test at 5 % of significance.

RESULTS AND DISCUSSION

The chemical characterization of the hexanic seed extract revealed that 97.2 % of the constituents of *A. montana* seeds were identified (Table 1), with the major compounds being linoleic (37 %), oleic (36.7 %), palmitic (16.9 %) and stearic (5.2 %) acids.

The fatty acids detected here, commonly reported in extracts from various plant species, have documented insecticidal and acaricidal potential, acting either synergistically (Romo-Asunción et al. 2016) or individually (Liu et al. 2019). The insecticidal activity of the hexanic

extract of *A. montana* on *A. craccivora* may be primarily attributed to these fatty acids, which can block spiracles and impair respiration. Celestino et al. (2016) similarly reported that the mortality of *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae) was associated with the obstruction of respiratory openings.

Table 1. Chemical composition of the seed hexane extract from *Annona montana*.

Fatty acid	Concentration (%)	Chemical compound
C16:0	16.9	Palmitic acid
C18:0	5.2	Stearic acid
C18:1	36.7	Oleic acid (ω-9)
C18:2	37.0	Linoleic acid (ω-6)
C18:3	0.9	γ-linolenic acid
C18:3	0.1	α-linolenic acid
C20:0	0.2	Arachidonic acid
C20:1	0.1	Gadoleic acid
C22:0	0.1	Behenic acid
Identified	97.2	-

The phytochemical analysis of the ethanolic leaf extract identified 83.31 % of the compounds (Table 2), with the most abundant being α-tocopherol (19.97%), β-sitosterol (10.16%), cyclohexanecarboxylic acid, heptadecyl ester (6.26 %), methyl linolenate (5.35 %) and phytol (4.43 %).

Previous studies have also reported tocopherols in *A. montana* (Leung et al. 2022), as well as phytosterols and terpenoids (Zahid et al. 2018), phytol (Chaves et al. 2003) and derivatives of flavonoids and phenolic compounds (Tauchen et al. 2016). The bioactivity of alkaloids against *S. zeamais* has likewise been established (Ribeiro et al. 2014b).

Table 2. Chemical composition of the foliar ethanolic extract of *Annona montana.*

Retention time (min)	Concentration (%)	Chemical compound
19.160	0.35	Spathulenol
23.460	0.66	Loliolide
24.814	2.73	Isophytol
26.321	0.54	Farnesyl acetone
27.354	1.70	Palmitic acid
27.880	0.40	3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraenoic acid
27.961	2.42	Ethyl palmitate
30.109	4.43	Phytol
30.639	0.78	(Z)-7-tetradecenal
31.027	1.55	Ethyl linoleate
31.143	5.35	Methyl linolenate
31.641	0.60	Ethyl stearate
34.336	0.80	Clonitazene
34.642	0.59	3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraen-1-ol
36.596	0.91	Fumaric acid
36.878	1.08	2-palmitoylglycerol
38.167	0.63	Ethyl docosanoate
38.477	3.91	Dothiepin
39.672	1.52	1-heptacosanol
41.080	0.92	Ethyl tetracosanoate
41.146	3.39	Solanesol
41.278	0.84	Squalene
42.527	0.82	Triacontyl heptafluorobutyrate
44.210	0.89	β-tocopherol
44.445	2.62	γ-tocopherol
44.711	6.26	Cyclohexanecarboxylic acid, heptadecyl ester
44.990	1.12	6,7,8-trimethoxy-1,2-dimethyl-1,2,3,4-tetrahydroisoquinoline
45.635	19.97	α-tocopherol
47.159	1.78	Campesterol
47.649	2.90	Stigmasterol
48.894	10.16	β-Sitosterol
69.090	0.69	*E,E,Z*-1,3,12-nonadecatriene-5,14-diol
-	83.31	

At their respective LC_{50} values, both ethanolic and hexanic extracts were equally toxic to *A. craccivora* nymphs. Interestingly, the LC_{90} for both extracts was identical (0.41 mg mL^{-1}), resulting in mortality of nymphs of 90 % (Table 3).

The insecticidal and acaricidal potential of extracts and derivatives from *Annona* species has been investigated against several pest arthropods within an integrated pest management context with particular emphasis on *A. craccivora* (Gomes et al. 2019, Dutra et al. 2020). Bandeira et al. (2017) also reported the toxicity of *A. montana* extracts on *A. craccivora* nymphs. In the present study, both hexanic seed extracts and ethanolic leaf extracts showed insecticidal effects on cowpea aphid nymphs.

Similar negative impacts of *A. montana* extracts have been reported on *S. zeamais* (Ribeiro et al. 2014b), *S. frugiperda* (Blessing et al. 2010), *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) and *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) (Ribeiro et al. 2014a). Furthermore, as observed by Lin et al. (2009) in cotton aphids, nymphs treated in this study showed desiccation and detachment from leaf surfaces following exposure to *A. montana* extracts.

When adult *A. craccivora* were treated at LC_{50} values, the mortality rate reached 29 % with the hexanic extract (0.11 mg mL^{-1}) and 61 % with the ethanolic extract (0.07 mg mL^{-1}) (Table 4). Thus, the ethanolic extract was more effective, with both treatments differing significantly from the control. The mode of action likely involves cuticle disruption and spiracle obstruction, leading to asphyxiation and increased vulnerability to environmental stress.

In the choice repellency test, where insects could choose between treated and control discs, no interaction was detected between treatment and time. However, the extract treatment significantly reduced the aphid preference at all observation times (30, 60, 90 and 120 min) (Figure 1).

Both the ethanolic and hexane extracts of *A. montana* repelled cowpea aphid adults across all evaluated time intervals. It is important to emphasize

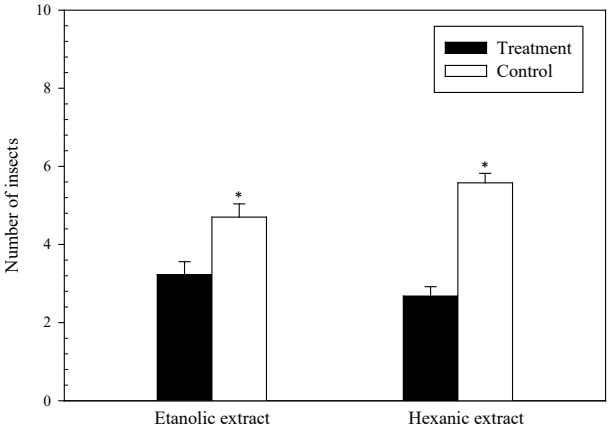

Figure 1. Number of adult aphids (mean ± SE*) recorded on cowpea leaf discs treated with ethanolic leaf extract (LC_{50} = 0.07 mg mL^{-1}) and hexane seed extract (LC_{50} = 0.11 mg mL^{-1}) of *Annona montana* (choice test) (25 ± 2 ºC; 60 ± 10 % of RH; 12-h photophase). Means differed by the t-test ($p \leq 0.05$).

Table 3. Lethal concentrations of 50 % (LC_{50}) and 90 % (LC_{90}) (mg mL^{-1}) of ethanolic leaf and hexanic seed extracts of *Annona montana* on *Aphis craccivora* nymphs (25 ± 2 ºC; 60 ± 10 % of RH; 12-h photophase).

Extract	Number of aphids	Slope (± SE)[(a)]	LC_{50} (CI 95 %)[(b)]	LC_{90} (CI 95 %)	$\chi^{2(c)}$	p-value
Ethanolic leaf extract	1.715	1.69 ± 0.74	0.07 (0.05; 0.10)	0.41 (0.34; 0.51)	146.37	1
Hexanic seed extract	1.888	2.19 ± 0.31	0.11 (0.09; 0.12)	0.41 (0.36; 0.48)	25.48	1

[(a)] Standard error; [(b)] confidence interval; [(c)] chi-squared.

Table 4. Mortality (mean ± SE) of adult *Aphis craccivora* exposed to LC_{50} of ethanolic leaf and hexanic seed extracts of *Annona montana*, applied by spraying (25 ± 2 ºC; 60 ± 10 % of RH; 12-h photophase).

Treatment	Mortality (%)*
Ethanolic leaf extract - LC_{50} of 0.07 mg mL^{-1}	61.00 ± 4.82 a
Hexanic seed extract - LC_{50} of 0.11 mg mL^{-1}	29.00 ± 7.06 b
Control	2.00 ± 1.33 c
CV (%)**	27.73

* Means followed by the same letter do not differ according to the Tukey test ($p \leq 0.05$). Data were transformed to $\sqrt{(x+1)}$. ** CV: coefficient of variation.

Table 5. Number of adult aphids (mean ± SE*) recorded on cowpea leaf discs treated with ethanolic leaf extract and hexanolic seed extract of *Annona montana* in a no-choice test (25 ± 2 ºC; 60 ± 10 % of RH; 12-h photophase).

Treatment	Times (min)*			
	30	60	90	120
Hexanic seed extract (LC_{50} of 0.11 mg mL^{-1})	4.33 ± 0.74 a	5.60 ± 0.56 a	4.90 ± 0.46 a	3.90 ± 0.72 a
Ethanolic leaf extract (LC_{50} of 0.07 mg mL^{-1})	7.20 ± 0.66 b	7.50 ± 0.40 b	7.10 ± 0.74 b	7.40 ± 0.54 b
Control	9.67 ± 0.22 c	9.90 ± 0.10 c	9.80 ± 0.13 c	9.70 ± 0.21 c
CV (%)**	21.67			

* Means followed by the same letter do not differ according to the Tukey test ($p \leq 0.05$). Data were transformed to $\sqrt{(x+1)}$. ** CV: coefficient of variation.

Table 6. Mortality (%) (mean ± SE*) of *Aphis craccivora* nymphs after application to cowpea leaves of the ethanolic leaf extract and the hexanic seed extract of *Annona montana* (translaminar effect) (25 ± 2 ºC; 60 ± 10 % of RH; 12-h photophase).

Treatment	Mortality (%)**
Hexanic seed extract - LC_{50} of 0.11 mg mL^{-1}	2.86 ± 0.81 a
Ethanolic leaf extract - LC_{50} of 0.07 mg mL^{-1}	0.99 ± 0.40 ab
Control	0.24 ± 0.17 b
CV (%)***	40.87

* Standard error. ** Means followed by the same letter do not differ according to the Tukey test ($p \leq 0.05$). Data were transformed into $\sqrt{(x+1)}$. *** CV: coefficient of variation.

that for vector insects such as *A. craccivora*, repellency can prevent colony formation and consequently reduce virus transmission rates to host plants (Soares et al. 2021).

In the no-choice repellency test, where insects had no alternative between treatments, a significant interaction was observed between the extracts and evaluation periods. Both the ethanolic and hexanic extracts were characterized as repellent, when compared with the control at all time intervals (Table 5). Notably, the hexane seed extract was less attractive to the insects, indicating a repellency stronger than for the ethanolic extract. The repellent activity observed in this study supports the potential of *A. montana* extracts to reduce virus transmission by *A. craccivora*.

The insecticidal and repellent activities of *Annona* species have been investigated against various agricultural pests. Terpenic compounds (Kaur et al. 2017) and fatty acids (Romo-Asunción et al. 2016) have shown toxicity to economically important arthropods, including *A. craccivora* (Aziz et al. 2018). The prevalence of terpenoids and fatty acids detected in this study is therefore likely responsible for the repellency observed in the choice test.

Phytol, a major constituent of the ethanolic leaf extract, is known for its repellent properties (Jiménez-Durán et al. 2021). Similarly, Andrade et al. (2016) reported phytol-associated repellency against *Diaphorina citri* Kuwayma (Hemiptera: Liviidae). Sang et al. (2021) further demonstrated the repellent effect of phytol against stored-product pests such as *Tribolium castaneum* (Herbst), *Lasioderma serricorne* (Fabricius) and *Liposcelis bostrychophila* Badonnel.

The translaminar effect of *A. montana* extracts on *A. craccivora* was reduced at 72 hours after application. Nonetheless, the hexanic seed extract still differed significantly from the control, whereas the ethanolic leaf extract showed intermediate values and did not differ statistically from either treatment (Table 6).

Although a translaminar activity has been reported for organic extracts from *Annona* species, including *A. montana* (Brito et al. 2020) and commercial botanical insecticides (Soares et al. 2021), this effect was not pronounced in the present study, as mortality rates of *A. craccivora* were low.

The extracts were capable of penetrating the leaf tissues and reaching insects without direct contact, confirming a depth effect. However, further research is recommended to assess and validate the translaminar action of *A. montana* extracts, given the limited toxicity observed.

CONCLUSIONS

1. Linoleic (37 %), oleic (36.7 %), palmitic (16.9 %) and stearic (5.2 %) acids are the predominant

constituents of the hexanic seed extract of *Annona montana*, whereas α-tocopherol (19.97 %), β-sitosterol (10.16 %), cyclohexanecarboxylic acid, heptadecyl ester (6.26 %), methyl linolenate (5.35 %) and phytol (4.43 %) are the major compounds of the ethanolic leaf extract;

2. *Aphis craccivora* nymphs are equally susceptible to the ethanolic leaf extract and the hexanic seed extract of *A. montana*;
3. At their respective median lethal concentrations, the ethanolic leaf extract is more toxic to *A. craccivora* adults than the hexanic seed extract;
4. Both extracts exhibited repellency against *A. craccivora* adults in choice and no-choice tests;
5. The extracts showed translaminar activity against *A. craccivora* nymphs, although this effect was limited at 72 hours after application.

ACKNOWLEDGMENTS

To the Universidade Federal de Roraima (UFRR) and its Graduate Program in Agronomy (Posagro). To the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), for granting a scholarship; Universidade Federal do Vale do São Francisco (Univasf), for the collaboration in the development of this research; and Dr. Edvan Alves Chagas, from the Agroforestry Research Centre at the Empresa Brasileira de Pesquisa Agropecuária (Embrapa), for the botanical identification of the studied species.

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