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EXTRACTS OF NATIVE FOREST SPECIES OF THE SOUTHERN AMAZON IN THE CONTROL OF *Aphis craccivora* KOCH (HEMIPTERA: APHIDIDAE)

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Abstract

The objective of this study was to evaluate the mortality of *Aphis craccivora* Koch (Hemiptera: Aphididae) submitted to different extracts with different concentrations of leaves of the native forest species: *Tetragastris altissima* (Aubl.) Swart (Burseraceae), *Metrodorea flavida* K. Krause (Rutaceae) and *Cheiloclinium cognatum* (Miers) AC Sm (Celastraceae) under laboratory conditions. Adults of *Aphis craccivora* were collected in *Gliricidia sepium* (Jacq.) Kunthe Walp. (Fabaceae), and separated into groups of ten individuals, transferred to Petri dishes and exposed to topical application of aqueous, alcoholic and leaf infusion extracts of *Cheiloclinium cognatum*, *Metrodorea flavida* and *Tetragastris altissima*. The extracts were applied at concentrations of: 3, 6, 9, 12 and 15%, with 1% dimethylsulfoxide (DMSO), using a control treatment 1 (distilled water + DMSO 1%) and control treatment 2 (fipronil). Evaluations were performed at 24, 48 and 72 hours after the application of the extracts by counting the dead insects. The experimental design was completely randomized, with five replicates, three types of extracts and five concentrations with controls. In all the studied species, the aqueous and infusion extracts presented a lower mortality (less than 52%), whereas the alcoholic extract in higher concentrations of *Cheiloclinium cognatum*, *Metrodorea flavida* showed a mortality of up to 100%. The species *Tetragastris altissima* reached an average mortality of 92%. The lethal concentration for the alcoholic extract of *Cheiloclinium cognatum* was LC_{50} 6.43% and LC_{90} 12.22%, *Metrodorea flavida* LC_{50} was 3.08% and LC_{90} 7.05% and that for *Tetragastris altissima* LC_{50} 5.58% and LC_{90} 17.47%, after 72 hours. The use of the alcoholic extract of the species *Metrodorea flavida* at a concentration of 9% in the control of *Aphis craccivora* is indicated.

Keywords: Aphid. Insecticide Botanic. Plant Extracts.

1. Introduction

Aphis craccivora Koch (Hemiptera: Aphididae), commonly known as black aphid, causes damage to different species of plants and is considered an important pest, mainly because during feeding, it injects toxins and can be a virus transmitter. The aphids live in colonies, under leaves, new shoots and flowers (Fazolin et al. 2016), and can cause the leaves to shrink and the deformation of the shoots due to their high capacity to suck sap (Gallo et al. 2002). This high feeding capacity is based on the presence of a differentiated arrangement in the region of the digestive tract, known as filter chamber, allowing the continuous suction of sap, eliminating excess liquid sucked, a sugary substance called honeydew (Camargo et al. 2011). This substance facilitates establishment of a fungus, commonly referred to as fumagina (*Capnodium* sp.),

characterized by the darkening of the attacked tissues (Laamari et al. 2008) damaging the mechanisms of photosynthesis and respiration due to the coverage of part or all of the leaf surface.

Aphis craccivora is one of the most common species of aphids and considered an important pest in the tropics (Latinovic et al. 2017). It feeds on several families of plants, but preferring species of the family Fabaceae, being considered one of the most important cause of damages in the cowpea (*Vigna unguiculata* (L.) Walp), one of the main crops grown on family farms (Bandeira et al. 2015; Melville et al. 2016; Rodrigues et al. 2012). This species has already been recorded in weeds (*Solanum americanum* Mill. and *Amaranthus hybridus* L.) (Sturza et al. 2011), orchids (*Catasetum* sp.) (Leite et al. 2017) and alfalfa (*Medicago sativa* L.) (Cunha et al. 2016).

Black aphid is usually controlled with commercially available chemical insecticides with broad action spectrum such as pyrethroids and neonicotinoids (Agrofit 2018). However, inappropriate use may cause adverse effects on the environment, such as mortality of beneficial organisms, and frequent use of insecticides with the same mechanism of action increases selection pressure in the population, selecting individuals resistant to insecticide used or to others with the same principle. It also presents high toxicity to humans, domestic animals, and wildlife. There is, therefore, a need for the development of alternative control methods.

In this way, the interest for the development of alternatives pest control measures is growing, either using natural enemies or insecticides of botanical origin (Carvalho et al. 2008), since plant have a diversity of active compounds (Navarro-Silva et al. 2009). Plant extracts may have different potentials of activity against different pests (Rizvi et al. 2012), which may interfere with the development, negatively affecting the females' posture, as well as with the population growth rate (Carvalho et al. 2014), actions of repellency, deterrence to oviposition and food (Silva et al. 2012; Fonseca et al. 2018), causes various effects on insects, including mortality. Species of Anonaceae family, for example, possess acetogenins, substances that act in the mitochondria, inhibiting the NADH – ubiquinona oxidorredutase and causing the death of the insects (Krinski et al. 2014).

The Amazon, because of its rich and diverse flora, presents many species that are considered potential sources of substances that can be used as insecticides. Their constituents can potentially be used as botanical insecticides, prompting studies with species native to Southern Amazonia. Therefore, in this research the choice of species was based on preliminary tests that demonstrated the presence of secondary metabolites with possible insecticidal action, in addition, they are species that occur frequently in this region.

The objective of this study was to evaluate the mortality of *Aphis craccivora* submitted to three types of extracts with different concentrations, obtained from leaves of native forest species: *Cheiloclinium cognatum* (Miers) A.C.Sm (Celastraceae), *Metrodorea flavida* K. Krause (Rutaceae) and *Tetragastris altissima* (Aubl.) Swart (Burseraceae), at different periods of exposure and under laboratory conditions.

2. Material and Methods

The experiment was carried out in the laboratories of the State University of Mato Grosso, Campus Alta Floresta-MT. Three species of plants were evaluated: *Cheiloclinium cognatum*, *Metrodorea flavida* and *Tetragastris altissima*, collected in May 2017 in the city of Alta Floresta-MT (56°3’43,972”W, 9°57’1,312”S).

The plant extracts were prepared by drying the material in a forced air circulation oven at 65 °C for 72 hours, followed by crushing in a Willey type mill. Subsequently, 100g of dried leaves were added to 500 mL of cold distilled water (aqueous extract), alcohol 92.8º (alcoholic extract) and distilled water at 90ºC (extract by infusion), corresponding to the concentration of 20% (w/v). From this concentration, the other concentrations were produced via dilution in distilled water. The solutions were stored in glass jars wrapped with aluminum foil to protect them from light, kept at room temperature for 72 hours, filtered and stored again in closed containers, and finally kept under refrigeration and protected from light until analysed.

The bioassay was performed with adult aphids collected from natural infestation sites in the forest specie *Gliricidia sepium* (Jacq.) Kunthe x Walp. (Fabaceae), who mainly attacked new shoots and flowers. The infested leaves and flowers were collected, and individuals with a size of approximately 2 mm were separated, corresponding to the adult phase. These were transferred in groups of ten, for each Petri dish, constituting one replicata. The plates were lined with moistened filter paper to preserve the turgidity of the food. As food, we used leaves from the species *Gliricidia sepium,* a cotton swab dipped in distilled water was

added to the petiole to avoid leaf dryness during the evaluation period of the experiment and moistened when necessary.

With the use of a fine-tipped brush, the adult aphids were placed on the Petri dish on the sheet and topical application was performed with the aid of a hand sprayer, with 0.2 mm of extract at concentrations of 3%, 6%, 9%, 12% and 15%, with 1% concentration of dimethylsulfoxide (DMSO) added at each concentration for the solubilization of the extracts. The control treatment 1 (distilled water + 1% DMSO) and control 2 (chemical insecticide (Fipronil, ia: 2.5% p/v)) were also used. The petri dishes containing the aphids were then sealed with plastic PVC film and conditioned in a Biochemical Oxigen Demand (BOD) chamber, with a temperature of 25 °C and a photoperiod of 12 hours.

The experiment was conducted in a completely randomized experimental design with five replicates, and each experimental unit consisted of ten aphids, totalling 50 aphids per treatment. Each treatment consisted of a different concentration, totalling 17 treatments and 850 aphids. The evaluations were performed daily until the 3rd day (24, 48 and 72 hours) after the application of the extracts.

Mortality data were transformed and adjusted via the formula $\sqrt{x+1}$ to meet the normality parameter, submitted to analysis of variance (ANOVA) in a 3x7 factorial scheme (three forms of obtaining and five concentrations with another control treatment 1 (distilled water + DMSO) and a control 2 (chemical insecticide). Means were compared by the Scott Knott test ($p < 0.05$), using the package ExpDes.pt (Ferreira et al. 2013) software R version 3.4 (R Development Core Equipe 2017).

Lethal concentrations (LC_s) were obtained by Probit analysis, using the Ecotoxicology package (Gama 2015) of software R, with mortality correction performed by Abbott's formula (1925) (EQUATION 1) and compared based on the respective confidence intervals at the 95% probability level (Finney 1971; Carvalho et al. 2017). The LC_{50} and LC_{90} values were calculated only for extracts that caused a mortality equal to or greater than 90% and variation of mortality between the tested concentrations. Lethal concentrations were calculated only for the period in which all the extracts of the evaluated forest species presented mortality rates of 90% to compare between the different species.

$$\text{MCorr} = \frac{\text{MT} - \text{MC}}{100 - \text{MC}} * 100$$

EQUATION 1:

Where: MCorr = corrected mortality (%); MT = treatment mortality (%) and MC = control mortality (%).

3. Results

There was an interaction between the extracts (aqueous, alcoholic and infusion) of the species *Cheiloclinium cognatum*, *Metrodorea flavida* and *Tetragastris altissima* and their concentrations on the mortality of *Aphis craccivora*. The mean accumulated mortality values of *Aphis craccivora* after 24, 48 and 72 hours of exposure to the aqueous, alcoholic and leaf infusion extracts of *Cheiloclinium cognatum* are presented in table 1.

Comparing the concentrations within each exposure period to the extracts of *Cheiloclinium cognatum* (Table 1), in the 24 hours period, the aqueous extract showed no difference in relation to the control 1, causing an average mortality of up to 10%. After 48 and 72 hours, there was a difference in relation to the control 1 at all concentrations evaluated, except at the concentration of 9%. In the 48 hours period, mortality was up to 16%, and after 72 hours, mortality reached 24%. In all the evaluated periods, the average mortality of the aqueous extract was different from that of the control 2.

For the alcoholic extract, there was a difference in relation to the control 1 in all the evaluated periods. In the 24 hours period, mortality was higher in the concentrations from 9%, ranging from 24 to 100%. In the period of 48 and 72 hours, there was no difference in mortality between the concentrations, with mean mortality ranging from 10 to 100% after 48 hours and from 20 to 100% after 72 hours. In all evaluated periods, the mean accumulated mortality in the concentrations of 12 and 15% of the alcoholic extract was similar to that of the control 2 (Table 1).

In the infusion extract, there was a difference in relation to the control 1 in all evaluated periods, after 24 hours in the concentrations of 6 and 9% (8 and 10% of mortality) and in the other periods in all concentrations. In the period of 48 hours, there was no significant difference between the concentrations, with a mean mortality of up to 18%, but after 72 hours, mortality reached 18 to 30% at concentrations from

9%. In all evaluated periods, the average mortality of the infusion extract was different from that of the control 2 (Table 1).

Table 1. Mean accumulated mortality (%) of *Aphis craccivora* after 24, 48 and 72 hours of application of aqueous, alcoholic extracts and infusion of *Cheiloclinium cognatum* leaves at different concentrations.

Conc[1] (%)	24 Hours*			48 Hours*			72 Hours*		
	Aqu[1]	Alc[1]	Inf[1]	Aqu[1]	Alc[1]	Inf[1]	Aqu[1]	Alc[1]	Inf[1]
Control 1	0^{B}	0^{C}	0^{C}	0^{C}	0^{D}	0^{C}	4^{C}	4^{D}	4^{D}
3	10^{Ba}	2^{Cb}	0^{Cb}	16^{Ba}	12^{Ca}	8^{Ba}	26^{Ba}	20^{Ca}	14^{Ca}
6	6^{Ba}	6^{Ca}	8^{Ba}	12^{Ba}	10^{Ca}	14^{Ba}	18^{Ba}	28^{Ca}	16^{Ca}
9	4^{Bb}	24^{Ba}	10^{Bb}	6^{Cb}	30^{Ba}	18^{Ba}	20^{Bb}	64^{Ba}	30^{Bb}
12	4^{Bb}	96^{Aa}	2^{Cb}	12^{Bb}	100^{Aa}	18^{Bb}	24^{Bb}	100^{Aa}	28^{Bb}
15	8^{Bb}	100^{Aa}	4^{Cb}	10^{Bb}	100^{Aa}	10^{Bb}	24^{Bb}	100^{Aa}	18^{Bb}
Control 2	100^{A}	100^{A}	100^{A}	100^{A}	100^{A}	100^{A}	100^{A}	100^{A}	100^{A}
CV (%)		30.72			29.43			24.45	

[1]Concentration (Conc), Aqueous Extract (Aqu), Alcoholic Extract (Alc) and Infusion (Inf). *For statistical analysis, the data were transformed into $\sqrt{x+1}$. Averages followed by the same letter, uppercase in the column and lower case in the row for each evaluation period, do not differ statistically from one another by the Scott Knott test ($p < 0.05$).

Regarding the method of obtaining *Cheiloclinium cognatum* extracts, concentrations of 3 and 6% showed no mortality difference in the evaluated periods, except in the concentration of 3% in the 24 hours period, with a higher mortality in the aqueous extract. In all periods evaluated at concentrations of 9, 12 and 15%, the alcoholic extract presented a higher average mortality, although it did not differ from that of the infusion extract at the 9% concentration after 48 hours. On the other hand, the aqueous and infusion extracts did not significantly differ (Table 1).

The mean accumulated mortality of concentrations 3, 6, 9, 12 and 15% after an exposure of 24, 48 and 72 hours to the extracts of *Metrodorea flavida* on *Aphis craccivora* is shown in table 2.

Table 2. Mean accumulated mortality (%) of *Aphis craccivora* after 24, 48 and 72 hours of application of aqueous extracts, alcoholic and infusion of leaves of *Metrodorea flavida* in different concentrations.

Conc[1] (%)	24 Hours*			48 Hours*			72 Hours*		
	Aqu[1]	Alc[1]	Inf[1]	Aqu[1]	Alc[1]	Inf[1]	Aqu[1]	Alc[1]	Inf[1]
Control 1	2^{B}	2^{C}	2^{C}	6^{B}	6^{B}	6^{B}	14^{C}	14^{C}	14^{B}
3	2^{Ba}	4^{Ca}	2^{Ca}	12^{Ba}	10^{Ba}	8^{Ba}	52^{Ba}	58^{Ba}	32^{Bb}
6	4^{Bb}	62^{Ba}	2^{Cb}	12^{Bb}	64^{Aa}	6^{Bb}	38^{Bb}	82^{Aa}	28^{Bb}
9	2^{Bb}	94^{Aa}	2^{Cb}	12^{Bb}	96^{Aa}	10^{Bb}	44^{Bb}	98^{Aa}	26^{Bb}
12	0^{Bb}	90^{Aa}	4^{Cb}	6^{Bb}	90^{Aa}	12^{Bb}	26^{Cb}	98^{Aa}	26^{Bb}
15	4^{Bc}	100^{Aa}	12^{Bb}	10^{Bb}	100^{Aa}	18^{Bb}	52^{Bb}	100^{Aa}	42^{Bb}
Control 2	78^{A}	78^{B}	78^{A}	100^{A}	100^{A}	100^{A}	100^{A}	100^{A}	100^{A}
CV (%)		25.35			26.94			26.9	

[1]Concentration (Conc), Aqueous Extract (Aqu), Alcoholic Extract (Alc) and Infusion (Inf). *For statistical analysis, the data were transformed into $\sqrt{x+1}$. Averages followed by the same letter, uppercase in the column and lower case in the row for each evaluation period, do not differ statistically from one another by the Scott Knott test ($p < 0.05$).

Analyzing the extracts of *Metrodorea flavida* (Table 2), in the periods of 24 and 48 hours, the aqueous extract did not differ in relation to the control 1. Additionally, there was no difference between the concentrations, with a mortality of up to 4% after 24 hours and up to 12% after 48 hours. In the 72 hours period, the concentrations tested reached a mortality of 52%, with a difference in relation to the control 1, except in the concentration of 12%.

For the alcoholic extract in all the evaluated periods, the concentrations differed from those in the control 1, except for the concentration of 3% after 24 and 48 hours. In the 24 hours period, the mortality rates differed among the tested concentrations, with 62% in the concentration of 6%, similar to the control

2, which obtained 78%. In the 24 hours period, at the concentration of 9%, mortality rates ranged from 94 to 100% and were higher than the control 2. After 48 hours, at a concentration of 6%, mortality rates were higher than 64% and similar to that of the control 2 (100% mortality). In the 72 hours period, mortality ranged from 58 to 100%, and concentrations of 6% (above 80% mortality) were not significantly different from the control 2 (Table 2).

In the evaluated periods, the infusion extract showed no difference among the concentrations or in relation to the control 1, except in the concentration of 15% in the 24 hours period, which reached a mortality of 12%. After 48 hours, mortality varied from 6 to 18%, reaching 32% after 72 hours. In addition, in all the periods evaluated in the different concentrations tested, there was no similarity to the control 2.

There were no differences in mean mortality at the concentration of 3% for the extracts of *Metrodorea flavida*, except after 72 hours, where the aqueous and alcoholic extracts presented a higher mortality. The alcoholic extract presented a higher average mortality from the concentration of 6% in all evaluated periods. However, the aqueous and infusion extracts presented similar results, differing only in the concentration of 15% after 24 hours, where the infusion extract obtained higher mortality when compared to the aqueous extract (Table 2).

Table 3 shows the average accumulated mortality of *Aphis craccivora* after 24, 48 and 72 hours of application of aqueous extracts, alcoholic and infusion of leaves of *Tetragastris altissima* at different concentrations.

Table 3. Mean accumulated mortality (%) of *Aphis craccivora* after 24, 48 and 72 hours of application of aqueous extracts, alcoholic and infusion of leaves of *Tetragastris altissima* in different concentrations.

Conc[1] (%)	24 Hours*			48 Hours*			72 Hours*		
	Aqu[1]	Alc[1]	Inf[1]	Aqu[1]	Alc[1]	Inf[1]	Aqu[1]	Alc[1]	Inf[1]
Control 1	2^{B}	2^{D}	2^{B}	4^{B}	4^{D}	4^{B}	10^{C}	10^{C}	10^{C}
3	4^{Ba}	4^{Da}	6^{Ba}	18^{Ba}	12^{Ca}	24^{Ba}	40^{Ba}	42^{Ba}	38^{Ba}
6	10^{Ba}	0^{Db}	2^{Bb}	18^{Ba}	$6D^{a}$	14^{Ba}	38^{Ba}	44^{Ba}	34^{Ba}
9	6^{Ba}	12^{Ca}	0^{Bb}	12^{Ba}	20^{Ca}	10^{Ba}	38^{Bb}	82^{Aa}	32^{Bb}
12	8^{Bb}	36^{Ba}	4^{Bb}	18^{Bb}	44^{Ba}	6^{Bb}	48^{Bb}	92^{Aa}	28^{Bc}
15	2^{Bb}	38^{Ba}	2^{Bb}	10^{Bb}	40^{Ba}	12^{Bb}	32^{Bb}	84^{Aa}	44^{Bb}
Control 2	74^{A}	74^{A}	74^{A}	100^{A}	100^{A}	100^{A}	100^{A}	100^{A}	100^{A}
CV (%)		39.6			35.75			27.25	

[1]Concentration (Conc), Aqueous Extract (Aqu), Alcoholic (Alc) and Infusion (Inf). *For statistical analysis, the data were transformed into $\sqrt{x+1}$. Averages followed by the same letter, uppercase in the column and lower case in the row for each evaluation period, do not differ statistically from one another by the Scott Knott test ($p < 0.05$).

In the analysis of extracts of *Tetragastris altissima* (Table 3), in the 24 hours period, the aqueous extract presented a mortality of up to 10%, while in the period of 48 hours, mortality was up to 18%, in the two periods evaluated, no differences occurred between the concentrations. Only from 72 hours onwards, the aqueous extract showed a significant difference in mortality in relation to the control 1, varying from 32 to 48%. The mean mortality of the aqueous extract was not similar to that of control 2 in any of the evaluated periods.

The alcoholic extract in all periods presented a difference in relation to the control 1, being in the period of 24 hours from the concentrations of 9% with mortalities above 12% and after 48 hours in the concentrations of 3, 9, 12 and 15% with mortalities from 12 to 44%. In both periods, there was no similarity to control 2. There was a significant increase in mortality in the evaluation of 72 hours, providing a mortality from 42 to 92%, and in the concentrations from 9%, mean mortality was similar to that of control 2 (Table 3).

In the infusion extract in the 24 and 48-hours evaluation, there was no difference in relation to control 1 or between the concentrations, with mortality up to 6% in the first period and up to 24% in the second evaluation period. However, after 72 hours, the mean mortality at different concentrations differed from that of control 1, varying from 28 to 44%, with no difference between the concentrations. In all periods, the mean mortality of the infusion extract was not similar to that of control 2 (Table 3).

Regarding the methods of extracting the extracts, we observed no difference in mean mortality at the 3% concentration in all evaluated periods. We also observed no difference in mortality at the concentration of 6%, except in the 24 hours period, in which the aqueous extract presented a higher mortality. For the concentration of 9% in the period of 24 hours, the alcoholic extract obtained higher mortality, not differing from that of the aqueous extract, this was also the case for the period of 72 hours. At concentrations of 12 and 15%, the alcohol extract also caused a higher mortality in all the evaluated periods, and there was no difference between the aqueous and the infusion extracts, except for the concentration of 12% in the period of 72 hours, in which the aqueous extract presented a higher mortality (Table 3).

Table 4 shows the concentration-response results of the alcoholic extract for *Cheiloclinium cognatum*, *Metrodorea flavida* and *Tetragastris altissima* regarding *Aphis craccivora.*

Table 4. Lethal concentration of alcoholic extract of leaves *Cheiloclinium cognatum*, *Metreodora flavida*, *Tetragastris altissima* in *Aphis craccivora*, in the 72 hours period.

Spe.[1]	N	Slope (±EP)	LC_{50} (IC_{95}) % (*)	LC_{90} (IC_{95}) % (*)	X^2	GL	p-valor
Cc	250	4,598±1,511	6,43 (5,25; 7,54)^{b}	12,22 (10,14; 16,61)^{ab}	28,947	3	0,9999
Mf	250	3,562 ±0,476	3,08 (1,80; 4,02)^{a}	7,05 (5,53; 10,64)^{a}	2,614	3	0,5449
Ta	250	2.586±0,824	5,58 (3,77; 7,15)^{ab}	17,47 (12,45; 37,65)^{b}	15,341	3	0,9984

[1]Species (Spe.); *Cheiloclinium cognatum* (Cc), *Metreodoraflavida* (Mf), *Tetragastrisaltissima* (Ta); N: Number of insects used; CI: Confidence interval; X^2: Chi-square; (*): Significant difference based on confidence intervals at 95% probability.

The median lethal concentration (LC_{50}) of the alcoholic extract of *Cheiloclinium cognatum* was 6,43%, that of *Metreodora flavida* was 3,08%, and that of *Tetragastris altissima* was 5,58% (Table 4). Angular coefficients were different, with the coefficient of *Cheiloclinium cognatum* (4,598) higher than the other species, followed by *Metreodora flavida* (3,562) and *Tetragastris altissima* (2,586) (Table 4), showing that small changes in concentrations for *Cheiloclinium cognatum* result in a faster response of the mortality of aphids. However, the *Metreodora flavida* species had lower LC_{50} and LC_{90} values, which implies that this species shows higher mortality of *Aphis craccivora* with lower concentrations than the other species studied. The LC_{50} of the species *Tetragastris altissima* was similar to that of the other ones, based on the confidence interval at the 95% probability level. For the LC_{90}, an inversion was observed, with the species *Cheiloclinium cognatum* presenting similar results when compared to the other species (Figure 1).

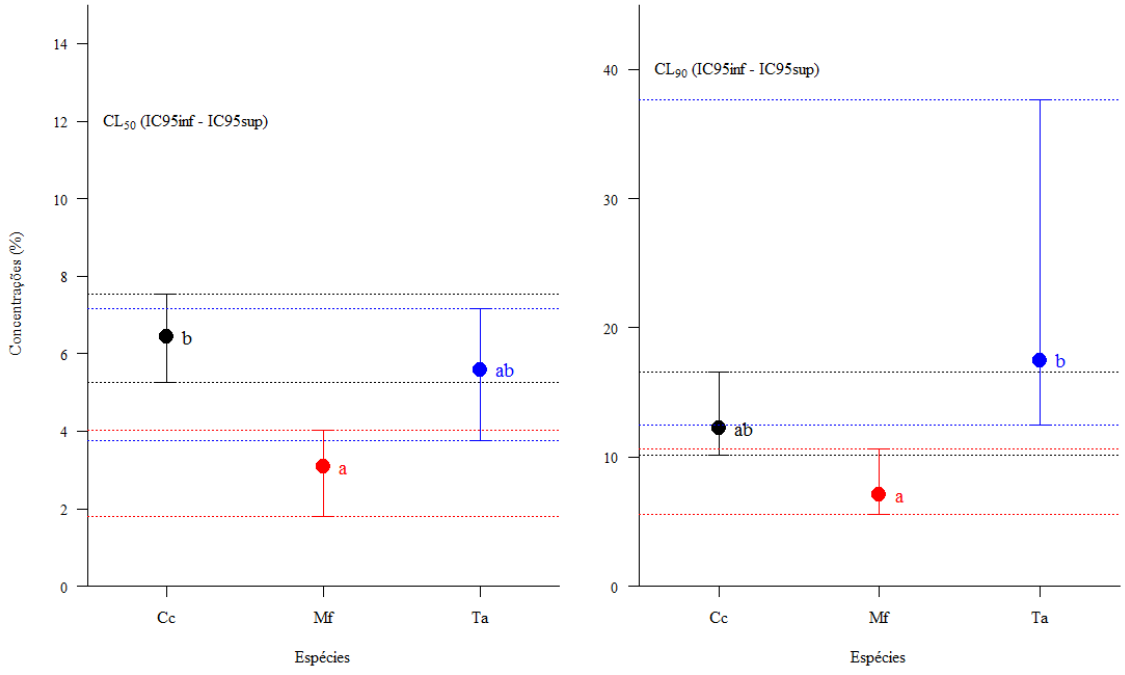

Figure 1. Comparison of lethal concentrations (LC_{50} and LC_{90}) of the alcoholic extracts of *Cheiloclinium cognatum* (Cc), *Metreodora flavida* (Mf), *Tetragastris altissima* (Ta). Mean [lower IC95 and higher IC95] accompanied by the same letter do not differ from each other based on the overlap of confidence intervals.

Figure 2 shows that the slope of the *Cheiloclinium cognatum* curve is greater than that of the *Metreodora flavida*, in addition, when the curves of the species *Cheiloclinium cognatum* and *Tetragastris altissima* overlap, LC_{90} inversion occurs.

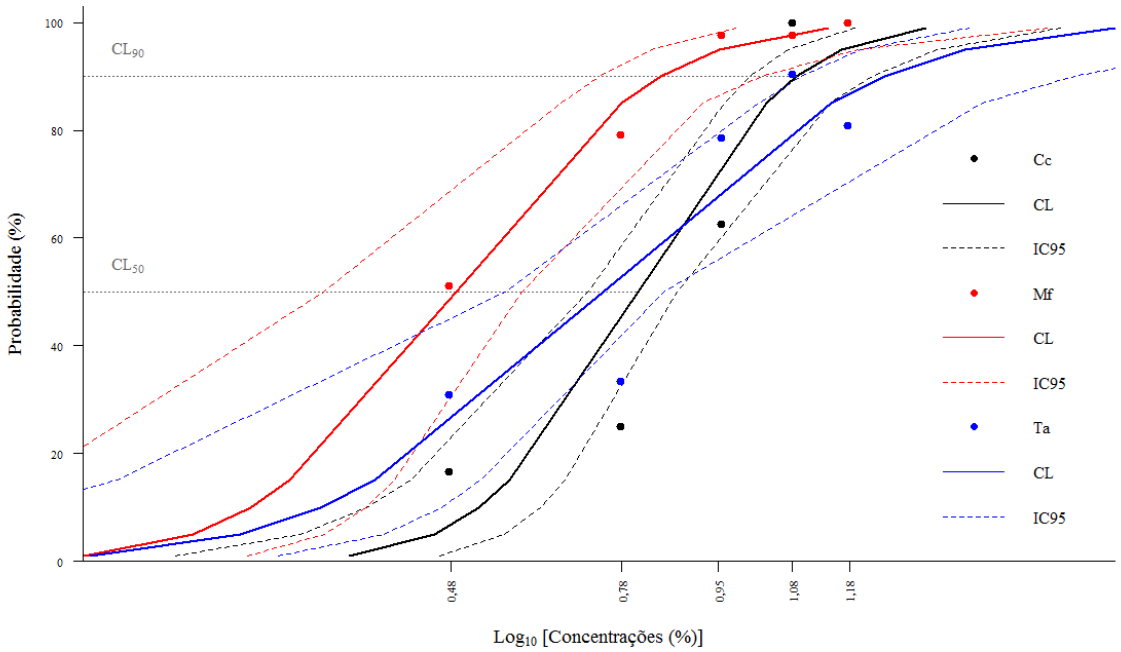

Figure 2. Concentration-mortality curves of the alcoholic extracts of leaves of *Cheiloclinium cognatum* (Cc), *Metreodora flavida* (Mf), *Tetragastris altissima* (Ta) for *Aphis craccivora*, after correction of mortality by the Abbott formula.

4. Discussion

In general, mortality of *Aphis craccivora* after the topical application of aqueous, alcoholic and infusion extracts of the species *Cheiloclinium cognatum*, *Metrodorea flavida* and *Tetragastris altissima* increased with the increase of the evaluation period. In all studied species, the aqueous and infusion extracts presented lower mortality (less than 52%), whereas the alcoholic extracts in the highest concentrations of *Cheiloclinium cognatum* and *Metrodorea flavida* had a mortality of up to 100% and for the species *Tetragastris altissima,* mortality reached 92%.

This higher mortality observed in the alcoholic extract in all the evaluated species can be attributed to the solvent used. Alcohol may have contributed to the extraction of metabolites that are not obtained with the water solvent or are extracted in larger amounts. The chemical nature of these compounds varies from simple to highly polarized, with a great variety of bioactive compounds in the plants and different amounts present, besides the possibility of interaction of the compounds with carbohydrates, proteins and other components. Some of these complexes, as well as some phenolics with high molecular weight, are highly insoluble in water (Andreo and Jorge 2006).

The type of solvent and the concentration are relevant factors that can influence the extraction of metabolites (Andreo and Jorge 2006). Silva et al. (2017a) verified that there were differences in the results when they compared different types of extracts (solvent: distilled water; alcoholic solvent: absolute ethanol; and hydroalcoholic solvent: water and absolute ethanol (1: 1)).

In this study, for the aqueous extract and infusion, the evaluated species presented a mortality of up to 52% in concentrations of up to 15%. Ghanim and Ghani (2014), studying the effects of aqueous extracts of plants on *Aphis gossypii* Glover under laboratory conditions, found that at the concentration of 6%, after 72 hours of application, the aqueous extract of leaves of *Pelargonium zonale* caused a mortality of 96%, and *Melia azedarach* fruit extract obtained 91,8%. The species evaluated by these authors showed higher mortality, demonstrating that in the conditions evaluated in this study, the aqueous and infusion extracts were not efficient for aphid nymphs.

The alcoholic extract had a mortality of 100% after 24 hours for the *Cheiloclinium cognatum* and *Metrodorea flavida* species, and for *Tetragastris altissima,* mortality reached 92% after 72 hours. Rabelo and Bleicher (2014) in an experiment carried out in a greenhouse, also studied alcoholic extracts of seeds of atemoia (*Annona cherimolamill x Annona squamosa L.)* and ata (*Annona squamosa*) (Anonaceae) at 0,5% and verified efficiencies of 98,18 and 99,27%, respectively, after 48 hours of application, attributing such effect to the active control of the black aphid. As for Rabelo and Bleicher (2014), the alcoholic extract of leaves of the species studied also present a higher mortality, but in higher concentrations and different plant parts.

The calculated lethal concentrations for the species studied were as follows: LC_{50}=6,43% (*Cheiloclinium cognatum),* LC_{50}=3,08% (*Metrodorea flavida),* LC_{50}=5,58% (*Tetragastris altissima).* These results reinforce the evidence that for the species *Metreodora flavida,* the lowest concentration tested was able to control at least 50% of the *Aphis craccivora* population, while the species *Cheiloclinium cognatum* and *Tetragastris altissima* require higher concentrations in the 72 hours period. A study carried out by Bandeira et al. (2017) obtained an LC_{50} of 7,69% after 48 hours of application of the hydroethanolic extract of *Annona montana*, on *Aphis craccivora* under greenhouse conditions. The lethal concentrations calculated for the alcoholic extract of the species studied after 72 hours under laboratory conditions were smaller when compared with those obtained by Bandeira et al. (2017), but in a longer evaluation period.

The insecticidal potential of *Metrodorea flavida* can be attributed to the fact that this species belongs to the family Rutaceae, commonly known as the citrus family. These plants have highly fragrant flowers, some are constituents of essential oils such as citronella. Rutaceae is one of the families that are the main sources of terpenes with insecticidal activity, for example, limonoids (Viegas Jr 2003), phenols, flavonoids, tannins (Loizzo et al. 2018). Spletozer et al. (2015) qualitatively analyzed the presence of secondary metabolites in *Metrodorea flavida* and phytochemical tests were positive for tannins, alkaloids, saponins and flavonoids. Also, this family presents species with insecticidal properties, for example, the essential oil of leaves of *Murraya exotica* L. which is a promising source against *Aedes aegypti* L., *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say (Krishnamoorthy et al. 2015), macerated leaves of *Ruta graveolens* L., which have effects on larvae of *Spodoptera frugiperda* (J. E. Smith) (Tagliari et al. 2010), the essential oil of *Citrus aurantium* L., which presents fumigation toxicity against *Spodoptera littoralis* (Boisd.) Larvae (Laarif et al. 2013) and others (Tagliari et al. 2010; Barakat 2011; Baskar et al. 2012; Krishnappa and Elumalai 2012).

Cheiloclinium cognatum, as mentioned above, has shown that small changes in concentrations result in a more rapid response. This species was reported with potential, because when it is cultivated *in vitro*, it is an important source of raw material to obtain pentacyclic triterpenes and quinona methides (Pina et al. 2017). It is inferred here that the presence of these substances may have caused a higher mortality when the concentration of the extract increased. Numerous species of the Celastraceae family are known, especially in China and Latin America, for their use as insecticide in traditional agriculture (Spivey et al. 2002). Several species of this family have been studied for pharmacological purposes since they present sesquiterpenos, alkaloids, flavonoids and other important secondary metabolites (Silva et al. 2014), being considered a source of important secondary bioactive metabolites.

The species *Tetragastris altissima* took longer to present higher mortality compared to the other species, that is, it presented a low mortality in the initial period (24 hours). A study carried out with resin extract of *Protium* sp. (Breu-white), a species belonging to the same family (Burseraceae), at concentrations of 10% on *Brevicoryne brassicae* (L.) nymphs, also provided a lower mortality rate of 15,9% within 24 hours (Silva et al. 2017b). This shows that this species has a slower effect on aphids. However, it still is a potential species, because studies with resins obtained from different parts of Burseraceae species, such as leaves, fruits and wood, demonstrate a chemical composition, mainly terpenic, with large amounts of monoterpenes, sesquiterpenes and triterpenes. In addition, other secondary metabolites, such as coumarins, flavonoids and lignoids, have also been reported for this family (Rudiger et al. 2007; Gadir and Ahmed 2014).

The alcoholic extract resulted in a higher mortality, and considering that the aphids reproduce quickly, the control is sought in a shorter period. In addition, the immediate action of the product is also an important aspect, reducing the risk of loss of the product via rain events. Therefore, the alcoholic extract at the concentration of 12% for *Cheiloclinium cognatum* caused a mortality above 90%. The species *Metrodorea*

flavida also presented a mortality above 90% within 24 hours from a concentration of 9%. The species *Tetragastris altissima* took more time to present similarities to the control, with a mortality above 80% at the concentration of 9%, with results after 72 hours.

The species evaluated in this study are promising. They can be studied at the level of identification of classes and substances present, besides being indicated studies of other parts of the plants to identify, where these species present larger amounts of the metabolites and also the isolation to identify which substance is responsible for the effect on aphids.

5. Conclusions

The mortality of *Aphis craccivora* increases with the increasing period of exposure to leaf extracts of the species *Cheiloclinium cognatum*, *Metrodorea flavida* and *Tetragastris altissima*. The lethal concentration for the alcoholic extracts of *Cheiloclinium cognatum* are LC_{50} 6,43% and LC_{90} 12,22%, while for *Metrodorea flavida,* they are LC_{50} 3,08% and LC_{90} 7,05% and *Tetragastris altissima* LC_{50} of 5,58% and LC_{90} of 17,47%, after 72 hours. The use of the alcoholic extract of the species *Metrodorea flavida* in the concentration of 9% in the control of *Aphis craccivora* is indicated.

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