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Original Article

Efficiency of essential oils in the control of the black bean aphid *Aphis craccivora* Koch (Hemiptera: Aphididae)

Eficiência de óleos essenciais no controle do pulgão preto do feijoeiro *Aphis craccivora* Koch (Hemiptera: Aphididae)

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Abstract

The black aphid *Aphis craccivora* Koch is one of the main pests of the caupi-bean crop *Vigna unguiculata* (L.) Walp. Due to the need to find effective and safe methods of control, there has been an increase in research seeking natural alternatives. Thus, the objective of this work was to evaluate the potential of essential oils from jatoba *Hymenaea courbaril*, copaiba *Copaifera langsdorffii* and aroeira *Schinus terebinthifolius* to control nymphs and adults of *A. craccivora*. The oils were extracted from the leaves by the hydrodistillation method, diluted to 0.1% in distilled water with 2% dimethyl sulfoxide (DMSO). Each treatment had four repetitions, plus a control with distilled water + 2% DMSO. The biotests were conducted in two stages: the first was conducted in the laboratory, under controlled conditions of temperature, relative humidity and photophase, and the second was conducted in the greenhouse, using only the treatment with the best laboratory test results. After 24, 48, 72, 96 and 120 hours of exposure, the insect mortalities were checked. In the first phase of the experiment, the aroeira oil showed 83.33% and 75.75% efficiency of mortality in nymphs and adults, respectively. In the greenhouse tests, this same oil showed 73.52% in nymphs and 62.85% in adults, opening new perspectives regarding its use as a natural insecticide for the control of the black aphid of the bean.

Keywords: caupi-bean, aphids, mortality.

Resumo

O pulgão preto *Aphis craccivora* Koch é uma das principais pragas da cultura do feijão-caupi *Vigna unguiculata* (L.) Walp. Em virtude da necessidade de encontrar métodos eficazes e seguros de controle, tem-se aumentado as pesquisas buscando alternativas naturais. Dessa forma, o objetivo deste trabalho foi avaliar o potencial dos óleos essenciais de jatobá *Hymenaea courbaril*, copaíba *Copaifera langsdorffii* e aroeira *Schinus terebinthifolius* para controlar ninfas e adultos de *A. craccivora*. Os óleos foram extraídos das folhas pelo método de hidrodestilação, diluídos a 0,1% em água destilada com Dimetilsufóxido a 2% (DMSO). Cada tratamento possuiu quatro repetições, além da testemunha com água destilada + DMSO 2%. Os biotestes foram conduzidos em duas etapas: a primeira foi realizada em laboratório, sob condições controladas de temperatura, umidade relativa do ar e fotofase e a segunda foi realizada em casa de vegetação, utilizando apenas o tratamento com melhor resultado do teste em laboratório. Depois de transcorridos 24, 48, 72, 96 e 120 horas de exposição, foram verificadas as mortalidades dos insetos. Na primeira fase do experimento, o óleo de aroeira apresentou 83,33% e 75,75% de eficiência de mortalidade em ninfas e adultos, respectivamente. Nos testes em casa de vegetação, esse mesmo óleo apresentou 73,52% em ninfas e 62,85% e em adultos, abrindo novas perspectivas quanto à sua utilização como inseticida natural para o controle do pulgão preto do feijoeiro.

Palavras-chave: feijão-caupi, afídeos, mortalidade.

1. Introduction

The caupi-bean *Vigna unguiculata* (L.) Walp., known in the Brazilian northeast as “feijão-de-corda”, is a rustic leguminosae with great productive capacity, cultivated mainly by small and average farmers in northern and northeastern Brazil (Fontes et al., 2011), in which its high nutritional value contributes to be one of the main elements of the diet of the population (Souza et al., 2019).

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Despite its high economic and social importance, this crop still has low productivity due to several factors, such as the use of cultivars with low productive potential, soils with insufficient nutrient availability, lack of use of appropriate technologies, and problems with weeds, pests, and diseases (Nodari and Guerra, 2015).

Insect pests, in turn, are among the biotic factors that most compromise the productivity of the cowpea bean, since they cause direct and indirect damage, in addition to regular and intense attacks (Ferreira et al., 2020). The black aphid bean *Aphis craccivora* Koch 1854 stands out, an insect that causes a drop in productivity due to the damage it causes to the crop from the emergence of seedlings to the reproductive phase (Vieira et al., 2019).

Both the nymphs and the adult aphid insects are capable of causing damage to the crop because of their feeding habit of sucking the sap from the plant, causing nutritional deficiency and loss of secondary compounds. In addition, this insect is a vector of viruses and the presence of this insect is related to the development of the *Capnodium* fungus, popularly known as fumagin, which develops on the sugar substance produced by the aphid (Cosmo and Galeriani, 2020).

The current management of aphids consists mainly in the use of chemical insecticides (Moraes and Marinho-Prado, 2016), and this type of control can lead to a series of problems, among them, the resistance of the pest to the products, intoxication and environmental imbalances (Neves et al., 2020), thus, several studies are conducted in order to find alternative sources to the use of synthetic chemical insecticides (Avelino et al., 2019), becoming a viable alternative for organic producers (Radünz et al., 2024).

Thus, the present work aims to evaluate the toxicity of the essential oils of jatoba *Hymenaea courbaril* L., copaiba *Copaifera langsdorffii* Desf. and mastic *Schinus terebinthifolius* Raddi, for the control of nymphs and adults of the black aphid, in laboratory and greenhouse conditions.

2. Material and Methods

2.1. Location

The research was conducted in the Laboratory of Agricultural Entomology of the Federal University of Cariri, in the Center for Agricultural Sciences and Biodiversity (CCAB), in the municipality of Crato-CE, under controlled conditions of temperature (25 ± 2º C), relative humidity (70 ± 10%), obtained by means of a thermo-hygrometer and photophase of 12 hours in an air-conditioned chamber type B.O.D. (Biochemical Oxygen Demand), Eletrolab brand, EL202, São Paulo, Brazil and in a greenhouse of Embrapa Algodão, in the municipality of Barbalha, from March to December 2022.

2.2. Obtaining the leaves and insects for the experiments

The plant materials, leaves and branches, from aroeira trees, copaiba and jatoba for obtaining essential oils, were collected in the Araripe National Forest with the authorization of the competent body for the area through the System of Authorization and Information on Biodiversity (SISBio). The black aphids used in the tests were collected in the experimental field of CCAB, which were duly identified with the aid of an optical microscope, using the identification key at species level proposed by Blackman and Eastop (2000). The cowpea plants, in turn, were grown in 2-liter plastic pots, where three bean seeds were sown per pot, using two parts of soil to one part of manure and, after germination, thinning was performed, leaving only one plant per pot.

2.3. Essential oil extraction and yield

For oil extraction, the leaves of the plants were washed in running water, dried at room temperature and cut into small pieces with the help of machetes and pruning shears. The essential oils were extracted using the hydrodistillation method in a Clevenger distiller according to the method described by Alencar et al. (1984). About 300g of leaves were weighed and placed submerged in 3,000 mL of distilled water in a 5,000 mL capacity flat bottom flask, establishing an extraction period of 120 minutes. The extraction procedure was repeated in order to obtain a satisfactory amount. After the extraction period, the essential oils were removed from the apparatus using a Pasteur pipette, stored in hermetically sealed glass flacons, covered with aluminum foil, protected from light, and kept in a domestic refrigerator at 5°C for later chromatographic analysis and testing in the greenhouse and laboratory.

The identifications of the essential oil components were performed by gas chromatography with a mass selective detector (GC-MS) Agilent 5975C TAD Series GC/MSD, equipped with a non-polar chromatographic column, stationary phase (5% phenyl)-methylpolysiloxane (DB-5), following the chromatographic conditions described by Silva et al. (2016). Quantitative analyses were performed under the same conditions as those described for GC-MS in a Thermo Trace-GC Ultra equipment. The identifications were made by GC-MS through comparison with the mass spectra obtained by the equipment with those from libraries (MassFinder 4, Dr. Hochmuth scientific consulting, Hamburg, Germany); NIST08 Mass Spectral Library (ChemSW Inc. Fairfield, CA, USA); Wiley Registry™ of Mass Spectral Data 9th Edition (Wiley, Hoboken, NJ, USA). It was also compared with the spectra published by Adams (2007) and their retention indices calculated by co-injection of the essential oils sample with solution of C9-C30 hydrocarbon standards, calculated by the van Den Dool and Kratz (1963) equation. GC quantifications were done in triplicate to obtain standard deviation.

2.4. Toxicity of essential oils on A. craccivora under controlled conditions

To evaluate the insecticidal effect of the essential oils on aphids under controlled conditions, they were diluted to 0.1% in distilled water with 2% Dimethylsufoxide (DMSO). The solution was stirred manually in a 200 mL volumetric flask in order to obtain a homogeneous solution. The experimental design was entirely randomized and represented by the oils of the three plant species and the five exposure periods of the grouts, in a 3x5 factorial scheme.

The control treatment, in turn, consisted of distilled water + solvent, and each treatment and control had four repetitions, tested on nymphs and adults of the black aphid.

The sampling unit consisted of a leaf disk of the cowpea bean placed with the abaxial side up inside a 9.5 cm diameter and 1.5 cm high glass Petri dish. Cotton moistened with distilled water was placed under the leaf and around the petiole to avoid rapid dehydration and prevent the aphids from escaping. To standardize the insects to be used in the bioassays, adult aphids remained for 24 hours on plates containing the leaf discs in a B.O.D. chamber at a temperature of 25 ± 2°C, relative humidity of 70 ± 10% and photoperiod of 12 hours. The nymphs produced during this period were used for the application of treatments, using nymphs with larger body size as a criterion for selection.

0.5 mL of the respective grouts were applied to the leaves with a 50 mL sprayer and after drying for 30 minutes in open air, 10 nymphs were deposited on each sample with the help of a soft, fine bristle brush moistened with distilled water to avoid any type of injury to the insect. Then the plates were covered with plastic film, perforated with a thin pin to avoid excessive humidity, and placed in the B.O.D. chamber under the conditions described above. Readings in the treatments were taken at 24, 48, 72, 96 and 120 hours after exposure of the nymphs to the oils, considering dead those that did not react to mechanical stimulus from a fine bristle brush. This same evaluation was performed with adult black aphid insects according to the methodology described above.

2.5. Toxicity of essential oils on A. craccivora in greenhouse

To evaluate the insecticidal effect of the essential oils under greenhouse conditions, the treatments used were those that obtained the best results in the laboratory tests, which in this case was the mastic tree oil, with four repetitions each, in addition to the control treatment.

Bean plants previously sown, with 30 days after sowing, were kept in the greenhouse of Embrapa Algodão, in which they were infested by aphids and after 24 hours, these were removed, remaining only 10 nymphs produced in this period in each plant, aiming to standardize the age of the insects. In addition, white plastic plates were placed at the base of the stem in order to better visualize the dead insects that were detached from the plant.

0.5 mL per leaf was applied using a 50 mL sprayer. Subsequently, the pots were wrapped in a “voil” type fabric and tied with string to avoid infestation by other pests and to control the population of the species under study. Readings were taken in the treatments after 24, 48, 72, 96, and 120 hours of insect exposure to the oils. Insects that did not react to mechanical stimulation with a fine bristle brush dampened with distilled water were considered dead. This same test was also performed with adult aphid insects, following the same methodology.

2.6. Statistical analysis

The data obtained were submitted to variance analysis using the Operational Program SISVAR-UFLA (Ferreira, 2011). In addition, the mortality efficiency of insects was calculated using Abbott’s (1925) formula: E (%)= ((Nc - Nt)/Nc)x100. Where: E = efficiency; Nc = number of live individuals in the control treatment and Nt = number of live individuals in the treatments. Regarding hydrodistillation, the yields of essential oils were calculated from the formula: R (%) = (mass of oil obtained (g)/mass of leaves (g)) x 10.

3. Results and Discussion

3.1. Yield of essential oils

To have a satisfactory yield, considering that each plant species produces variable amounts of essential oils, depending on the location and soil and climate conditions, two extractions were made from the leaves of aroeira trees, two of copaiba and four of jatoba. The yields were 0.25%, 0.18% and 0,05%, respectively, and the essential oils of aroeira and copaiba were close, producing 1.38 and 1.26g of oil, respectively (Table 1). The jatoba oil, on the other hand, produced a smaller amount of oil, 0.84g, even using about three times more material than the other species and performing two more extractions.

The yield of jatoba oil (0.05%), despite being the one that obtained the lowest result among the species under study, corroborated with the values found in the literature, which range from 0.03 to 0.6% (Mercês, 2015). Moreover, this value remained very close to that obtained in the work of Sales (2014), 0.052%, even though its extraction was performed through the fruit peels of *H. courbaril*.

The yield of aroeira oil, in turn, obtained the highest result among the extracted oils (0.25%), but this value was lower than those found in the literature, which range from 0.45% (Tomazoni et al., 2017) to 0.8% (Santos et al., 2013). This variation in yield, as well as occurs in the chemical composition of oils, occurs due to differences between the various environmental factors, in addition to the extraction technique used and the time of collection of plant material, since the highest production of secondary metabolites occurs under high levels of solar radiation, as a result of biosynthetic reactions that occur through photosynthetic processes (Taiz and Zeiger, 2009), and the material in this study was collected at 9:00 am on a cloudy day.

Table 1. Yield of essential oils of the three species under study. Crato - CE, 2022.

Species	Scientific name	Leaf mass (g)	Oil mass (g)	Yields (%)
Aroeira	*Schinus terebinthifolius*	545.59	1.38	0.25
Copaíba	*Copaifera langsdorffii*	694.30	1.26	0.18
Jatobá	*Hymenaea courbaril*	1.455	0.84	0.05

Regarding the oil of copaiba, Pereira et al. (2008) found oil yield values of 2.29, 0.20 and 1.04%, extracted from the pericarp of the plant, with the first value resulting from three hours of extraction, the second from another three hours and the third from another three hours, totaling a yield of 3.53% after nine hours of extraction and 240 hours of contact of the plant material with water between one distillation and another, proving that the time directly influences the result of the yield.

3.2. Laboratory testing

When testing the insecticidal effect of essential oils on aphid nymphs and adults under laboratory conditions, it was possible to see that mortality in all treatments increased as exposure time passed, with higher mortality in insects submitted to mastic oil, which reached a percentage of 83.33% in nymphs and 75.75% in adults at 120 hours. Moreover, at 48 hours this treatment was able to control more than half of the nymph population (Figure 1).

According to the analysis of variance, it was found that all treatments and their interactions were significant at 1% probability, and the coefficient of variation was 12.32%, indicating a very homogeneous set of data.

In the experiment developed by Santos et al. (2013), the essential oil of *S. terebinthifolius* also obtained satisfactory results in controlling the coffee berry borer *Hypothenemus hampei* Ferrari, whose concentration of 10^{-2} (0.01%) reached 90% mortality after 24 hours of exposure and with 48 hours reached total efficiency in combating the larva of the coleopteran. In addition, the authors observed that there was practically no variation between the exposure periods (24 and 48 hours), indicating that the topical exposure of the coffee berry borer to the oil causes rapid mortality. Compared to the results of the present study, this high level of mortality in the first periods of exposure can be explained by the fact that the authors used acetone to dilute the oil, and that its application was topical and not residual, as occurred in this phase of the study.

At 24 hours, the jatoba oil presented a satisfactory mortality rate for the two phases of the insects' biological cycle. However, as the exposure period went by, the results were not satisfactory, reaching 63.63% of mortality in nymphs and 40% in adults.

When using the essential oil of *H. courbaril* var. *courbaril* in the control of the spider mite *Tetranychus urticae*, Mercês et al. (2018) found that from the lowest concentration tested of 2 $\mu L.mL^{-1}$, which is equivalent to 0.02%, there was a reduction in the mite population, and the concentration of 100 $\mu L.mL^{-1}$ (1%) reached 100% mortality, in which its CL_{50} was in the value of 43.7 $\mu L.mL^{-1}$ (0.43%). As for the control of the corn weevil *Sitophilus zeamais*, the authors found that the concentration of 60 $\mu L.mL^{-1}$ (0.6%) controlled 50% of the population, showing that there is great variation in efficiency depending on the species of insect to be controlled.

In turn, copaiba essential oil obtained unsatisfactory results, reaching the lowest values of mortality efficiency, of 30 and 24.24%, for nymphs and adults, respectively. This low efficiency was also verified in the work of Avelino et al. (2019), in which they found that nymphs of *A. craccivora*, in cowpea crops, exposed to 0.05% of this oil reduced a total of 53.52%.

3.3. Laboratory testing

Regarding the tests performed in the greenhouse, the aroeira oil was used, since it showed the best results under controlled conditions. Thus, it is possible to recognize from the values of mortality efficiency (Figure 2) that the use of this oil on insects of *A. craccivora* caused higher values of mortality in immature insects, causing 73.52 and 62.85% efficiency of mortality in nymphs and adults, respectively, after the period of 120 hours of exposure, and as the time of exposure to oil increased, the efficiency of mortality increased.

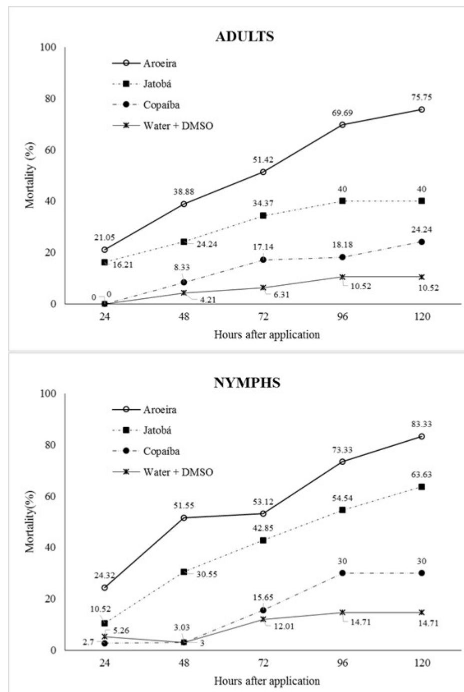

Figure 1. Mortality curves (%) of nymphs and adults of the black aphid *Aphis craccivora* submitted to 0.1% essential oils of Aroeira *Schinus terebinthifolius*, Copaíba *Copaifera langsdorffii* and Jatobá *Hymenaea courbaril* in five periods of exposure under laboratory conditions. Barbalha - CE, 2022.

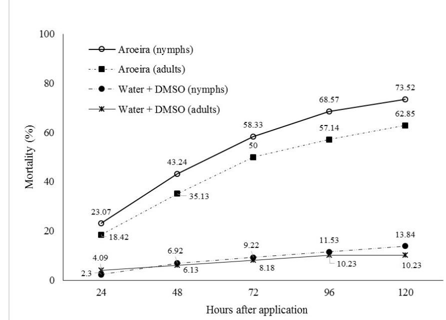

Figure 2. Mortality curves (%) of the black aphid *Aphis craccivora* submitted to 0.1% of *Schinus terebinthifolius* essential oil in five periods of syrup exposure in hours and mortality efficiency in percentage under greenhouse conditions. Barbalha - CE, 2022.

The analysis of variance for the second phase of the experiment proves that the treatments (oil and exposure period) and their interaction have significant values at 1% probability, and the coefficient of variation reached (14.88%) confirms that the data set is homogeneous.

Vieira et al. (2019) when seeking alternative methods for the control of *A. craccivora* under greenhouse conditions, tested extracts of leaves of the cotton plant *Prosopis juliflora* at concentrations of 10, 25, 50 and 100 mL/L (1; 2.5; 5 and 10%) and obtained as the highest mortality rate only 36.21%, evidencing that the low concentration of the extract was not enough to generate efficient results.

When comparing the results of the test performed under laboratory conditions with the results of the test under greenhouse conditions, it is possible to assess that the analysis performed in the laboratory reached more efficient values than in the greenhouse, this can be explained due to the fact that under uncontrolled conditions the efficiency of the essential oil is reduced, since factors such as radiation, temperature and wind influence the degradation content and volatility of the compounds of essential oils (Sales, 2018), this being one of the major factors that hinder their direct use as components to act as potential agricultural insecticides (Moura et al., 2020).

Furthermore, it is possible to see that the treatments with nymphs had a higher mortality rate than the adult insects. One of the justifications for this is that the essential oils, tested under contact action in the second phase of the experiment, are more effective on insects with a less rigid exoskeleton. The insect exoskeleton consists of several layers, the outermost, epicuticle, is formed mainly by lipids, and also has a portion consisting of hydrocarbons (wax layer), aiming its waterproofing (Chapman, 2013).

In the work developed by Lucca (2009) with the cabbage aphid *Brevicoryne brassicae* L., it was also found higher mortality averages in nymphs than in adults, in which the essential oil of fennel *Foeniculum vulgare* Mill. at 1% controlled 66.7% of nymphs and 47.8% of adults.

3.4. Chromatographic analysis

From the chromatographic analysis of the aroeira essential oil (Table 2), 28 chemical constituents were found, showing α-pinene as the majority compound with 61.73%, followed by α-selinene (7.50%), β-selinene (4.39%) and β-elemene (3.17%).

Table 2. Qualitative analysis by gas chromatography coupled to mass spectrometry (GC-MS) and quantitative analysis by gas chromatography (GC) of the essential oil from the leaves of *Schinus terebinthifolius*. Recife - PE, 2022.

Constituent	Calculated retention rate	Literature retention rate[1]	%	Standard Deviation
α-pinene	931	932	61.73	2.24
Camphene	944	946	0.47	0.09
β-pinene	973	974	1.14	0.21
Myrcene	990	988	2.75	0.49
α-phellandrene	1.002	1.002	0.11	0.04
ρ-cymene	1.022	1.020	0.11	0.04
Limonene	1.026	1.024	2.52	0.35
(Z)-β-ocimene	1.037	1.032	0.11	0.05
(E)-β-ocimene	1.048	1.044	0.20	0.05
γ-terpinene	1.057	1.054	0.12	0.03
Terpinolene	1.087	1.086	0.56	0.07
Isoledene	1.374	1.374	0.15	0.04
α-copaene	1.376	1.374	0.27	0.02
β-elemene	1.393	1.389	3.17	0.40
(E)-caryophyllene	1.420	1.417	0.66	0.07
Aromadendrene	1.440	1.439	1.69	0.09
α-humulene	1.455	1.452	0.19	0.04
Allo-aromadendrene	1.462	1.458	0.17	0.03
Germacrene D	1.482	1.480	0.99	0.11
β-selinene	1.488	1.489	4.39	0.36
α-selinene	1.497	1.489	7.50	0.56
α-muurolene	1.501	1.500	0.43	0.04
Germacrene A	1.506	1.508	0.07	0.02
γ-cadinene	1.515	1.513	0.22	0.02
7-epi-α-selinene	1.519	1.520	0.18	0.05
δ-cadinene	1.525	1.522	0.80	0.07
n-hexadecane	1599	1.600	0.69	0.32
(Z)-asarone	1615	1.616	0.33	0.05
			91.73	

[1]Adams (2007).

Tomazoni et al. (2017) also found α-pinene (27.85%) as the majority constituent in the chemical composition of the oil extracted from dried leaves of *S. terebinthifolius*, in addition to β-pinene (8.37%) and spatulenol (9.09%). Santos et al. (2013), identified 37 chemical constituents present in the essential oil of mastic tree, in which the main components were germacrene D (25%), (E)-β-caryophyllene (17.5%) and δ-elemene (10.5%). In another study, germacrene D (37.55%), (E)-caryophyllene (13.61%), α-cadinol (4.29%) and α-pinene (3.81%) were the most abundant constituents found in fresh leaves of red aroeira (Clemente, 2006).

Comparing the data described in the literature with those found, it is possible to determine that there is a variation among the major constituents present in the different samples of essential oils of the species under study. These variations can occur due to the state of maturation and development of the plant, seasonality, circadian rhythm, water availability, available nutrients, temperature, altitude, atmospheric pollution, induction by mechanical stimuli and/or pathogen attack (Gobbo-Neto and Lopes, 2007).

The constituents α-pinene and β-pinene are bicyclic monoterpene compounds, formed by various chemical structures based on two isoprene units (C_{10}). α-pinene is a monounsaturated phytoconstituent that has a characteristic resinous, wood-like odor. This compound is highly volatile under normal conditions because of its high vapor pressure. In addition, they are widely found in the essential oils of conifers, rosemary, and lavender (Ho et al., 2006).

Regarding its biological activity, larvicidal (Govindarajan et al., 2016), antibacterial (Eduardo et al., 2018), antifungal (Nóbrega, 2019), acaricidal (Iori et al., 2005) and insecticidal (adulticidal) properties are already evidenced (Viegas Júnior, 2003).

4. Conclusions

The essential oil of aroeira is the most efficient in inducing the mortality of the black aphid of the bean, both in laboratory and in greenhouse conditions, opening new perspectives regarding its use as a natural insecticide in the control of this pest.

In the laboratory aphid mortality is higher than in the greenhouse, since under controlled temperature, humidity, and photophase conditions the essential oils are not degraded and volatilized as quickly.

The α-pinene component, found in the aroeira tree oil, is the majority, and therefore there is a need for further studies with this isolated constituent to verify if its isolated action is responsible for controlling insects.

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