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Research Article

Bioactivity of andiroba oil on whitefly, *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae)

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Abstract. The whitefly, *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) is a phytophagous insect that is difficult to control and can cause great damage to several crops of economic importance, as it can use more than 600 botanical species as hosts. This study aimed to evaluate the insecticidal and deterrent effect of andiroba (*Carapa guianensis* Aubl., Meliaceae) fixed oil on *B. tabaci* biotype B in kale (*Brassica oleracea* var. acephala). The andiroba oil samples, Manaus and Silves were diluted in acetone P.A. at concentrations of 0.5%, 1%, and 1.5%, and compared with the commercial insecticide Evidence® 700WG (imidacloprid). For the deterrence test, the concentrations were applied on healthy seedlings and placed in a cage with adult insects. Then the oviposition of whiteflies on the leaves was counted. For the mortality test, applications were performed on seedlings with leaves infested with *B. tabaci* nymphs to evaluate the nymphicidal effect. Analysis of variance was performed using the F test, and comparison of means using the Tukey test ($p<0.05$), using the statistical program R®. Concentrations of 1% and 1.5% reached a deterrent effect of 82.13% and 88.55%, respectively. All concentrations caused mortality greater than 60%. These results attest to the potential of andiroba oil as an alternative control for the whitefly.

Keywords: Vegetal oil, Deterrent effect, Insecticidal plant, Alternative control, *Carapa guianensis*.

Introduction

Kale (*Brassica oleraceae* var. acephala) is one of the main vegetables grown in the state of Amazonas, Brazil, with a production of 9,919 t in 2022, in an area of 102 ha (IDAM 2023). Among the pests that attack and limit kale production in the state is the whitefly *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae), a cosmopolitan pest that has more than 600 plant species as hosts (Butter & Dhawan 2021). They attack several crops of economic importance and can cause field losses of up to 100% (Karthik 2020).

The whitefly is a phytophagous and sap-sucking insect (Bernardino et al. 2019), with the main damage related to the transmission of several phytoviruses (Polston et al. 2014). Whitefly is considered the second most widespread and economically important invasive pest in the world, with reports of resistance to 56 different insecticides in 165 countries (Willis 2017). Its genetic plasticity makes this pest difficult to control as it can quickly develop resistance to insecticides from different chemical groups (Lourenção et al. 2015). Therefore, plant extracts with toxic properties to this pest are the target of research in search of an alternative control that works together with integrated pest management (IPM) (Emilie et al. 2015).

Andiroba (*Carapa guianensis* Aubl.) (Meliaceae) is a promising plant for pest control because its fixed oil has insecticidal and deterrent properties (Santos et al. 2015). The oil is easily extracted from its seeds and is abundantly found on the medicinal plant market, with several indications, including healing, anti-itching, and anti-inflammatory effects. It also exhibits antiparasitic, antifungal, and bactericidal effects (Ribeiro et al. 2021).

The objective of this work was to evaluate the insecticidal and deterrent activities of the fixed oil of *C. guianensis* produced in the Amazon region, in the control of *B. tabaci* nymphs, under semifield conditions.

Material and Methods

Bioassays were carried out in the Laboratory of Agricultural Entomology and Acarology (LEA) (24.02 ± 0.20 °C; 55.13 ± 0.98% RH) and in the greenhouse of the Experimental Area of the Faculty of Agrarian Sciences (FCA) (38.43 ± 0.59 °C; 55.08 ± 1.62% RH), both belonging to the Federal University of Amazonas (UFAM), Manaus, AM campus, Brazil.

The DNA analyzes to confirm the species and the biotype of the insects were conducted at the Laboratory of Entomology and Biotechnology of Embrapa Arroz e Feijão, in Santo Antônio de Goiás, GO, Brazil, using the PCR (polymerase chain reaction) technique and comparing the results of the biotype analyzed with the works of Bosco et al. (2006) and Marubayashi et al. (2013). The specimens for DNA analysis were collected from rearing stock in Manaus, AM (repeat 1) and in a rural vegetables-producing property in Iranduba, AM (repeat 2), with the voucher specimens deposited in the LEA entomological collection.

Three samples of fixed andiroba oil were acquired, and their respective plant materials were collected, confirmed as *C. guianensis* and deposited in the herbarium of the Institute of Biological Sciences (ICB) at UFAM. The first sample was collected in the municipality of Manaus, AM, property Sítio Vô Agenor (02°46'53.2"S, 60°08'11.4"W), the second in the municipality of Silves, AM, property Sítio Amaral (02°47'16.4"S, 58°24'19.4"W), these two being obtained by traditional extraction, where the seeds went through a cooking process, to then be broken and form a mass from which the oil was drained; the third sample was a commercial product produced by a local company, with seeds from planting in the municipality of Silves, AM, Comunidade Sagrado Coração de Jesus (02°47'32.2"S, 58°24'21.9"W), but with its own patent-protected extraction process carried out at its headquarters in Manaus, AM. The collections of *B. tabaci* and *C. guianensis* were carried out upon request from the Biodiversity Authorization and Information System - SISBIO: 77294.

A GC-MS analysis was carried out at the Gas Chromatography Laboratory of UFAM (LABCG), to attest that the commercial oil had not been contaminated or adulterated, being compared with oil acquired by traditional extraction directly from the producer in the municipality of Silves, Sítio Amaral property, and used as a purity standard. The oils had to first go through a transesterification process (Metcalfe et al. 1966) at the Natural Products Chemistry Laboratory (LQPN) of the National Institute for Amazonian Research (INPA), Manaus, and then undergo GC-MS analysis at the LABCG, being compared with the works of Bataglion et al. (2014) and Dos Reis et al. (2021).

To begin the stock rearing, the whitefly adults were attracted from the FCA experimental area, using healthy kale grown in pots inside the greenhouse. Collard greens seedlings were continuously produced to replace stock plants and setup experiments, using seeds of the brand Horticeres® Sementes variety 'Folha Manteiga da Georgia', in commercial substrate Tropstrato HT Hortaliças® in 4 cm-diameter tubes (55 cm^3).

A cage was built to induce infestation (1.5 × 0.8 × 0.8 m) covered with voile fabric. Grids that could contain up to 60 seedlings with average leaf dimensions of 6.0 × 3.5 cm were placed inside the cage. Through lateral openings, the host plants from the rearing stock with unsexed *B. tabaci* adults were introduced and manually shaken to disperse the adult insects that established themselves on the healthy seedlings that were in the grid. Only infested seedlings remained in the cage allowing whitefly to oviposit for a 24 h period, with the aim of homogenizing the age of the nymphs, adapted from Pena et al. (2009). After the nymphs hatched, two leaves of each seedling were selected. Areas containing about 50 nymphs per leaf (100 nymphs/seedling) were marked for the assembly of the bioassays.

The oil was diluted in 100% acetone P.A. - ACS (790G) Dinâmica® (Souza & Fávero 2015). Applications were made with the aid of a Burkard Scientific® Potter's Tower (Auto-Load, Harpenden, Herts, England). The seedlings were placed in the center of the base of the device where the aspersion occurred, with an application of 2 mL of each extract per leaf. After this process, the seedlings returned to the greenhouse and were evaluated after seven days.

A concentration of 1% was used for pilot tests with the three oil samples, where the best among them was selected for mortality and deterrence tests. The concentrations used for mortality and deterrence tests were 0.5%, 1%, and 1.5%, with a control of 100% acetone P.A., with Evidence® 700WG (imidacloprid) being the cryptic control.

The bioassays for deterrence were evaluated by counting the eggs laid, calculating the percentage of deterrence using the adapted formula from Obeng-Ofori (1995): PD = [(NC - NT) / (NC + NT) × 100] (where PD = deterrence percentage; NC = number of eggs in the control and NT = number of eggs in the treatments with the oil). The mortality tests were evaluated based on counting the number of observed dead nymphs. Corrected natural mortality was calculated for each treatment using the Abbott (1925) formula: Mc (%) = [(%Mo - %Mt) / (100 - %Ma)] × 100, (where: Mc = corrected mortality; Mo = observed mortality; Ma = Mortality in the control with only acetone).

The median lethal concentration (LC_{50}) was then estimated using concentrations of 0.5%, 0.75%, 1%, 1.25% and 1.5%. The LC_{50} was obtained by Probit analysis (Finney 1971), using the R® statistical program.

The experimental design adopted was completely randomized with 5 replications (500 nymphs per treatment). The normality test was performed using the Shapiro-Wilk test, and when a non-normal distribution was found, the data were transformed into arcsen {[(x+0.5) /100] 0.5}. The analysis of variance was performed using the F test, and the comparison of means using the Tukey test ($p<0.05$), employing the R® statistical program.

Results and Discussion

All specimens of adult females analyzed by DNA testing collected in Manaus and Iranduba were identified as the species *B. tabaci* Middle East Asia Minor 1 - MEAM1 (biotype B) (Fig. 1).

GC-MS analyzes found no significant differences between the commercial oil and the traditional oil, and no evidence of adulteration or contamination of the commercial oil was found (Fig. 2). The works of Bataglion et al. (2014) and Dos Reis et al. (2021) corroborate these results by carrying out GC-MS analyzes with andiroba oils purchased in the Manaus market and by extracting it from seeds collected in the field, respectively, and also identifying patterns similar to those found in this analysis.

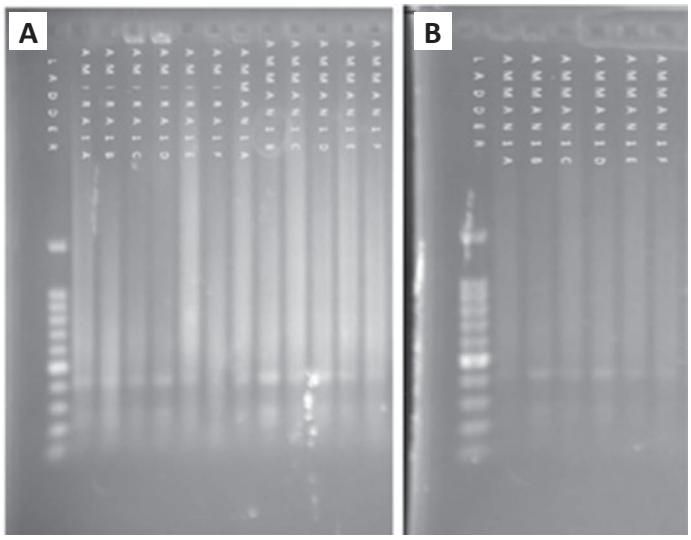

Figure 1. Photodevelopment of whitefly, *Bemisia tabaci* samples sent to the Entomology Laboratory for molecular identification, repeat 1: whitefly Manaus (A) and repeat 2: whitefly Iranduba (B).

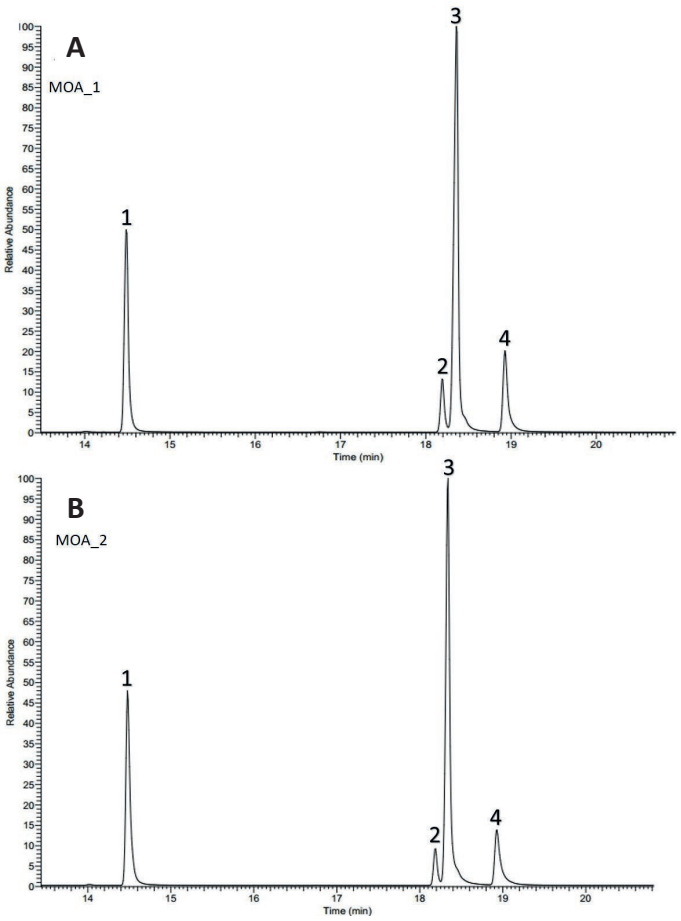

Figure 2. GC-MS total ion chromatograms from a) traditional andiroba oil: 1- Palmitic acid, 2- Linoleic acid, 3- Oleic acid, 4- Stearic acid; b) Canto da Luz commercial andiroba oil: 1- Palmitic acid, 2- Linoleic acid, 3- Oleic acid, 4- Stearic acid.

At a concentration of 1%, traditional oils (Manaus and Silves) caused severe burns to the leaves, making any type of evaluation impossible. However, the commercial oil did not cause any injury and caused mortality considered promising, providing a better result than

the commercial product registered for pest control (cryptic control), statistically differing from both this and the Acetone P.A. control (Tab. 1), this being the oil used in the other tests. Several authors corroborate this result when reporting the insecticidal potential of andiroba oil against other pests (Prophiro et al. 2014; Farias et al. 2017).

Table 1. Comparison of mean (±EP) mortality of whitefly nymphs (*B. tabaci*) between *Carapa guianensis* oil and Evidence WG700 (registered commercial product).

Structures/Species	Mortality (%)
C. guianensis (andiroba commercial) 1%	54.81±.25 a
Evidence WG700 (1g/L)	40.56±.35 b
Acetone P.A.	18.86±.32 c

Means followed by the same letter do not differ statistically (p ≤0.05) by Tukey's test.

All treatments tested for deterrent effect showed reductions in oviposition in relation to the acetone P.A. control according to the increase in concentrations (Tab. 2) (Fig. 3). However, the concentration of 0.5% caused a deterrent effect of only 7.44%, being considered relatively low, but statistically differing from the acetone P.A. control. At the concentration of 1%, there was a marked increase in deterrent effect of almost 70% in relation to the control. This was even greater at the concentration of 1.5% with 76.15% deterrent effect, both being statistically equal and effective in preventing egg laying in kale.

Table 2. Mean (± EP) eggs laid and deterrent effect of *B. tabaci* using *Carapa. guianensis* oil under different concentrations.

	Concentrations			
	Acetone P.A.	0.5%	1%	1.5%
Eggs laid*	98.8±0.58 a	85.8±1.39 a	17.8±0.86 b	13.4±0.50 b
Deterrent Effect (%)	0%	7.44%	69.68%	76.15%

Means followed by the same letter do not differ statistically (p ≤0.05) by Tukey's test.

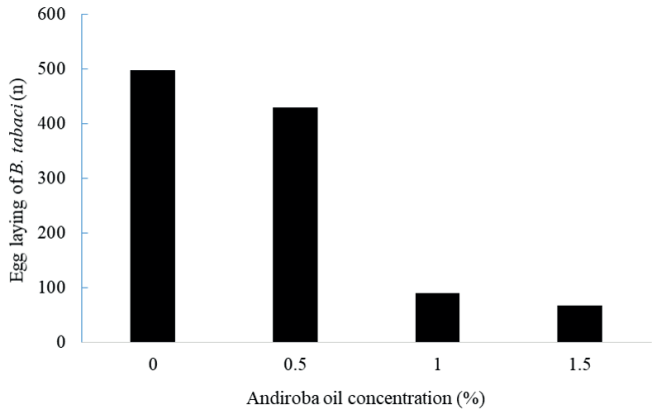

Figure 3. Total eggs laid by the whitefly, *B. tabaci*, in relation to different concentrations of andiroba oil, *C. guianensis.*

Other authors have studied the effect of andiroba oil and found positive results. Machado da Rosa et al. (2013) tested the repellency of *C. guianensis* oil on the fruit fly (*Anastrepha fraterculus* (Wiedemann, 1830)) (Diptera: Tephritidae) in a feijoa orchard (*Acca sellowiana* (O.Berg) Burret) by trapping with hydrolyzed protein and andiroba oil at concentrations of 0.5%, 1%, and 2%. They verified that the reduction in the capture of flies was proportional to the increase in concentrations, as occurred in this work in relation to the reduction in oviposition by *B. tabaci* with increasing concentrations of the oil.

Fernandes et al. (2016) found a repellent effect of andiroba oil on species of flies of the family Calliphoridae, at a concentration of 50% of the oil mixed with liquid vaseline, which is much higher concentrations than used in the present work. Brilinger et al. (2019) tested andiroba oil on *A. fraterculus* in apples, at a concentration of 25%, much higher than that used in this work, and observed the deterrent effect of the oil due to the inhibition of insect oviposition. These results corroborate those achieved in the present work employing *C. guianensis* oil, which indicates that this oil has properties capable of preventing the oviposition of *B. tabaci*, demonstrating a potential use in an alternative measure of control against this pest.

All concentrations used resulted in nymphal mortality greater than 65%, statistically differing from the control, but not from each other (Tab. 3). Farias et al. (2017) tested the effectiveness of andiroba oil *in vitro* on *Damalinia caprae* (Gurlt, 1843) (Phthiraptera: Trichodectidae), using distilled water and Tween 80 as dispersants. They observed that all concentrations caused 100% mortality, within the first hour by the concentrations of 100%, 50%, and 30%, three hours later for concentrations of 10% and 5%, and six hours later for 2.5%. All of these concentrations are higher than those used in this work, indicating that an increase in concentration could increase mortality. However, the tests were carried out *in vitro,* while in this work, the tests were on kale leaves. The use of high concentrations of andiroba oil can cause phytotoxicity that damage the commercial product. Thus, research needs to verify the resistance limit of kale to increased concentrations of this oil.

Table 3. Mean (±EP) mortality of *B. tabaci* using different concentrations of *Carapa guianensis* oil.

Concentrations	Mortality (%)	
C. guianensis 1.5%	68.33±0.49	a
C. guianensis 1%	67.91±0.64	a
C. guianensis 0.5%	65.30±0.29	a
Acetone P.A. (100%)	6.07±0.37	b

Means followed by the same letter do not differ statistically (p ≤0.05) by Tukey's test.

Santos et al. (2016) assessed 200 µL of oil (undiluted) from *C. guianensis* in Petri dishes on the fall armyworm *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae) and achieved 64.7% and 97.50% in the control of the eggs and caterpillars, respectively. However, these results were achieved with the use of essential oil, which is extracted in smaller amounts from seeds and with the help of laboratory equipment that requires more time and is more difficult to extract than fixed oil, which can be extracted even in an artisanal manner by producers in the field and with fewer resources, as in a social technology.

When estimating the LC_{50}, there was an increase in mortality as concentrations increased, resulting in a LC_{50} of 0.063% (Tab. 4). Prophiro et al. (2014) obtained an LC_{50} of 0.013% with *C. guianensis* oil on larvae of *Aedes aegypti* (L., 1762) (Diptera: Culicidae), however, this result was obtained in a laboratory with an accurate temperature and relative humidity control chamber (25 °C and 80% RH). The different experimental conditions related to the difference in susceptibility of the pests to the oil may have influenced the difference between the results, as the whitefly has already demonstrated great resistance to a wide range of products (Lourenção et al. 2015). The results obtained are considered promising and attest to the nymphicidal potential of andiroba oil on *B. tabaci*, aiming at alternative control for the management of this pest.

Table 4. Mean (±EP) mortality of *B. tabaci* and LC_{50} with the use of andiroba oil (*Carapa guianensis*) diluted in acetone P.A.

Concentrations	Mortality (%)	
C. guianensis (1.5%)	70.81±1.39	a
C. guianensis (1.25%)	68.14±0.56	ab
C. guianensis (1%)	65.81±0.72	bc
C. guianensis (0.75%)	65.68±0.55	bc
C. guianensis (0.5%)	63.72±0.25	c
Acetona P.A. (100%)	9.62±0.54	d
	LC_{50}= 0.063 (CI (97%) = 0.004 – 0.121)	

CI: Confidence Interval; Means followed by the same letter do not differ statistically (p ≤0.05) by Tukey's test.

Conclusion

Traditional fixed oils (Manaus and Silves) of *C. guianensis* caused severe lesions on kale leaves and are therefore not recommended in concentrations above 1%. Studies at lower concentrations are recommended. The commercial fixed oil of *C. guianensis* showed a deterrent effect on oviposition and an insecticidal effect (contact) on whitefly nymphs (*B. tabaci* Biotype B). This oil is of interest for future studies as a possible control alternative for whiteflies in the context of IPM in kale crops.

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Authors' Contributions

MVSO: planning, execution, tabulation, data analysis, writing, review and statistical treatment; CTSD: planning, execution, tabulation, review; MRP: planning, supervision, review, data analysis, statistical treatment; NMS: planning, supervision, writing and review.

Conflit of Interest Statement

The authors declare no conflicts of interest.

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