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Composition and Bioactivity of Essential Oil From the Leaves of *Genipa americana* Against the Coconut Mite *Aceria guerreronis*

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Abstract

Genipa americana L. has a diversity of secondary metabolites, including iridoids, phenolic compounds, and alkaloids. Pharmacological and biological properties have also been reported. This study has aimed to evaluate the chemical composition of the essential oil (EO) obtained from the leaves of *G. americana* and its bioactivity against *Aceria guerreronis* Keifer (Acari: Eriophyidae), a serious pest of coconut production areas worldwide. EO from the leaves was extracted and analyzed by Gas Chromatography-Mass Spectrometry and flame ionization detection (GC-MS/FID). For the bioassays, the adult coconut mites were subjected to increasing concentrations of EO. The analyses showed a predominance of sesquiterpenes, followed by monoterpenes, aldehydes, and fatty acids. The EO (LC_{50} = 0.41 mg mL^{-1}; LC_{90} = 6.43 mg mL^{-1}) showed toxicity and repellent effects against the coconut mite. The tested EO has the potential to develop as a natural product, with acaricidal activities against *A. guerreronis*, in order to assist in the control of the coconut mite.

Keywords: *Cocos nucifera*, repellency, terpenes, toxicity

1. Introduction

Genipa americana L. (Rubiaceae) is widely distributed in tropical Central and South America and it presents an economic and environmental importance (Bailão et al., 2015; Sá et al., 2015). It possesses value for the recovery of degraded areas, composition in permanent preservation areas, and in agroforestry systems (Bailão et al., 2015; Sá et al., 2015). Satisfactory quantities of iridoids and tannins are found in the leaves and fruits, which display pharmacological actions (Silva et al., 2015). In addition, monoterpenoids, phenolic compounds, and steroids have been identified and isolated from the fruits of *G. americana* (Alves et al., 2017; Bentes & Mercadante, 2014; Conceição et al., 2011; Ono et al., 2007). However, there is little research that has evaluated the chemical composition of the leaves, in fact, only two new iridoids from the leaves of the *G. americana* were recently reported (Alves et al., 2017).

Pests are a natural part of ecosystems, but they can be problematical in field crops and ornamental plants (Ajayi, Adedire, & Lajide, 2012; Ebadollahi et al., 2017). *Aceria guerreronis* Keifer (Acari: Eriophyidae) is a species considered as a serious pest of coconut production areas worldwide, including the Americas, Africa, and some Asian countries. It occurs in high population levels in northeastern Brazil (Lawson-Balagbo et al., 2008; Souza et al., 2012). The coconut mite populations are found underneath the perianth and on the meristematic tissues of the coconut fruits (Navia et al., 2013; Lima et al., 2012; Melo et al., 2014). The mite causes chloroses that are triangular in shape, and as they develop, they become brown, causing superficial and longitudinal cracks of a dark brown color to fruits (Moreira & Nascimento, 2002).

Although chemical control is the only known means of controlling the pest effectively, it is not sustainable due to the necessity of frequent and regular applications, and a high cost (Siriwardena et al., 2015). Consequently, control that is associated with other measures, such as cultural control, botanical acaricides, and natural biological control can be adopted, in order to maintain this species at acceptable population levels (Moreira & Nascimento, 2002). In addition, the control of this species is difficult because of its high mobility, microscopic

size, and hidden lifestyle, making such an assessment time-consuming, tedious, and often inaccurate (Siriwardena et al., 2015).

There is a rapid growth in the screening of plant materials to find new bio-pesticides, as essential oil and its main components are effective in pest management; and besides, terpenoids have repellent properties and strong acaricidal activities (Ebadollahi et al., 2017; Sena-Filho et al., 2017; Wagan, Cai, & Hua 2018). When considering the medicinal and biological importance of *G. americana*, this study has aimed to evaluate the chemical composition of essential oil from its leaves and its bioactivity (toxicity and repellency) against *A. guerreronis*.

2. Method

2.1 Plant Material

Fresh, mature and healthy leaves of *G. americana* were collected in the city of Nossa Senhora das Dores, Sergipe, Genipap Genebank (10°29′30″S; 37°11′36″W; 204 m altitude) between the months of March and April 2016. The fresh leaves were cut and hydrodistilled immediately.

2.2 Essential Oil Extraction

The essential oil (EO) was extracted by hydrodistillation using a Clevenger-type apparatus. The fresh leaves (1 kg) were distilled for 180 min using hexane as a solvent. The EO obtained was dried with powdered anhydrous sodium sulfate and stored at 4 °C in a sealed amber bottle before chemical analysis by gas chromatography coupled to mass spectrometry (GC/MS) and flame ionization detector (FID).

2.3 GC-MS/FID Analysis

The GC analyses were performed by using a GC-MS/FID (QP2010 Ultra, Shimadzu Corporation, Kyoto, Japan) that was equipped with an autosampler (AOC-20i, Shimadzu). The separations were accomplished using an Rtx®-5MS Restek fused silica capillary column (5%-diphenyl-95%-dimethyl polysiloxane) of 30 m × 0.25 mm i.d., with a 0.25 μm film thickness, at a constant helium (99.999%) flow rate of 1.2 mL min^{-1}. An injection volume of 0.5 μL was employed, with a split ratio of 1:10. The oven temperature program started at 60 °C, was held for 4 min, then increased at a rate of 3 °C min^{-1} to 300 °C, followed by an increase of 20 °C min^{-1} to 280 °C. The MS and FID data were simultaneously acquired by employing a Detector Splitting System; the split flow ratio was 5:1 (MS:FID). A 0.4 m × 0.15 mm i.d. restrictor tube (capillary column) was used to connect the splitter to the MS detector; a 0.6 m × 0.22 mm i.d. restrictor tube was used to connect the splitter to the FID detector. The MS data (total ion chromatogram, TIC) was acquired in full scan mode (*m*/*z* of 40-550) at a scan rate of 0.3 $scans^{-1}$ using electron ionization at 70 eV. The injector temperature was set at 280 °C and the ion source temperature at 200 °C. The FID temperature was 300 °C, and the gas supplies for the FID were hydrogen, air, and helium, at flow rates of 30, 300, and 30 mL min^{-1}, respectively. The quantification of each constituent was estimated by FID peak-area normalization (%). The compound concentrations were calculated from the GC peak areas and were arranged in the order of GC elution. The retention index (Adams, 2007) was obtained by co-injecting the oil sample with a C7-C30 linear hydrocarbon mixture; the identification was made based on a comparison of the retention index and the mass spectra with those in the literature (Adams, 2007; Linstron & Mallard, 2005).

2.4 Toxicity Bioassay

The adult mites of *A. guerreronis* were collected from unsprayed coconut infested fruits in Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA) Tabuleiros Costeiros, Aracaju-SE (10°57′03.3″S, 37°03′07.4″W). The coconut fruits were approximately 10 weeks old and they were collected for the construction of arenas from the same coconut plantation. The EO was sprayed through a Potter tower (Burkard, Rickmansworth, UK) onto discs (1 cm diameter) that were prepared using the meristematic tissues of the new coconut fruits and then placed in Petri dishes (15 cm diameter, 2 cm deep) containing a mixture of 5% agar, 0.3% methylparaben (Nipagim^{TM}) as a fungicide, and distilled water, as described by Oliveira et al. (2017).

The concentrations used were selected from initial concentration-mortality bioassays (Silva et al., 2013), ranging between the lower (0%) and the higher (100%) limits of mortality of the coconut mite. Increasing concentrations of EO (0.06, 0.1, 0.2, 1.0, 2.0, 4.0, and 5.0 mg mL^{-1}) were diluted in acetone and then the spraying was conducted at a pressure of 5psi/pol^2 with a 9.3 mL spray aliquot, which resulted in a residue of 1.38 mg/cm^2, in line with the recommendations of the IOBC/WPRS (International Organization for Biological Control of Noxious Animals and Plants/West Palearctic Regional Section) (Hassan et al., 1994). Afterward, the sprayed discs were exposed to the environment for 30 minutes to dry. The arenas of the control treatment were sprayed with acetone and 20 adults were transferred with the help of a brush. Eight arenas (replicates) were used for each

concentration of the EO tested and they were maintained at 24 °C. The mite mortality was assessed after 24 h exposure and those mites that did not respond to the brush stimulus were considered dead.

2.5 Repellency Bioassay

The arenas were prepared as previously described. However, each arena consisted of a treated and an untreated area, and these were covered with two layers of adhesive tape during the spraying, as described by Teodoro et al., (2009). The arenas were sprayed with LC_{50} and LC_{90} solutions of the EO. After the drying of the solutions, a white glue point was placed in the center of the disc ($1.0 \times 1.0 \times 0.5 cm^3$). Coconut mites were individually positioned under the white glue point and their positioning evaluations were performed after 1, 24, and 48 h. For each LC essential oil (LC_{50} and LC_{90}), 60 replicates were performed.

2.6 Data Analysis

The lethal concentration and the confidence limits (CL) of *G. americana* EO were calculated by Probit analysis using SAS Software (SAS Institute, 2002). The frequency analyses used the Chi-square test and PROC FREQ SAS Software (SAS Institute 2008) was used to compare the percentages of mites choosing the sprayed and unsprayed disc halves.

3. Results and Discussion

3.1 Chemical Composition

The characterization of the chemical profiles via the GC-MS/FID analyses of EO of *G. americana* resulted in the identification of 42 compounds. The presence of short-chain fatty acids, aldehydes, monoterpenes, diterpenes, and sesquiterpenes was noticed (Table 1). The major compounds identified from the EO from the leaves were (2*E*,4*E*)-decadienal (6.01%), (*E*,*E*)-α-farnesene (5.10%), hexyl benzoate (5.61%), pentadecanal (11.55%), and linoleic acid (15.48%). Chemodiversity has been reported, mainly being iridoids, followed by flavonoids, alkaloids, carboxylic acids, monoterpenes, and phytosterols isolated from the fruits and seeds of *G. americana* and they were tested for biological activities (Alves et al., 2017; Ramos-de-la-Peña et al., 2016; Souza et al., 2018). The presence of kaura-16-ene and hexadecanoic acid shed a light on the biosynthetic pathway and the enzyme expression during the growing period of the leaves. In addition, the presence of a diversity of a low and a medium-chain of reactive aldehyde was identified. These volatiles are toxic in a high concentration, and the presence of low and medium fatty acids was interesting when searching for rich lipids from the plants, since these compounds are toxic and are repellent to the coconut mite *A. guerreronis* (Silva et al., 2013; Teodoro et al., 2017).

Table 1. Chemical composition (%) identified in the essential oil extracted from the leaves of *Genipa americana*

t_R (min)	Compounds	RI exp.	RI lit.	Composition
6.250	(3*Z*)-hexen-1-ol	840	850	0.66
6.550	Hexan-1-ol	852	863	3.87
9.615	(2*E*)-heptenal	947	947	tr
11.080	2-pentylfuran	982	984	0.19
11.355	Hexanoic acid	989	967	tr
13.635	(*E*)-β-ocimene	1039	1044	4.67
14.085	(2*E*)-octenal	1049	1049	0.44
14.820	Octan-1-ol	1065	1063	0.94
16.095	Linalool	1092	1095	0.69
16.280	(6*Z*)-nonenal	1096	1097	0.26
19.015	(2*E*)-nonenal	1152	1157	0.27
20.165	Naphthalene	1175	1178	0.65
20.185	Octanoic acid	1176	1167	tr
20.760	Methyl salicylate	1187	1190	0.31
22.010	β-cyclocitral	1213	1217	0.30
23.970	(2*E*)-decenal	1254	1260	3.72
26.560	**(2*E*,4*E*)-decadienal**	1309	1315	6.01
28.520	Eugenol	1351	1356	0.15
28.725	γ-nonalactone	1355	1358	0.45
29.340	α-copaene	1368	1374	0.44
29.705	(*E*)-β-damascenone	1376	1383	0.59
31.050	(*E*)-β-damascone	1406	1413	tr
31.325	(*E*)-caryophyllene	1412	1417	1.70
32.660	Geranylacetone	1443	1453	0.38
33.830	Prenyl benzoate	1469	1485	0.47
34.220	(*E*)-β-ionone	1478	1487	0.97
35.030	**(*E*,*E*)-α-farnesene**	1497	1505	5.10
35.790	δ-cadinene	1515	1522	tr
37.450	(*E*)-nerolidol	1555	1561	0.35
37.830	**(3Z)-hexenyl benzoate**	1564	1565	5.61
38.035	Hexyl benzoate	1569	1579	1.20
39.300	Tetradecanal	1600	1611	0.76
41.825	Tetradecan-1-ol	1664	1671	0.20
43.360	**Pentadecanal**	1704	1717	11.55
45.370	Benzyl benzoate	1757	1759	0.15
47.030	Hexadecanal	1802	1811	0.18
48.110	Hexahydrofarnesyl acetone	1832	1845	0.93
50.635	Heptadecanal	1903	1922	0.57
50.850	Methyl hexadecanoate	1910	1921	0.25
55.235	Kaura-16-ene	2041	2042	0.71
56.655	Methyl linolenate	2084	2084	0.15
58.245	**Linoleic acid**	2148	2132	15.48

Note. RI exp., retention indices on the RTX-5MS column calculated according to van den Dool and Kratz (1963). RI lit., retention indices according to Adams (2007). tr, trace amounts of the compound were detected. Bold, major compounds.

3.2 Toxicity to the Coconut Mite Aceria guerreronis

The EO from *G. americana* exhibited acaricidal activities with LC_{50} and LC_{90} values of 0.41 mg mL^{-1} and 6.43 mg mL^{-1}, respectively (Table 2). Several studies have reported applications of natural products of plant origin as promising sources of acaricide, for integrated pest management in the replacement of conventional synthetic pesticides (Araújo et al., 2012; Miresmailli & Isman, 2014). Crude vegetable oils have been characterized and tested against *A. guerreronis*, such as vegetable oil babassu, degummed soybean, and coconut oils (Oliveira et al., 2017). A further study conducted and evaluated the bioactivity of cottonseed oil to control *A. guerreronis*.

Linoleic and oleic acids have been shown to be bioactive against pests, displaying toxic and repellent activities (Teodoro et al., 2017). Here, in this study, the synergistic action of terpenes and linoleic acid that was found in the leaves of *G. americana* may have contributed to the highly observed acaricidal activities.

Table 2. Lethal concentrations (LC_{50} and LC_{90}) ($mg.mL^{-1}$ with 95% confidence interval in parentheses) of *Genipa americana* essential oil on the coconut mite *Aceria guerreronis* based on the concentration-mortality bioassays, after 24 hours of exposure

Treatment	LC_{50} (95% CI)	LC_{90} (95% CI)	χ^2	P
	---------- mg mL^{-1} ----------			
EO	0.41 (0.34-0.49)	6.43 (4.59-9.82)	0.949	0.713

Note. Mean values obtained (n = 8 replicates with 20 adult mites per replicate). The lethal concentrations (LC_{50} and LC_{90}) and the values (mg mL^{-1}) were estimated using Probit analysis. CI = Confidence interval at 95% probability, χ^2 = Chi-square, P = Probability ($p \leq 0.05$).

Acaricidal activities and the repellence of EO on phytophagous mites have been demonstrated (Araújo et al., 2012; Tak and Isman, 2017). Interestingly, in accordance with the results, the EO of *Vitex gardneriana* showed strong acaricidal activities on *A. guerreronis* (Sena-Filho et al., 2017). However, the LC_{50} estimated for the EO of *G. americana* (0.41 mg mL^{-1}) was twice as low as the estimated LC_{50} for the EO of *V. gardneriana* (0.85 mg mL^{-1}), therefore, being more toxic. Although the EO of *G. americana* is demonstrating its potential as a natural pesticide promisor for the control of *A. guerreronis*, it is still needed to be understood about its selectivity toward non-target organisms and natural enemies, mainly the predatory mites belonging to the family Phytoseiidae.

3.3 Repellence of Essential Oil to Aceria guerreronis

The LC_{50} and LC_{90} of the EO of *G. americana* were repellents to the coconut mites at all times of application of the compound (Fig 1). The effect was increased over 48 hours of evaluation. Thus, the sublethal effects of the oil were maintained throughout the experiment. Therefore, the substances present in the EO of *G. americana* can interfere with the behavior of *A. guerreronis*, because the arthropods are able to detect toxic substances and move away from the treated areas (Cordeiro et al., 2010). The cottonseed and palm oils have also presented repellent activities to *A. guerreronis* (Teodoro et al., 2017; Freitas et al., 2019).

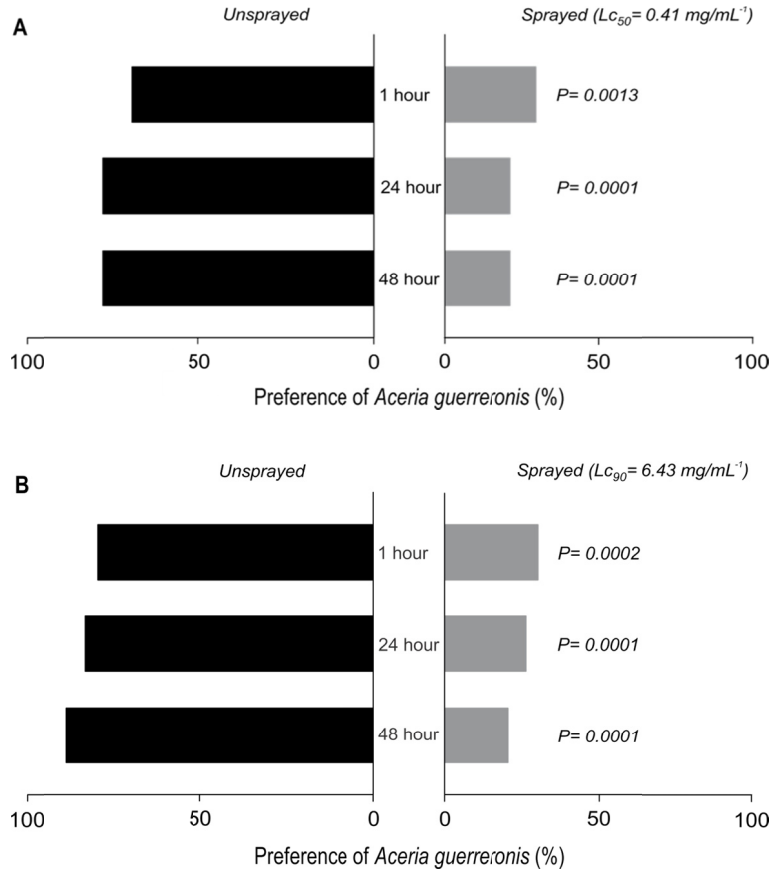

Figure 1. Repellency of LC_{50} (1A) and LC_{90} (1B) of the *Genipa americana* essential oil on the *Aceria guerreronis* coconut mite in percentages, after 1, 24, and 48 hours of spraying. Bar with the untreated area (black) and treated (grey)

All of this data tends to confirm that the bioactive compounds of EO seem to play an important role as an acaricide repellent. They may be effective in reducing the infestation of coconut mites, whose control is difficult because the colonies are protected by the bracts (Melo et al., 2014). Moreover, the *G. americana* extracts have biological properties that are associated with several molecules, such as steroids, iridoids, and monoterpenoids (Conceição et al., 2011; Ono et al., 2007; Codignoto et al., 2017). These results indicate that the EO from the leaves *G. americana* can be used to decrease the *A. guerreronis* infestation in coconut plantations. Further field studies, as well as information on the sublethal effects and selectivity, are required.

4. Conclusion

The EO of *G. americana* showed acaricidal effects after 24 hours of exposure, being also repellent to the coconut mite at the concentrations tested. It was possible to identify alcohols, aldehydes, monoterpenes, and sesquiterpenes in the chemical composition of the EO. It was reported for the first time of the presence of diterpenes in its composition. This EO has great potential for a natural acaricide development to contribute to future integrated management programs of pest mites.

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