

Bioactivity of iridoids of *Genipa americana* against the coconut mite *Aceria guerreronis* Keifer (Acari: Eriophyidae)



Bioactividad de iridoides de *Genipa americana* contra el ácaro del cocotero *Aceria guerreronis* Keifer (Acari: Eriophyidae)

<https://eqrcode.co/a/RuUdIh>

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ABSTRACT: Genipap (*Genipa americana* L.) is a medicinal tree native to South America and the Amazon regions, where it is used as a popular traditional medicine. It is also highly consumed by people in the form of jams, juices, preserves, and wines. The aim of this study was to evaluate the toxicity, persistence, and the repellence properties of leaf methanolic extracts of genipap (LME) and its major iridoids (genipin and geniposide), against the key coconut pest mite *Aceria guerreronis* Keifer. The concentration-mortality bioassays revealed a strong acaricidal activity of LME against the coconut mite ($LC_{50} = 0.60 \text{ mg.ml}^{-1}$; and $LC_{90} = 16.69 \text{ mg.ml}^{-1}$). In addition, standard concentrations (0.6 mg.ml^{-1}) of either genipin or geniposide killed 50 % and 62 % of the *A. guerreronis*, respectively, after 24 hours of exposure, highlighting the role of these iridoids in the acaricidal activity of LME. The persistence bioassays of LME revealed toxicity towards *A. guerreronis* for up to 36 hours after spraying. Furthermore, LC_{50} and LC_{90} of LME repelled the coconut mite after 1, 24, and 48 hours of exposure. Therefore, LME can be considered an alternative to assist in the integrated control of the coconut mite in coconut plantations.

Keywords: coconut mite, *Genipa americana*, methanolic extract, repellency, toxicity.

RESUMEN: La planta de jagua (*Genipa americana* L.) es un árbol medicinal nativo de América del Sur y de la región Amazónica, donde se utiliza en la medicina popular y sus frutos son consumidos en forma de dulces, jugos, mermeladas y vinos. El objetivo de este trabajo fue evaluar la toxicidad, persistencia y repelencia del extracto metanólico de las hojas de *Genipa americana* (LME) y sus principales iridoides (genipina y geniposideo), contra la plaga clave de la palma de coco *Aceria guerreronis* Keifer. Los bioensayos de concentración-mortalidad revelaron una fuerte actividad acaricida de LME de *G. americana* al ácaro de la necrosis del cocotero ($CL_{50} = 0,60 \text{ mg.ml}^{-1}$ e $CL_{90} = 16,69 \text{ mg.ml}^{-1}$). Además, las concentraciones padrones ($0,6 \text{ mg/ml}^{-1}$) de extracto de genipina y geniposideo mataron 48 % y 52 %, respectivamente, de *A. guerreronis* después de 24 horas de exposición y se destacó la función de estos iridoides en la bioactividad de LME. Los bioensayos de persistencia de LME de *G. americana* revelaron toxicidad para *A. guerreronis* hasta 36 horas después de la pulverización. Además, las CL_{50} y CL_{90} del LME de *G. americana* repelieron el ácaro de la necrosis del cocotero después de 1, 24 y 48 horas de exposición. Así, el LME de esta planta puede ser considerado como una alternativa para auxiliar en el control integrado del ácaro de la necrosis *A. guerreronis* en plantaciones de coco.

Palabras clave: toxicidad, repelencia, extracto metanólico, *Genipa americana*, ácaro de la necrosis.

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INTRODUCTION

Genipap, (*Genipa americana* L.) (Rubiaceae), is a tree widely distributed in the tropical regions of Central and South America. It is used in folk medicine (1,2,3,4,5). Many phenolic compounds are described in the literature from its fruits, for example, leucoanthocyanidins, catechins, flavanones, anthraquinones, coumarins, and flavonols, as well as triterpenoids and steroids (6). The presence of flavonoids and iridoids in the leaf extract has also been associated with biological activities (30).

In fact, a plethora of iridoids have been isolated from *G. americana*, such as genameside A-D, geniposidic acid, geniposide, gardenoside, genipin-gentiobioside, genipin, gardendiol, deacetyl asperulosidic acid methyl ester, shanzhiside, genipacetal, genipamide, and genipaol (2, 7, 6). Additionally, a total of 17 compounds have also been identified. Geniposide was the most abundant iridoid compound, whereas 5 - caffeoylquinic acid was the most outstanding phenolic compound (3); notably, all of them possess a high biological activity against insect pests (8).

The coconut mite, *Aceria guerreronis* Keifer (Acari: Eriophyidae), is one of the most serious pests in coconut producing areas worldwide (9,10, 11). Colonies of *A. guerreronis* develop while protected under the perianth of the coconut fruits, where they feed on the meristematic tissues. This causes an extensive damage visible as a triangular white spot next to the edge of the perianth, which progressively becomes necrotic and leads to reduced fruit commercial values (11,12).

In Brazil, pesticides are applied monthly or at shorter intervals to control the coconut mite and potentially induce to high production costs, secondary pest outbreaks, as well as environmental, food, and human contamination (9, 12). Recently, the use of plant natural products to assist in pest management programs has gained increasing attention (13, 14).

Although several studies have reported the acaricidal effects of secondary metabolites from a myriad of plants species, the bioactivity of iridoids from the leaves of *G. americana* has yet to be determined. Due to the chemical diversity

of the compounds present in genipap and their potential biological activities, the aim of this study was to evaluate the toxicity, persistence, and repellence of their leaf methanolic extracts (LME) and their major iridoids (genipin and geniposide) against the coconut mite *A. guerreronis*.

MATERIAL AND METHODS

Plant material

In April 2016, fresh leaves of *G. americana* of the CR2 genotype were collected from the Genipap Genebank located in the Nossa Senhora das Dores municipality (10°29'30"S-37°11'36"W- 204 m.a.s.l.), Sergipe State, Brazil. To obtain the leaf methanolic extract (LME), 500 g of the leaves were macerated in 1 liter of MeOH for 48 hours. Afterwards, the extract was filtered and concentrated at 40°C. For the identification of the iridoids in the leaves, thin layer chromatography (TLC) plates on Si gel (MERCK-Germany, 105553) were developed by the solvent systems: EtOAc-HCOOH-AcOH-H₂O (100: 11: 11: 26, v:v), and EtOAc-HCOOH-AcOH-H₂O (100: 0.5: 0.5: 0.5, v:v). Briefly, an aliquot of 10 µl of genipin, geniposide, and LME was developed on the plate of chromatography. The detection was performed using vanillin-sulphuric acid reagent. The compounds genipin (690277-8) and geniposide (24512-63-8) used in the additional bioactivity bioassays were purchased from Sigma-Aldrich (98 % purity).

Toxicity bioassay

Young and healthy coconut fruits (from Embrapa Tabuleiros Costeiros) were collected for the confection of arenas; the mites, likewise, were obtained from the young infested fruits. The epidermis of the perianth was covered by a mixture of 5 % agar, 0.3 % methylparaben (Nipagim®) as a fungicide, and distilled water. Discs (1 cm diameter) were opened with the help of a strainer to expose the area of the fruit epidermis, which served as an experimental units.

The concentrations used were selected in the initial concentration-mortality bioassays and ranged between the lower (0 %) and higher (100

%) limits of mortality (15). According to the recommendations of the IOBC/WPRS (International Organization for Biological Control of Noxious Animals and Plants/West Palearctic Regional Section) (16), increasing concentrations of LME (0.06, 0.1, 0.2, 1.0, 2.0, 4.0, and 5.0 mg.ml⁻¹) were dissolved in acetone and subsequently sprayed through a Potter Tower (Burkard, Rickmansworth, UK) at a pressure of 5psi, with a 9.3 ml spray aliquot, which resulted in a residue of 1.38 mg cm⁻²; the arenas of the control treatment were sprayed only with acetone. The sprayed discs were exposed to the environment for 30 minutes to dry; subsequently, 20 *A. guerreronis* adults were transferred to each arena with the help of a fine brush. Eight arenas were used for each concentration of LME and they were maintained at 28 ± 2°C, with a relative humidity of 65 ± 5 %, and a 12 h photoperiod. Mortality was assessed after 24 h of exposure and the mites that did not respond to the brush stimulus were considered dead. Based on the estimated LC₅₀ value of LME, the toxic effects of standard concentrations (0.6 mg.ml⁻¹) of either genipin or geniposide were assayed, aiming at investigating the role of these iridoids in the acaricidal activities of the extract. Abbott's formula (17) was used for mortality correction. The corrected mortality (PT) values were computed as: $PT = [(Po - Pc)/(100 Pc)] \times 100$, where Po = observed mortality and Pc = control mortality.

Persistence bioassay

The persistence of LME was evaluated by spraying LC₉₀ on the arenas using the same procedures as described above and adapting the methodology of others bioassays (18,19). Twenty adults of the coconut mite were placed on an arena at 6 h intervals (i.e., 6, 12, 18, 24, 30, 36, 42, and 48 h after spraying), with five replications. The control group only used the solvent acetone. Mortality was recorded 24 h after mite transference.

Repellency bioassays

For the repellency bioassays, the arenas were prepared as previously described; however, each

arena consisted of a half treated and a half untreated area, this latter area was covered with two layers of adhesive tape during the LME spraying (20). The arenas were sprayed with LC₅₀ and LC₉₀ of the LME. After the drying of the solution, a white glue point was placed in the center of the disc (1.0 x 1.0 x 0.5 mm). The adult coconut mites were individually positioned onto the white glue point and their positions were evaluated after 1 h, 24 h, and 48 h. Three replicates were performed for each treatment, and the replicates consisted of 20 mites, totaling 60 mites per treatment.

Data analysis

The lethal concentrations of LME to the *A. guerreronis* were determined by the Probit analysis using SAS software (SAS Institute, 2002). The data on mortality of mites when treated with the main compounds were analyzed using an analysis of variance (ANOVA); the means were compared with Tukey test ($p < 0.005$). We conducted a linear regression to assess mortality persistence of the LME lethal doses, and the binomial test, at 5 % probability, was used to analyze the differences of the mite numbers choosing the treated or the untreated areas (repellency).

RESULTS AND DISCUSSION

Toxicity

Due to the negative effects related to using synthetic products, especially halogenated and organophosphorus pesticides, as well as to the increasing attention given to the use of natural products to assist in pest management and the demonstrations of phytochemicals as a great source for pest control, various research groups have profiled secondary metabolites from a variety of plant species (13,14). Data of these current studies have indicated that LME is toxic to the coconut mite (LC₅₀ = 0.60 mg.ml⁻¹, LC₉₀ = 16.69 mg.ml⁻¹) and show genipap as promising to control this important coconut pest (Table 1).

In previous screening studies, extracts of lipidomic profiles from the families Arecaceae, Fabaceae, and Malvaceae, such as babassu, coconut, cottonseed, and degummed soybean

Table 1. Lethal concentrations of LME to the coconut mite *A. guerreronis* based on the concentration-mortality bioassays, after 24 hours of exposure. / Concentraciones letales de LME al ácaro del cocotero *A. guerreronis* basado en bioensayos de concentración-mortalidad después de 24 horas de exposición.

Lethal concentrations	mg.ml ⁻¹	(95 % CI)	χ^2	P	n
LC ₁₀	0.02	(0.006-0.04)			
LC ₂₅	0.10	(0.05- 0.16)			
LC ₅₀	0.60	(0.41- 0.89)	9.523	0.089	20
LC ₇₅	3.46	(2.04- 7.82)			
LC ₉₀	16.69	(7.46-63.01)			
LC ₉₉	250.25	(65.61- 2408)			

Mean values obtained (n=8 replicates with 20 adult mites per replicate). Lethal concentrations were estimated using the Probit analysis. CI= Confidence interval at 95 % probability, χ^2 = Chi-square, P= Probability.

oils, were assayed against *A. guerreronis*; and they all demonstrated toxicity to this mite (21, 22). Interestingly, linoleic and oleic acids, which have been shown to be bioactive against the coconut mite (21, 22), were the major compounds in the lipidomic profiles of soybean and cottonseed oil.

The phytochemical profiles of the LME of genipap detected the presence of iridoids as main compounds. After 24 h of exposure, the iridoids genipin and geniposide, at 0.6 mg.ml⁻¹, inflicted 48 % and 52 % mortality on *A. guerreronis*, respectively, which differed significantly from mortality in the control ($p < 0.05$) (Figure 1). The biological and insecticidal activities of genipap are associated with several molecules, such as steroids, iridoids, and monoterpenoids, and they have been demonstrated by other authors (23, 7, 2, 8). These findings suggest that the major iridoids genipin and geniposide contributed to the bioactivity of LME towards *A. guerreronis*.

Persistence

The persistence bioassays revealed over 60 % mortality of *A. guerreronis* when exposed to the LC₉₀ of LME after 6 h of spraying. In general, the loss of toxic effects was evident as time after spraying elapsed, falling to less than 50 % only after 18 h, and the acaricidal effects disappeared completely after 42 h. This contrasted with the plain mortality in control (Fig 2). The low persistence may be explained by the compound oxidation, since the presence of phenolics was significant in the extract (24). The acaricide

residual activities are crucial for the management of *A. guerreronis*. Indeed, the understanding of persistence is important because, as this pest inhabits the perianth of the fruits, its control is difficult and frequent applications are needed (25, 26).

Repellency

The LC₅₀ and the LC₉₀ of LME were repellent to the coconut mite after 1, 24, and 48 hours after application (Figure 3A; Figure 3B), indicating that the mites were able to detect and avoid the treated areas. The observed repellent activities may be attributed to the rich chemical composition of the iridoids and to the fact that they act as a defense against the herbivores (2, 3). Plant repellency has been pointed out as an efficient strategy for avoidance of pest infestations in agroecosystems, being capable of reducing oviposition, injuries, and productivity losses; with the consequent reduction of the costs in the control of some of these pests (27, 14).

Thus, in addition to toxicity, it is important to understand the sublethal effects, such as repellency, in order to control pest dissemination. Several studies have demonstrated the repellent activities of extracts of plants against pest mites (14, 21, 22).

The results of this study suggest that the major iridoid compounds of *G. americana* play an important role in toxicity, with high repellency and low persistence to *A. guerreronis*. Other studies implicating genipin and geniposide also showed a variety of biological properties (28, 29,

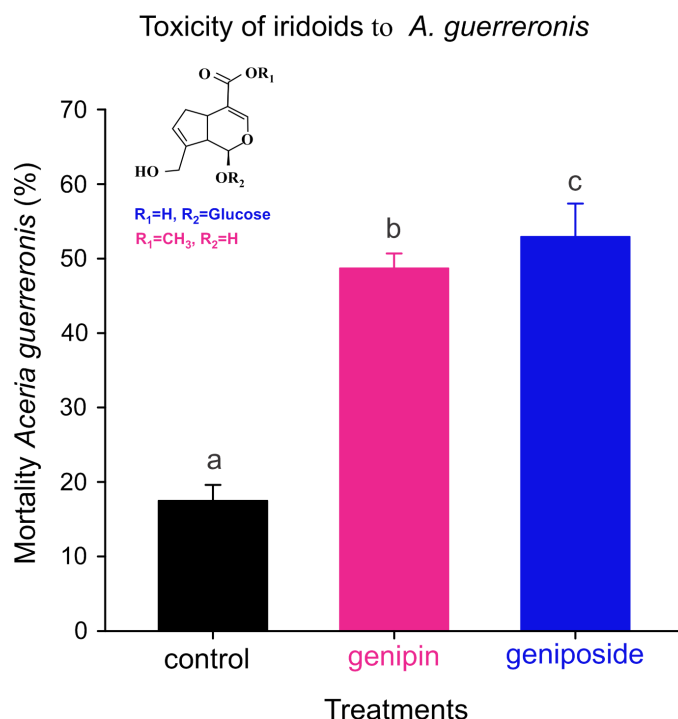


Fig. 1. Mortality of *A. guerreronis* when exposed to the main compounds of *G. americana*. The bars represent the mean of five replicates (\pm standard error); Different letters on each bar indicate significant differences between treatments according to Tukey's test ($p < 0.05$). / Mortalidad de *A. guerreronis* cuando se expuso a los principales compuestos de *G. americana*. Las barras representan la media de cinco réplicas (\pm estándar error); diferentes letras sobre cada barra indican diferencias significativas entre tratamientos por Test de Tukey ($p < 0,05$).

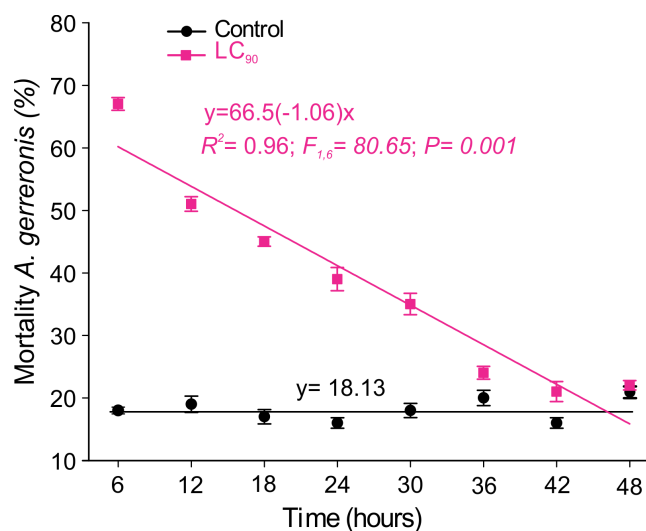


Fig. 2. Persistence of the acaricide activities of the leaf methanolic extracts of *G. americana* when the adults of *A. guerreronis* were exposed for 24 h to LC₉₀ at different time intervals after the application. The symbols represent the mean of five replicates and the vertical bars represent the standard error / Persistencia de actividad acaricida del extracto metanólico de las hojas de *G. americana* cuando se expusieron adultos de *A. guerreronis* por 24 h a la CL₉₀, en diferentes intervalos de tiempo después de la aplicación. Los símbolos representan la media de cinco réplicas y las barras verticales representan el error estándar.

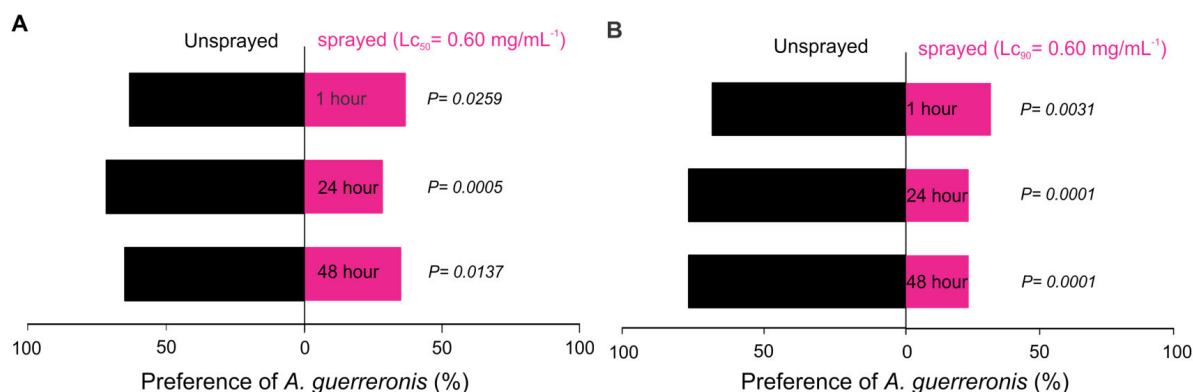


Fig. 3. Repellency of LC₅₀ (A) and LC₉₀ (B) of the leaf methanolic extracts of *G. americana* to the coconut mite *A. guerreronis*, after 1, 24, and 48 hours of spraying. / Repelencia de CL₅₀ (A) y CL₉₀ (B) de extracto metanólico de las hojas de *G. americana* al ácaro del cocotero *A. guerreronis* después de 1, 24 y 48 horas de pulverizado.

30). Overall, the results indicate that LME is a potential candidate for the development of plant-based pesticides to be used in the integrated management of *A. guerreronis*. Also, the major iridoids genipin and geniposide explain, at least in part, the bioactivity of LME towards the mite *A. guerreronis*. Further laboratory studies are necessary to assess the possible sublethal effects of LME on the natural enemies of *A. guerreronis*.

CONCLUSIONS

LME showed an acaricidal effect after 24 hours of exposure, repelling at the concentrations tested on the coconut mites. The major iridoids, genipin and geniposide, played a relevant role in its bioactivity. LME had a low persistence to *A. guerreronis*, suggesting that the mortality was concentrated in the first 12 hours after spraying. Nevertheless, LME can be considered as an alternative for assisting in the integrated control of the coconut mite in coconut plantations.

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The authors declare that they have no conflict of interest.

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