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Xylopia ochrantha essential oil against the peanut beetle

Óleo essencial de Xylopia ochrantha *frente ao besouro-do-amendoim*

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Palavras-chave
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Abstract

Ulomoides dermestoides is a cosmopolitan pest of various types of stored grain, causing significant economic and food losses. Plants are important sources of bioactive substances and, as such, are targets for the development of new pesticides. This study aimed to evaluate the larvicidal effect of *Xylopia ochrantha* Mart. essential oil against *Ulomoides dermestoides*. Dried aerial parts of *Xylopia ochrantha* were subjected to hydrodistillation to obtain the essential oil. Chemical characterization was carried out using gas chromatography and revealed bicyclogermacrene (38.5%), germacrene D (31.6%), and *δ*-elemene (9.8%) as the major components. The LC_{50} was 190.1 (105.6–300.5) µL/mL after 20 days of observation, showing its potential use as a bioinsecticide. This is the first report of *X. ochrantha* essential oil against *U. dermestoides* larvae. This study values biodiversity, with a focus on conserving native species, pointing to a future of sustainability.

Resumo

Ulomoides dermestoides *é uma praga cosmopolita de diferentes tipos de grãos armazenados, que causa perdas econômicas e de alimentos. As plantas são importantes fontes de substâncias bioativas e, como tal, são alvos no desenvolvimento de novos pesticidas. Este trabalho teve como objetivo avaliar o efeito larvicida do óleo essencial de* Xylopia ochrantha *Mart. frente ao* Ulomoides dermestoides. *Partes aéreas secas de* X. ochrantha *foram submetidas à hidrodestilação para obtenção do óleo essencial. A caracterização química foi feita por cromatografia gasosa e revelou biciclogermacreno (38,5 %), germacreno D (31,6 %) e* δ*-elemeno (9,8%) como componentes majoritários. A* CL_{50} *foi de 190,1 (105,6–300,5) µL/mL, após 20 dias de observação, mostrando seu uso potencial como bioinseticida. Esse é o primeiro relato do óleo essencial de* X. ochrantha *contra larvas de* U. dermestoides. *Esse estudo valoriza a biodiversidade, com foco na conservação de espécies nativas, o que aponta para um futuro rumo à sustentabilidade.*

Introduction

The reduction in post-harvest losses is an important factor in the food production chain. According to the Food and Agriculture Organization (FAO), adopting simple and effective storage practices can enhance food and nutrition security (FAO, 2014; Da Rocha et al., 2017). In this context, arthropods threaten the quality standards of food storage, as they can cause significant losses in nutritional value when they infest (Alcala-Orozco et al., 2019). Among the insects that attack stored grains, *Ulomoides dermestoides* (Fairmaire, 1893) (Coleoptera: Tenebrionidae) poses a risk to commerce because it feeds on stored grains (Morillo-Garcia et al., 2016). Also known as the peanut beetle, the insect can be found in various types of grain, including maize, oats, soybeans, peanuts, and rice, among others. As such, it is considered an important pest of grain and stored products (Melo et al., 2017; Plata-Rueda et al., 2020). The

Coleoptera order has insects popularly known as beetles. The Tenebrionidae family has one of the greatest diversities of the Coleoptera order in the animal kingdom, comprising more than 20.000 described species, classified into nine subfamilies. Many species have become secondary entomological pests of great importance, especially those that feed on stored products, being considered pests of stored grains (Aloquio Junior, 2020).

Plants are important sources of bioactive substances, which exhibit bioactivity due to their diverse chemical structures, especially those derived from essential oils (EOs) that have a low predisposition to generate resistance phenomena in various organisms (Garcez et al., 2013; Alcala-Orozco et al., 2019). This fact suggests the possibility of utilizing plant species that can counteract the phenomenon of resistance and mitigate environmental contamination. Bioinsecticides prepared with essential oils may be considered an important and sustainable option for controlling stored grain pests.

The genus *Xylopia* L. comprises approximately 160 species, distributed throughout the Americas, Africa, and Asia. Brazil has about 25 of these species. Metabolites such as alkaloids, amides, lignoids, acetogenins, and terpenoids are produced by this genus (Silva et al., 2015). *Xylopia ochrantha* Mart. has been reported with molluscicidal and insecticidal properties (Araújo et al., 2019; Viana et al., 2023). The study of the biological activity of *X. ochrantha* is quite inviting, particularly in light of the researchers' recent publications.

This plant belongs to the Annonaceae family and is endemic to Brazil, found in Restinga de Jurubatiba National Park and other states, including Espírito Santo and Pará. Generally, they are located in coastal ecosystems as sandbanks (Costa and Dias, 2001). Restinga de Jurubatiba National Park, situated within the Atlantic Forest domain in Rio de Janeiro, Brazil, is a unique ecosystem characterized by sandbanks. This region boasts a rich diversity of fauna and flora, largely due to its complex environmental mosaics, which occur in sandy soils with a relatively low rate of free water (Gonçalves-Esteves et al., 2001; Santos et al., 2009; Cogliatti-Carvalho et al., 2010). There, many species have aroused interest in scientific research due to their potential biological use. To our knowledge, the biological activity of the EO of *X. ochrantha* has never been verified against *Ulomoides dermestoides* larvae. This work aimed to obtain the essential oil of *X. ochrantha*, to identify its chemical profile, and to verify its biological activity against *Ulomoides dermestoides* larvae

Material and Methods

Plant Material

Leaves of *Xylopia ochrantha* Mart., Annonaceae, were collected at Restinga de Jurubatiba National Park in Brazil (RJ). It was authorized by SISBIO/ICMBio (13.659–12) and SisGen (A0D648D). The plant material was identified, and a voucher specimen was deposited (RFFP 15.326) at Faculdade de Formação de Professores (UERJ, Brazil).

Essential Oil Extraction

The *X. ochrantha* leaves were dried in an oven with air ventilation at 37 °C for 48 hours. The dried leaves (1.621 g) were crushed in a blender (model SPL-052, Spolu-benesse, Itajobi, SP, Brazil) with distilled water. After that, a Clevenger-type apparatus was used to carry out hydrodistillation for 4h. The obtained essential oil was dried with anhydrous sodium sulfate (Sigma-Aldrich, St. Louis, MO, USA) and stored under refrigeration (-20 °C) in an amber flask for further identification of the chemical constituents and biological tests.

Essential Oil Chemical Characterization

A GC-MS QP2010 (Shimadzu, Kyoto, Japan) gas chromatograph was used to analyze the essential oil. GC-MS conditions were as follows: injector temperature, 260 °C; carrier gas, Helium; flow rate, 1 mL/min; and split injection with a split ratio of 1:20. The oven temperature was initially 60 °C and then increased to 290 °C at a rate of 3 °C/min. One microliter of the essential oil dissolved in dichloromethane (1000 ppm) was injected into an RTX-5MS column (RESTEK, Corp., Bellefonte, PA, USA; 0.25 mm ID, 30 m in length, 0.25 μm and film thickness). Mass spectrometry (MS) was performed using electron ionization at 70 eV, with a scan rate of 1 scan per second. The identification of compounds was performed by comparison of the arithmetic index (AI) with the retention times (RT) of a mixture of a series of aliphatic hydrocarbons (C7 – C30, Sigma-Aldrich, St. Louis, Mo, USA) (Van Den Dool and Kratz, 1963). The identification of the compounds was achieved by comparing their retention indices and mass spectra with those reported in the literature (Adams, 2007) and the NIST database.

Ulomoides dermestoides Bioassay

Ulomoides dermestoides insects were created as study models at the Laboratory of Insect Biology, belonging to the Department of General Biology of the Universidade Federal Fluminense (LABI/GBG/UFF) under registration A855382 of the Genetic Heritage Management Council (SISGEN). The colonies are stored in organizer boxes with perforations covered with filo tissue, kept at a temperature of 28 ± 2 °C, a relative humidity of 60%, and a photoperiod of 12 hours (Lim and Sudderuddin, 1977).

To determine the mortality of the essential oil on the insects, three groups of 15 larvae, aged 20 to 30 days after hatching, were randomly separated and exposed to the essential oil through contact application. The assay had seven treatments: a negative control (no treatment), a solvent control (acetone), pure oil (without dilution), and serial acetone dilutions at the following concentrations: 500 μL/mL, 250 μL/mL, 125 μL/mL, and 62.5 μL/mL To expose the insects to the compounds, 9 μL of each solution was applied to a 9 cm diameter Petri dish (internally coated with filter paper), following a distribution pattern of 3 μL in columns and 3 μL horizontally. The insects were placed on the Petri dish in direct contact with the filter paper and followed for 20 days.

Statistical Analysis

Estimating test Chi-squared, LC_{50} estimations were performed using Probit analysis in SAS software (SAS Institute Inc., 2018). ANOVA (two-way) followed by Tukey's test was performed using Graphpad Prism 8, and differences were considered significant when $p < 0.05$.

Results

The hydrodistillation of *X. ochrantha* dried leaves yielded 0.82 % (w/w) of a transparent essential oil. The gas chromatograph analysis revealed 15 compounds, with 92.93 % of these being successfully identified. The major compounds were identified as bicyclogermacrene (38.5%), germacrene D (31.6%), and δ-elemene (9.8%), representing 79.97% of the EO (Figure 1). The essential oil was found to contain sesquiterpenes as the primary chemical class. Together, they account for more than 75% of the EO's composition (Table 1).

There was no larval mortality in either the negative or solvent controls throughout the 20-day experiment. In contrast, *U. dermestoides* larvae exposed to pure essential oil and to the concentration of 500 µL/mL exhibited the first mortality events within 24 h of exposure. From the 7th day onward, mortality was recorded at all concentrations tested, with a progressive increase until the end of the bioassay (Table 2). After 20 days, the mortality rates were observed at pure essential oil (75.56 ± 3.06%) and 500 µL/mL (64.44 ± 1.15%), 250 µL/mL (53.33 ± 1.00%), 125 µL/mL (40.00±1.00%), and 62.5 µL/mL (37.78±1.15). The full mortality results are presented in Table 2. The median lethal concentration (LC_{50}) of the *X. ochrantha* EO from leaves in *U. dermestoides* was estimated at 190.1 (105.6-300.5) µL/mL (Figure 2). In addition, low survival rates were observed on the 20th day (Table 2), with the highest rate being 62.2% in the 62.5 µL/mL dilution and the lowest with pure EO, which showed 24.5% survival.

Discussion

It was not the first time the chemical profile of this species had been studied. Araujo and coworkers (2019) identified 27 compounds, and the main constituents were bicyclogermacrene (25.18 %) and germacrene D (20.90 %) (Araujo et. al., 2019). That indicates a similarity between the two essential oils collected at the

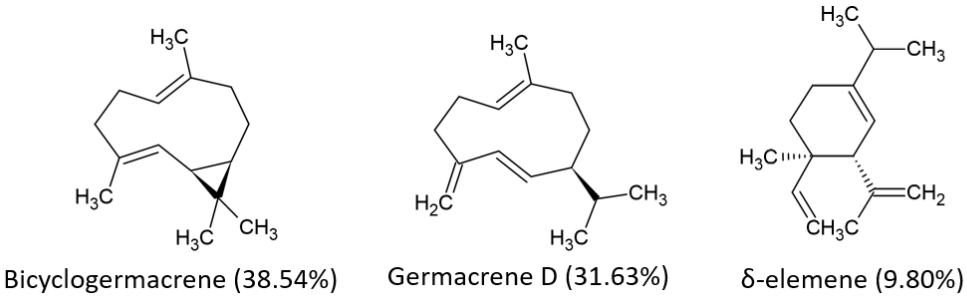


Figure 1. Major components of the essential oil of *Xylopia ochrantha* obtained from the leaves: bicyclogermacrene (38.5%), germacrene D (31.6%), and *δ*-elemene (9.8%).

Table 1. Chemical characterization of *Xylopia ochrantha* EO from leaves by GC-MS.

Peak	Retention time (min)	RI_{Rep}	RI_{Lit}	Substances	Relative abundance (%)
1	6.812	928	932	α-pinene	0.98
2	7.526	998	-	N.I.	3.56
3	7.588	1001	1002	*a*-phellandrene	4.04
4	19.492	1330	-	N.I.	2.67
5	19.635	1333	1335	**δ-elemene**	**9.80**
6	21.920	1387	1389	*β*-elemene	1.07
7	23.114	1416	1417	*β*-caryophyllene	2.35
8	25.417	1473	1478	*γ*-muurolene	0.75
9	25.638	1478	1480	**Germacrene D**	**31.63**
10	26.047	1488	-	N.I.	0.36
11	26.232	1493	1500	**Bicyclogermacrene**	**38.54**
12	27.157	1517	1522	*δ*-cadinene	0.92
13	28.695	1556	1559	Germacrene B	0.81
14	29.458	1576	1577	Spathulenol	2.04
15	31.477	1629	-	N.I.	0.49
Total identified					92.93
Monoterpenes hydrocarbons					15.89
Sesquiterpenes hydrocarbons					75.00
Oxygenated sesquiterpenes					2.04

Table 2. Mortality and survival of *Ulomoides dermestoides* larvae treated with *Xylopia ochrantha* EO after 20 days of exposure.

	Mortality (%)					Survival (%)
	1 D.A.T.	2 D.A.T.	7 D.A.T.	14 D.A.T.	20 D. A. T.	20 D. A. T
Negative Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	100±0.00
Solvent Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	100±0.00
pure EO (0.142 µL/cm²)	4.44±1.15	8.89±0.58	42.22±3.51[a]	64.44±2.08[a]	75.56±3.06[a]	24.44±3.06
500 µL/mL (0.071 µL/cm²)	2.20±0.58	2.20±0.58	15.56±2.08	31.11±0.58[b]	64.44±1.15[a]	35.56±1.15
250 µL/mL (0.036 µL/cm²)	0.00	0.00	6.67±0.00	26.67±1.00[c]	53.33±1.00[a]	46.67±1.00
125 µL/mL (0.018 µL/cm²)	0.00	0.00	6.67±1.00	24.44±1.15[d]	40.00±1.00[a]	60.0±1.00
62.5 µL/mL (0.009 µL/cm²)	0.00	0.00	8.89±1.15	15.56±1.15	37.78±1.15[a]	62.22±1.15

*Days after treatment (D.A.T.); When comparing with 1 D.A.T [a]p<0.0001; [b]p 0.0002; [c]p 0.0006; [d]p 0.0017.

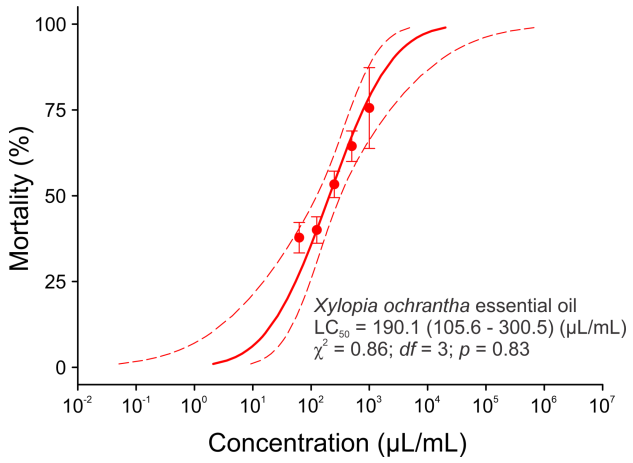


Figure 2. Dose response curve and median lethal concentration (LC_{50}) of the essential oil of *Xylopia ochrantha* from leaves in *U. dermestoides* larvae after 20 days.

same National Park. Sesquiterpenes are the most varied terpenoids, and their structural and stereochemical diversity is of great interest among researchers. Its functions are also varied and include repellents and natural pesticides (Vera, 2019). While hydrodistillation of *X. ochrantha* dried leaves yielded 0.82 % (w/w) of transparent EO, Araujo and coworkers (2019) obtained a yield of 0.2 % (w/w) from fresh leaves of *X. ochrantha*.

Various intrinsic and extrinsic factors may interfere with the composition of leaves' EO of *X. ochrantha* specimens, such as physiological variations, climate conditions, and genetic factors (Teles et al., 2013). A recent review study of the *Xylopia* genus conducted by Shakri et al. (2020) showed a great variety of the major components of the EO, mainly from the leaf part. Among them, bicyclogermacrene and germacrene D are among the most common.

A bioassay verified the action of *Cymbopogon citratus* EO against *U. dermestoides* with topical application to the insects to verify the LD_{50}, which was determined to be 5.17 µg/insect. The mechanism of action of *C. citratus* EO has not been fully elucidated, but the neurotoxic effect that led to rapid mortality is probably due to the presence of terpenoids (Plata-Rueda et al., 2020). The major components of EOs are generally accepted as being responsible for biological activity, and the magnitude of their effects is attributed to the major constituents, with modulation promoted by the minor constituents (Cervantes-Ceballos et al., 2015).

The toxic effect caused by contact with *X. ochrantha* EO may be related to the residual action of the major components present. That demonstrates its potential use as a possible bioinsecticide. This aspect opens up space for new approaches, utilizing natural products, which, through integrated management, can enhance the efficiency of controlling these pests (Torres et al., 2019). It is essential to ensure a sustainable future for humanity by producing chemicals, pharmaceuticals, and sustainable materials that meet human needs without depleting the resources that nature provides (Ferreira, 2022).

Conclusion

The experiment demonstrated the biological activity of *X. ochrantha* EO, as it was effective in promoting mortality among the treated insects. The toxic effect was evident in all dilutions at the end of the experiment, as mortality occurred at all concentrations tested. Thus, that points to the potential action as a natural bioinsecticide. Furthermore, it values biodiversity, as the research utilizes a Brazilian endemic plant from a protected area. The plant was harvested in accordance with sustainable management practices, as only the leaves were collected without cutting down the trees. It also gives visibility to this important ecosystem, which is the sandbank, a target for real estate speculation. The results were promising and suggest continuing to confirm its potential through future investigations. This type of research enables the development of

innovative products that are safer for human use and less harmful to the environment.

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Authorship Contributions

Conceptualization: RL, FD. Data curation: SSL, ER. Formal Analysis: SSL, ER. Funding acquisition: RL, FD, VVCR. Investigation: VVCR, SSL. Methodology: VVCR, SSL, SVM, MKC. Project administration: VVCR, SSL, RL, FD. Resources: FD, RL. Software: MFP. Supervision: FD, RL, SMG. Validation: SSL, VVCR. Visualization: ER, MFP. Writing – original draft: VVCR. Writing – review & editing: MFP, VVCR.

Conflict of Interest

The authors declare that there are no conflicts of interest to report.

Data Availability

The complete set of data analyzed during the current study is presented in the body of the manuscript.

Ethical Compliance

The authors have adhered to all ethical and legal guidelines for conducting this work. SISBIO/ICMBio (13.659–12) and SisGen (A0D648D).

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