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Review

Comparative study of the oil repellent activity of *Copaifera officinalis* Linnaeus and *Copaifera reticulata* Ducke front nymphs of *Periplaneta americana* Linnaeus

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To control urban pests, especially cockroaches of the *Periplaneta americana* species, various pesticides have been developed that are increasingly potent and effective. However, the unrestrained application of pesticides has had negative consequences, such as the disappearance of some useful insect species and, consequently, the appearance of new pests, both in the countryside and cities. Due to the current scenario, it was necessary to search for new alternatives for the control of these insects. Among the species studied, Copaíba stood out. The oils were analyzed using GC-MS, b-caryophyllene and a-bergamotene being the predominant compounds. Repellency tests were performed with three different concentrations of *C. officinalis* and *C. reticulata*, 500 µg/mL, 250 µg/mL and 125 µg/mL, in triplicate. It can be observed that the oil of *C. officinalis* was more repellent to the nymphs at concentrations of 500 µg/mL and 250 µg/mL, however, when the behavior in nymphs exposed to the concentration of 125 µg/mL was compared, it was noted that *C. reticulata* oil was more repellent at this concentration. *Copaifera* has shown promising activity as a repellent against arthropods owing to the complex chemical composition of its oils.

Keywords: *Periplaneta americana. Copaifera officinalis. Copaifera reticulata*. Repellent activity. Sesquiterpenes.

INTRODUCTION

One of the main challenges to public health in large cities is urban pest and insect control, especially in cockroaches the *Periplaneta americana* species. This species of insects, often found in household areas, is one of the most harmful to humans, as it has a high adaptability, reproductive capacity, and facility for finding shelter and food in urban areas, acting as a vector and reservoir of various human pathogens (Zorzenon, 2002).

To control these pests, more potent synthetic pesticides have gained popularity in the market and are widely traded (Flores *et al*., 2004). However, the unrestrained use of these products has negative consequences, such as the disappearance of some species of useful insects and, consequently, the appearance of new pests with resistance to these products. In addition, they are highly toxic to humans, which is common in domestic accidents involving this type of product (Cruz, 2002).

Thus, to solve this problem, the use of natural products, such as insecticides and repellents, is increasingly sought, especially those of plant origin, as they are less toxic, inexpensive, and have good efficacy. Among the species studied for this purpose, we highlight

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the species of *Copaifera*, a plant present in the Amazon region, whose oil extracted from its stem has, among numerous properties, repellent activity against insects (Nerio, Olivero-Verbel, Stashenko, 2010).

Oils from two Copaifera species*, Copaifera officinalis* and *Copaifera reticulata* were selected. The species was chosen because they are widely used in the Amazon region and because they have repellent activity. The aim of this study was to compare the repellent activities of oils from these two species.

METHODS

Insects

The *P. americana* insects used in the project were created and reproduced at the Toxicology Laboratory of the Pharmacy Course of the Federal University of Amapá, UNIFAP. The insects were kept at room temperature with low light in covered boxes that allowed the passage of air, separated by developmental stage, and fed dog food and water supplied through vegetables.

Obtaining the oils

The oil of *C. officinalis* (Lot L091) was purchased from J. R. D. Indústria Farmacêutica LTDA (Uniphar®), Anápolis-Goiás, Brazil.

The oil of *C. reticulata* was supplied by the Federal University of Western Pará (UFOPA) from the Tapajós National Forest (FLONA) located at km 83 of BR-163, in the municipality of Belterra-PA (latitude 3° S 07' 36.2528517843, longitude 54° W 99' 59.896691144').

Sample preparation for GC-MS analysis

The samples of *Copaifera* oils from both species were filtered through a Millepore 0.45 µm filter membrane and 100 µL aliquots were diluted in 2 mL hexane and analyzed in GC-MS.

GC-MS oil analysis

The analyses were performed using gas chromatography coupled with mass spectrometry (CG-MS; Shimadzu Corporation, QP2010SE). The chromatographic conditions were: capillary column Rtx-5, 30 m, 0.25 mm ID, 0.25 µm; Helium as carrier gas with a flow of 1.5 mL/min; Oven temperature at 120 °C for 2 min - increase of 3 °C/min to 160 °C for 2 min, followed by an increase of 8 °C/min until final temperature of 290 °C maintained for 2 minutes; Injector temperature 270 °C; detector temperature 290 °C; 1.0 µL injected volume in a 20:1 split ratio. The mass detector was operated in Scan mode of 40-400 (m/z) with a 70 eV electronic impact.

To identify the compounds, the fragmentation spectrum of peaks with a similarity above 90% compared to the NIST library of the equipment was used (Carvalho *et al*., 2015).

In vivo tests

This test was based on the method proposed by McDonald *et al*. (1970). The repellency test was performed with three different concentrations of *C. officinalis* and *C. reticulata* oil, 500 µg/mL, 250 µg/mL and 125 µg/mL, in triplicate, to determine a minimum effective concentration.

In this test, 2 mL of ethanol (control) was placed in one half of a 9 cm diameter filter paper disk, while in the other half, 2 mL of ethanolic solution containing *C. officinalis* or *C. reticulata* oil was placed, and the solvent was evaporated at ambient temperature. The filter paper disks were placed inside Petri dishes with 5 nymphs of the specie *P. americana* nymphs in triplicate for each concentration, and the behavior of the nymphs was observed for 4 h.

The movement of insects was analyzed every hour to evaluate their attraction to the control or treated paper on the plate. Based on the data recorded during the test, the preference index (PI) cited by Procópio *et al.* (2003) was calculated as follows:

$$PI = (\%ITP - \%IC) / (\%ITP + \%IC)$$

where %ITP = % insects on the test paper and %IC = % insects on the control paper. The index was classified as PI: from -1.00 to -0.10 means repellent oil, -0.10 to +0.10 means neutral oil and from +0.10 to +1.00 means attractive oil.

Statistical analysis

All experiments were performed in triplicate and compared using the one-way analysis of variance (ANOVA) test and Tukey's test in BioStat Software 5.3; $p < 0.05$.

RESULTS AND DISCUSSION

Copaíba oil is characterized as an oil resin consisting of a volatile component (sesquiterpenes) and a resinous component (diterpenes), which vary in content according to the species and environmental conditions (Galúcio *et al.,* 2016). The sesquiterpenes identified in the *C. officinalis* and *C. reticulata* oils are shown in Table I. The main hydrogenated sesquiterpenes identified represent 57.54% of *C. officinalis* oil and 70.25% and *C. reticulata* oil. The main components identified in *C. officinalis* oil were β-caryophyllene (38.97%), α-bergamotene (4.37%), β-bisabolene (3.85%), aromadendrene (3.31%), germacrene D (3.08%), γ-muurolene (2.14%). The main components present in *C. reticulata* oil are β-caryophyllene (39.91%), α-bergamotene (11.34%) and β-bisabolene (7.38%).

TABLE I – Major sesquiterpenes identified in *C. officinalis* and *C. reticulata* oils analyzed in GC-MS, with their respective percentage data

Retention index		Compounds	*C. officinalis*	*C. reticulata*
DB-5	Lit. (Adams, 2017)	% hydrogenated sesquiterpenes		
1405	1408	β-caryophyllene	38.97	39.91
1436	1411	α-bergamotene	4.37	11.34
1510	1505	β-bisabolene	3.85	7.38
1455	1436	aromadendrene	3.31	-
1480	1484	germacrene D	3.08	5.84
1479	1478	γ-muurolene	2.14	-
1455	1436	α-humulene	1.17	3.15
1376	1374	α-copaene	0.65	2.63
		Total	**57.54**	**70.25**

β-caryophyllene is a terpenoid present in abundance in copaiba oil. It is divided into caryophyllene, humulene, and copaene, which are volatile compounds, and diterpenes that have a resinous aspect, such as kaurenoic, copal, and polyalitic acids, which are non-volatile compounds (Arruda *et al.,* 2019). The described terpenes have repellent properties that are often associated with the chemical composition of their natural oils, which may explain their repellency against *P. americana* (Nerio, Olivero-Verbel, Stashenko, 2010; Keeler, Tu, 1991).

α-bergamothene has been identified in both species, and studies have suggested that it has repellent activity against mosquitoes of the genus Aedes (Conti *et al*., 2012).

The repellency of *C. reticulata* oil was greater than that of *C. officinalis* oil at the lowest concentration tested (125 µg/mL), since at this concentration, *C. officinalis* oil had neutral repellency (Frames I and II). The repellency results were satisfactory, although the concentration (250 µg/mL) of the *C. reticulata* oil obtained a result lower than expected (Frame II).

In the first 3 h of the test, neither oil showed statistically significant differences ($p>0.05$). In the 4th hour of the test, both were present ($p<0.049$) at a concentration of 250 µg/mL, demonstrating that the *C. officinalis* oil was more repellent than the *C. reticulata* oil. In the statistical analysis, it was also noted that the number of hours the insects were exposed to the oils did not influence their repellency.

Frame II shows that *C. reticulata* oil repelled more nymphs at 125 µg/mL than at 250 µg/mL. However, by analyzing the average number of insects repelled in each hour of the test (Frame III), we observed that only in the fourth hour of the test did the concentration of 125 µg/mL repel more than 250 µg/mL, which influenced the preference index value and percentage of repelled insects.

FRAME I – Preference index (PI) of each concentration of *C. officinalis* the percentage of insects in the control and treatment groups at each concentration tested

CONCENTRATION	PI (PREFERENCE INDEX)	% OF NYPHIES IN CG	% OF NYPHIES IN TG
500 µg/mL (1.11 g/m²)	- 0.86	93.33 %	6.66%
250 µg/mL (0.55 g/m²)	- 0.70	85 %	15%
125 µg/mL (0.28 g/m²)	+0.10	45%	55%

PI (preference index): repellency (-0.10 to -1.00), neutral (-0.10 to + 0.10) and attractive (+0.10 to + 1.00). CG (Control Group); TG (Treated Group).

FRAME II – Preference index (PI) of each concentration of *C. reticulata* the percentage of insects in the control and treatment groups at each concentration tested

CONCENTRATION	PI (PREFERENCE INDEX)	% OF NYPHIES IN CG	% OF NYPHIES IN TG
500 µg/mL (1.11 g/m²)	- 0.8	90%	10%
250 µg/mL (0.55 g/m²)	- 0.2	60%	40%
125 µg/mL (0.28 g/m²)	- 0.4	70%	30%

PI (preference index): repellency (-0.10 to -1.00), neutral (-0.10 to + 0.10) and attractive (+0.10 to + 1.00). CG (Control Group); TG (Treated Group).

FRAME III – Average number of insects repelled by *C. reticulata* oil in each test hour

CONCENTRATION	1st hour	2nd hour	3rd hour	4th hour
500 µg/mL (1.11 g/m²)	4.33	4.33	5	4.33
250 µg/mL (0.55 g/m²)	3	3.33	3.67	3.33
125 µg/mL (0.28 g/m²)	3	3.33	3	4.67

The greater repellency of the oil of *C. reticulata* might have been due to its higher sesquiterpene content, and it is important to note that its repellency was more pronounced at the lowest concentration (125 µg/mL) compared to the others. The different repellency concentrations can be attributed to oil extraction and volatilization (Boskovic *et al.,* 2015).

According to Shadia *et al*. (2007), β-bisabolene is the second most abundant sesquiterpene in white basil (*Ocimum americanum*) oil. In one study, the oil of *O. americanum* showed repellent and insecticidal activity against the pupae and larvae of *Agrotis ipsilon*. In a study carried out with *Thymus magnus* oil, β-bisabolene showed 39.8% repellency against *Aedes albopictus* females, whereas β-caryophyllene showed 18% repellency, being the second- and third-most repellent compounds of the oil, respectively. (Park, Koo, Kim, 2012).

Other compounds present in *C. officinalis* and *C. reticulata* oils, such as aromadendrene - identified in *C. officinalis* oil - is also described in the composition of oils from other species that also have repellent activity, such as *Salvia dorisiana*, which has in the composition of its oil 25.7% aromadendrene (Conti *et al.,* 2012).

Most insect repellents work by providing a vapor barrier that prevents the insect from coming into contact with the desired surface, which is made up of volatile substances such as the hydrogenated sesquiterpenes identified in both oils. These compounds are more volatile than oxygenated compounds owing to their lower molecular weights. The volatility of the majority of compounds in oils can explain their repellency (Gomes *et al.,* 2014).

The higher content of sesquiterpenes present in *C. reticulata* oil may explain its higher repellent activity, since it showed repellent activity even at the lowest concentration tested, whereas *C. officinalis* oil was neutral to nymphs at the lowest concentration (Gomes *et al.,* 2014).

The synergism between the compounds present in oils can also be attributed to their repellent activity, and not only to an isolated compound (Nerio, Olivero-Verbel, Stashenko, 2010). The study of synergistic effects between the constituents of oil and oil mixtures, as well as the search for new additives that can increase the protection period, are promising lines of study.

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REFERENCES

Arruda C, Mejía JAA, Ribeiro VP, Borges CHG, Martins CHG, Veneziani RCS, et al. Occurrence, chemical composition biological activities and analytical methods on Copaifera genus—A review. Biomed Pharm. 2019;109:1-20.

Boskovic M, Zdravkovic N, Ivanovic J, Janjic J, Djordjevic J, Starcevic M, Baltic MZ. Antimicrobial activity of thyme (Tymus vulgaris), and oregano (Origanum vulgare) essential oils against some Food-borne microorganisms. Proc Food Sci. 2015;5:18-21.

Carvalho HO, Lima CS, Sanches AA, Silva JO, Fernandes CP, Carvalho JCT. Study of the in vitro release profile of sesquiterpenes from a vaginal cream containing Copaifera duckei Dwyer (Fabaceae) oleoresin. J Appl Pharm Sci. 2015;5(4):001-006.

Conti B, Benelli G, Flamini, G, Cioni PL, Profeti R Ceccarini L, et al. Larvicidal and repellent activity of Hyptis suaveolens (Lamiaceae) essential oil against the mosquito Aedes albopictus Skuse (Diptera: Culicidae). Parasitol Res. 2012;110(5):2013-2021.

Conti B, Benelli G, Leonardi M, Afifi FU, Cervelli C, Profeti R, et al. Repellent effect of Salvia dorisiana, S. longifolia, and S. sclarea (Lamiaceae) essential oils against the mosquito

Aedes albopictus Skuse (Diptera: Culicidae). Parasitol Res. 2012;111(1):291-299.

Cruz, I. Manejo da resistência de insetos-praga a inseticidas, com ênfase em Spodoptera frugiperda (Smith). Embrapa M e S. 2002;21:1-65.

Flores AV, Ribeiro JN, Neves AA, Quiroz ELRD. Organoclorados: um problema de saúde pública. Amb Soc. 2004;2(2):1-15.

Galúcio CDS, Benites CI, Rodrigues RA, Maciel, MRW. Recuperação de sesquiterpenos do óleo-resina de copaíba a partir da destilação molecular. Quím Nova. 2016;39(7):795-800.

Gomes MRF, Dorneles GG, Schuh RS, Jacques ALB; Bertholdo-Vargas LR, Bordignon SAL, et al. Chemical composition of essential oils of *Drimys angustifolia* Miers and *Drimys brasiliensis* Miers and their repellency to drywood termite *Cryptotermes brevis* (Isoptera: Kalotermitidae). Rev Cien Farm Básica Apl. 2014;35(1):41-46.

Keeler RF, Tu AT. Toxicological of plant and fungal compounds. Handb Nat Toxins. 1991;6:665.

Mcdonald, LL, Guy RH, Speirs RD. Preliminary evaluation of new candidate materials as toxicants, repellents, and attractants against stored-product insects-1. US Agricultural Research Service. 1970;1(882):14.

Nerio LS, Olivero-Verbel J, Stashenko E. Repellent activity of essential oils: a review. Bior Techn. 2010;101(1):372-378.

Park YU, Koo HN, Kim GH. Chemical composition, larvicidal action, and adult repellency of Thymus magnus against Aedes albopictus. J Am Mosq Control Assoc. 2012;28(3):192-198.

Procópio SO, Vendramim JD, Ribeiro Júnior JI, Santos JB. Bioatividade de diversos pós de origem vegetal em relação à Sitophilus zeamais Mots. (Coleoptera: Curculionidae). Cienc Agrotecnol. 2003;27(6):1231-1236.

Shadia E, El-Aziz A, Omer EA, Sabra AS. Chemical composition of Ocimum americanum essential oil and its biological effects against, Agrotis ipsilon, (Lepidoptera: Noctuidae). Res J Agric Biol Sci. 2007;3(6):740-747.

Zorzenon FJ. Noções sobre as principais pragas urbanas. Biol. 2002;64(2):231-234.

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