

Caracterização Química e Potencial Inseticida do Óleo Essencial de *Ocimum gratissimum* L. (Lamiaceae) Contra *Nauphoeta cinerea* (Blaberidae)

Chemical Characterization and Insecticidal Potential of the Essential Oil of *Ocimum gratissimum* L. (Lamiaceae) Against *Nauphoeta cinerea* (Blaberidae)

Caracterización química y potencial insecticida del aceite esencial de *Ocimum gratissimum* L. (Lamiaceae) contra *Nauphoeta cinerea* (Blaberidae)

Recebido: 07/07/2020 | Revisado: 21/07/2020 | Aceito: 03/08/2020 | Publicado: 11/08/2020

Felicidade Caroline Rodrigues

ORCID: <https://orcid.org/0000-0003-0803-7046>

Federal University of Pernambuco, Brazil

E-mail: rodriguescaroline26@gmail.com

José Weverton Almeida-Bezerra

ORCID: <https://orcid.org/0000-0002-0966-9750>

Federal University of Pernambuco, Brazil

E-mail: weverton.almeida@urca.br

Kleber Ribeiro Fidelis

ORCID: <https://orcid.org/0000-0002-2507-1468>

Federal University of Pernambuco, Brazil

E-mail: kleberfidelis0@gmail.com

Adrielle Rodrigues Costa

ORCID: <https://orcid.org/0000-0003-1518-0115>

Regional University of Cariri, Brazil

E-mail: adrielle.arc@hotmail.com

Mikael Amaro de Souza

ORCID: <https://orcid.org/0000-0001-7085-5870>

Regional University of Cariri, Brazil

E-mail: mikael.amaro.08@hotmail.com

Maria Haiele Nogueira da Costa

ORCID: <https://orcid.org/0000-0002-0316-0830>

Regional University of Cariri, Brazil

E-mail: haielecosta@gmail.com

Luciano Temoteo dos Santos

ORCID: <https://orcid.org/0000-0002-9215-6832>

Federal University of Cariri, Brazil

E-mail: luciano.temoteosantos@gmail.com

Maria Aparecida Barbosa Ferreira Gonçalo

ORCID: <https://orcid.org/0000-0003-4974-2606>

Regional University of Cariri, Brazil

E-mail: cida_barba@hotmail.com

Saulo Almeida de Menezes

ORCID: <https://orcid.org/0000-0001-6657-585X>

Federal University of Pernambuco, Brazil

E-mail: saulomenezes99@gmail.com

Samara Mendes de Sousa

ORCID: <https://orcid.org/0000-0002-5429-3844>

Regional University of Cariri, Brazil

E-mail: samaramendes.185@outlook.com

Edna Karol Rodrigues de Araujo

ORCID: <https://orcid.org/0000-0001-6370-8418>

Faculty of Juazeiro do Norte, Brazil

E-mail: edna_karol@hotmail.com

Ana Vaeline Patrício Braga

ORCID: <https://orcid.org/0000-0001-8274-5011>

Faculty of Juazeiro do Norte, Brazil

E-mail: vaelline.braga@hotmail.com

Talina Guedes Ribeiro

ORCID: <https://orcid.org/0000-0001-5801-6679>

Regional University of Cariri, Brazil

E-mail: thalinaguedes@gmail.com

Angélica Rodrigues de Souza Costa

ORCID: <https://orcid.org/0000-0001-5872-9492>

Regional University of Cariri, Brazil

E-mail: angelicarodrigues.pb@gmail.com

Marcos Aurélio Figueiredo dos Santos

ORCID: <https://orcid.org/0000-0002-3409-5242>

Regional University of Cariri, Brazil

E-mail: marcos.figueiredo@urca.br

Ricardo Gomes dos Santos Nunes

ORCID: <https://orcid.org/0000-0002-5814-0335>

Regional University of Cariri, Brazil

E-mail: ricardo.gomes232@gmail.com

Maria Ivaneide Rocha

ORCID: <https://orcid.org/0000-0002-0312-9315>

Regional University of Cariri, Brazil

E-mail: ivaneidemaria@bol.com.br

Resumo

Plantas são consideradas uma fonte rica de compostos bioativos e uma alternativa de agentes no controle de insetos. Dentre elas, as representantes da família Lamiaceae são as mais conhecidas com esse potencial, pois produzem compostos voláteis com atividades inseticidas, conhecidos como óleos essenciais, como é o caso de *Ocimum gratissimum*. Desta forma, o presente trabalho teve como objetivo, determinar a composição química do óleo das folhas de *O. gratissimum* e seu potencial inseticida contra a barata *Nauphoeta cinerea*. Para tanto, o óleo essencial foi caracterizado quimicamente por meio de um Cromatógrafo Gasoso acoplado a um Espectrômetro de Massas (CG/EM). Quanto à ação inseticida, foram utilizadas ninfas (20 dias de idade) sendo empregada a metodologia de fumigação em concentrações variando de 50 a 1000 µg de óleo por mL de ar. As taxas de mortalidade foram observadas a cada 12 horas durante 1 dia. Os resultados apontaram que o óleo essencial apresenta propriedades inseticidas, visto que ele apresentou uma concentração mediana letal (CL₅₀) de 516 µg/mL. Tal propriedade pode estar relacionada à composição heterogênea do óleo, o qual apresentou 12 compostos terpênicos, sendo o 1,8 Cineol o composto majoritário (30,04%). Sendo assim, o óleo essencial de *O. gratissimum* apresenta atividade inseticida, podendo ser utilizado no combate pragas, como no caso de baratas.

Palavras-chave: Alfavaca-cravo; 1,8 Cineol; Atividade biológica; Bioinseticida.

Abstract

Plants are considered a rich source of bioactive compounds and an alternative agent for insect control. Among them, representatives of the Lamiaceae family are the best known for this potential, as they produce volatile compounds with insecticidal activities, known as essential oils, as is the case with *Ocimum gratissimum*. In this way, the present work had as objective, to determine the chemical composition of the oil of the leaves of *O. gratissimum* and its potential insecticide against the cockroach *Nauphoeta cinerea*. For that, the essential oil was chemically characterized by means of a Gas Chromatograph coupled to a Mass Spectrometer (GC/MS). As for the insecticidal action, nymphs (20 days of age) were used and the fumigation methodology was used in concentrations ranging from 50 to 1000 µg of oil per mL of air. Mortality rates were observed every 12 hours for 1 day. The results showed that the essential oil has insecticidal properties, since it had a median lethal concentration (LC₅₀) of 516 µg/mL. Such property may be related to the heterogeneous composition of the oil, which had 12 terpenic compounds, with 1.8 Cineole being the major compound (30.04%). Thus, the essential oil of *O. gratissimum* has an insecticidal activity and can be used to fight pests, as in the case of cockroaches.

Keywords: Alfavaca-cravo; 1.8 Cineole; Biological activity; Bioinsecticide.

Resumen

Las plantas se consideran una rica fuente de compuestos bioactivos y un agente alternativo para el control de insectos. Entre ellos, los representantes de la familia Lamiaceae son los más conocidos por este potencial, ya que producen compuestos volátiles con actividades insecticidas, conocidos como aceites esenciales, como es el caso de *Ocimum gratissimum*. De esta manera, el presente trabajo tuvo como objetivo determinar la composición química del aceite de las hojas de *O. gratissimum* y su potencial insecticida contra la cucaracha *Nauphoeta cinerea*. Para esto, el aceite esencial se caracterizó químicamente por medio de un cromatógrafo de gases acoplado a un espectrómetro de masas (CG/EM). En cuanto a la acción insecticida, se usaron ninfas (20 días) y la metodología de fumigación se usó en concentraciones que varían de 50 a 1000 µg de aceite por mL de aire. Las tasas de mortalidad se observaron cada 12 horas durante 1 día. Los resultados mostraron que el aceite esencial tiene propiedades insecticidas, ya que tenía una concentración letal media (CL₅₀) de 516 µg/mL. Dicha propiedad puede estar relacionada con la composición heterogénea del aceite, que presentó 12 compuestos terpénicos, siendo 1.8 Cineol el compuesto principal (30.04%).

Por lo tanto, el aceite esencial de *O. gratissimum* tiene una actividad insecticida y puede usarse para combatir las plagas, como en el caso de las cucarachas.

Palabras clave: Alfavaca-cravo; 1.8 Cineole; Actividad biológica; Bioinsecticida.

1. Introduction

Plants are considered a rich source of bioactive compounds and an alternative to agents in insect control (Adeniyi et al., 2010). In studies on the development of insecticides, the use of natural products is highlighted, this due to the great diversity and high efficacy of products of plant origin, in this area essential oils have gained special attention (Benelli & Pavela, 2018; Bezerra et al., 2020).

Essential oils are produced by secondary plant metabolism and are found, mainly, in leaf trichomes (Duarte et al., 2016; Bezerra et al., 2017), they are a complex mixture of volatile organic compounds, usually with molecular weight below 300 M showing various chemical classes, such as alcohol, esters, aldehydes, ketones, ethers, amides, phenols and more abundantly, terpenes (Dhif et al., 2016).

Essential oils have a great diversity of biological activities, including insecticidal activity and several studies have proven the effectiveness of their use and the diversity of species that can be used for this purpose, with *Mesosphaerum suaveolens* (L.) Kuntze (Bezerra et al., 2020), *Dysphania ambrosioides* (L.) Mosyakin & Clemants (Bezerra et al., 2019), *Clausena anisata* (Willd.) Hook.f. ex Benth. (Pavela et al., 2018), *Acemella oleracea* (L.) R.K.Jansen (Benelli et al., 2019a) and several others.

Lamiaceae is one of the largest and most diverse families of aromatic plants, consisting of about 4,000 species distributed around the world, with ornamental, pharmaceutical and aromatic properties, being a source of essential oils and terpenes with toxic action (Giatropoulos et al., 2018).

One of the Lamiaceae species that stands out the most is *Ocimum gratissimum* L., a plant widely distributed in tropical regions, widely used in folk medicine to treat diseases such as respiratory infection, diarrhea, pneumonia, skin diseases, cough, conjunctivitis and others (Adeniyi et al., 2010; Pandey et al., 2017). Several studies have investigated the potential of *O. gratissimum*, proving antimicrobial, antimalarial, pesticide, repellent and insecticide activities (Kpoviessi et al., 2014; Pandey et al., 2017; Pandey, 2017).

Given the above, the objective of this study is to evaluate the insecticidal potential of

O. gratissimum against *Nauphoeta cinerea*, as well as identifying the constituents responsible for such activity.

2. Materials and Methods

2.1 Methodology

This research is an analysis of qualitative evaluation, performed in the laboratory of microscopy, of the Regional University of Cariri- URCA. It is an investigation of the chemical constituents present in the essential oil of the species *O. gratissimum* and the potential insecticide of this oil against the cheap lobster *Nauphoeta cinerea*. These secondary metabolites were extracted by gas chromatography, to detect the terpenes present in the analyzes sample. In addition to phytochemistry, the insecticidal method was applied to determine its effect on the tested cockroaches.

2.2 Botanical material collection

The leaves of *O. gratissimum* were collected in the Horto de Medicinal Plants of the Regional University of Cariri - URCA, Crato, CE, Brazil, under the coordinates 07°14'19.2 "S and 39°24'52.8" longitude of Greenwich. A specimen was pressed, identified by Francisco Assis Bezerra da Cunha and deposited at the URCA Caririense Herbarium Dárdano de Andrade-Lima (HCDAL) with voucher #10,790.

2.3 Extraction of essential oil

The essential oil of *O. gratissimum* was extracted from the dried leaves by hydro distillation. After dehydration, the leaves were crushed and placed in a 3 L volumetric flask, to which 2 L of distilled water were added. Subsequently, the flask was attached to Clevenger and the temperature increased until the water boiled, after boiling, the extraction continued for a period of 2 h and the essential oil was collected with the aid of a glass pipette and stored in an amber bottle and refrigerated. Sodium sulfate to remove the aqueous phase present in the essential oil.

2.4 Chemical composition

2.4.1 GC analysis

The essential oil after preparation was submitted to GC analysis in a Varian 3800 Gas Chromatograph equipped with a capillary fused silica column ($25\text{ m} \times 0.25\text{ }\mu\text{m}$) coated with SE-54. The GC conditions used were: carrier gas He (1 mL/min); on column injector $200\text{ }^{\circ}\text{C}$; FID $250\text{ }^{\circ}\text{C}$; column temperature $60\text{ }^{\circ}\text{C}$ to $325\text{ }^{\circ}\text{C}$ at $4\text{ }^{\circ}\text{C}/\text{min}$. GC-MS analyses were performed on an HP 5973 - 6890 GC-MSD system operating in the EI mode at 70 eV, equipped with an HP-5 cross-linked capillary column ($30\text{ m} \times 0.25\text{ mm}$). The temperature of the column and the injector were the same as those from GC.

2.4.2 Identification of the components

Identification of the constituents of *O. gratissimum* essential oil was based on retention index (RI), determined with reference of the homologous series of *n*-alkanes, C₇-C₃₀, under identical experimental conditions, comparing with the mass comparison of the mass spectra with those of NBS Library (Massada, 1995) and those described by Adams (1995). The relative amounts of individual components were calculated based on the CG peak area (FID response).

2.5 Inventory and creation of *Nauphoeta cinerea*

The cockroaches were obtained from the Federal University of Santa Maria - UFSM, donated by Professor Dr. João Batista Teixeira da Rocha. The cockroaches were created at the Microscopy Laboratory (LABOMIC) of the Regional University of Cariri - URCA under conditions of temperature of $25 \pm 5\text{ }^{\circ}\text{C}$ and relative humidity of 50%. The diet of adult nymphs and cockroaches consisted of mice food and water (Bezerra et al., 2020).

2.6 Insecticide testing

The determination of insecticidal activity took place through fumigation, where filter paper soaked in essential oil and attached to a 330 mL volumetric flask. Subsequently,

nymphs with 20 days of life were subjected to different concentrations of the oil (50-1000 µg/mL) and the mortality observed after 24 h (Bezerra et al., 2017).

2.7 Statistical Analysis

After obtaining the data, their means and their respective standard deviations were calculated. These were subjected to a one-way analysis of variance followed by the Tukey test at 95% reliability in the software GraphPad Prism 6.

3. Results

3.1 Chemical Composition

As can be seen in Table 1, a total of 12 phytochemical constituents were identified in the essential oil of *O. gratissimum*, of these the main compound detected, where we can consider the major compound, was 1,8-cineole (Figure 1A) with presence greater than 30% in a retention time of 18.07 min, followed by the secondary constituents eugenol (Figure 1B) with 27.58% (RT: 37.48) and terpineol-4 with 14.45% (RT: 47.96) (Figure 1C). Where the observation of the figures it is possible to observe a chemical structure of the main phytochemical compounds detected.

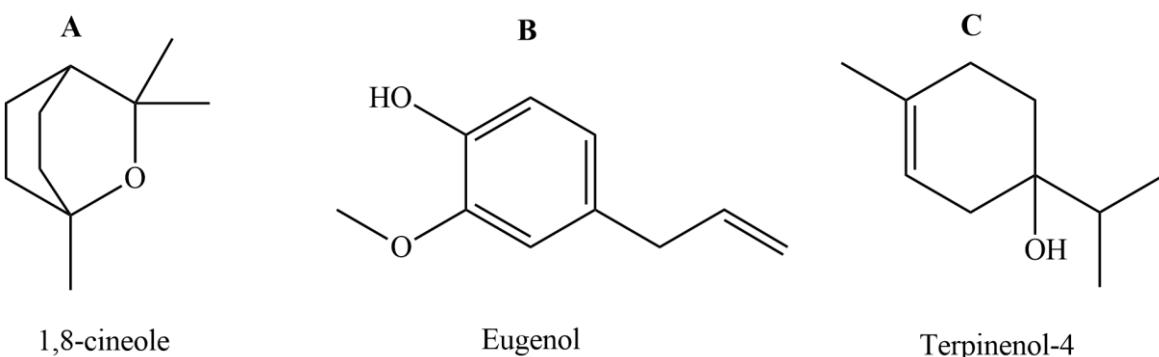
Table 1. Chemical components present in the essential oil of *Ocimum gastrissimum* L.

Constituientes	Fórmula Molecular	RT (min)	(%)
α-pinene	C ₁₀ H ₁₆	12,51	1,18
β-pinene	C ₁₀ H ₁₆	14,93	3,11
1,8 Cineole	C ₁₀ H ₁₈ O	18,07	30,04
α-terpineol	C ₁₀ H ₁₈ O	27,72	0,86
Eugenol	C ₁₀ H ₁₂ O ₂	37,48	27,58
Trans-caryophyllene	C ₁₅ H ₂₄ O	41,13	5,29
β-selinene	C ₁₅ H ₂₄	45,47	11,22
α-selinene	C ₁₅ H ₂₄	45,84	2,75
Naphthalene	C ₁₀ H ₈	46,63	1,49
(+)Spathulenol	C ₁₅ H ₂₄ O	47,87	0,45
Terpineol-4	C ₁₀ H ₁₈ O	47,96	14,45
Caryophyllene oxide	C ₁₅ H ₂₄ O	47,98	0,41
Total			98,83

Relative proportions of the essential oil constituents were expressed as percentages. RT: Retention Time

Source: Rodrigues (2020).

Figure 1. Main Compounds of *Ocimum gratissimum* essential oil.

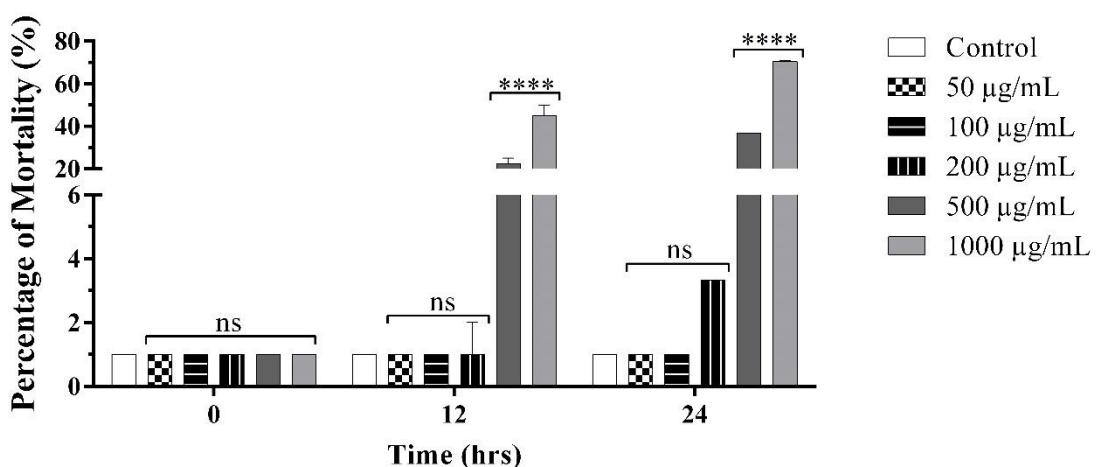


Source: Rodrigues (2020).

3.2 Insecticidal activity

Figure 2 represents the mortality rate of *N. cinerea* nymphs, where it is possible to observe that there was a significant increase in the percentage of mortality after a period of 12 h of exposure in the highest concentrations (500 and 1000 µg/mL), the percentage mortality increased after 24 h of exposure to treatment, with mortality >70%. The average lethal concentration (LC₅₀) was 516 µg/mL. Exposure to essential oil in concentrations > 500 µg/mL becomes toxic to the tested cockroaches.

Figure 2. *Nauphoeta cinerea* mortality graph.



Source: Rodrigues (2020).

4. Discussion

It has been reported that environmental conditions such as precipitation, temperature, light incidence and others, have a quantitative influence on the composition of essential oils of various aromatic plants (Lima et al., 2018).

From a chemical point of view, the essential oil of *O. gratissimum* has been extensively studied, differences in chemical composition are noted in almost all studies, while in our work the major compounds were 1,8-cineole, eugenol and terpineol-4 , in the study by Morh et al. (2016), the compounds that showed the highest percentage were Linalool (32.95%), 1.8-Cineole (21.91%) and Camphor (11.97%), eugenol and terpinol-4 were also identified, but in smaller quantities. Benelli et al. (2019b) identified thymol (50.0%) and p-cymene (16.8%) as major constituents. Other works also presented different compositions when compared to ours (Joshi, 2013; Mith et al., 2016; Lisboa et al., 2020).

Some studies have shown the potential insecticide and/or repellent of *O. gratissimum*, Benelli et al. (2019b) identified that both the essential oil and the extracts were active against the *Spodoptera littoralis*, the *Musca domestica* and the *Culex quinquefasciatus* (filariasis vector), this insecticidal activity was attributed to tymol, a major compound, which can easily cross the insect's cuticle, entering the body and promoting cell lysis. The essential oil of *O. gratissimum* also showed activity against *Spodoptera frugiperda*, one of the main pests for the corn harvest in Brazil, especially in the North, the compounds responsible for this activity being trans-anethole and limonene, acting in synergism (Cruz et al., 2017).

Although its insecticidal activity has been highly evaluated, there are no records of bioactivity of the essential oil of *O. gratissimum* against *Nauphoeta cinerea*, this being the first report. The activity of *O. gratissimum* against *N. cinerea* is due to the components of its essential oil, 1,8-cineole has several works with proven insecticidal activity (Liška et al., 2011; Shrestha et al., 2015; Bett et al ., 2016), as well as eugenol present in essential oils of various species (Amirmohammadi et al., 2012; Jankowska et al., 2018) and terpineol-4 (Mawussi et al., 2013; Lucia et al., 2013).

Essential oils are seen as alternatives to the use of conventional insecticides, Lamiaceae being one of the main botanical families with regard to aromatic compounds and with several species that have insecticidal activity (Park et al., 2016; Yeom et al., 2018). In addition, essential oils have advantages such as, they can be obtained on a large scale and from several plants, they have several modes of action which make the development less

resistant, they are relatively less toxic to mammals and do not leave residues due to its high volatility (Park et al., 2016).

5. Conclusion

The essential oil of the leaves of *Ocimum gratissimum* (clove) has a relevant insecticidal activity against *Nauphoeta cinera* (cockroach). In this way, its oil can be used for the production of bioinsecticides, in order to replace the synthetic insecticides that cause great ecotoxicological effect on the environment. Further studies are needed in order to assess how the oil behaves with other species of economic interest.

Acknowledgements

Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) e Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

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Percentage of contribution of each author in the manuscript

Felicidade Caroline Rodrigues – 15%

José Weverton Almeida-Bezerra – 10%

Kleber Ribeiro Fidelis – 5%

Adrielle Rodrigues Costa – 5%

Mikael Amaro de Souza – 5%

Maria Haiele Nogueira da Costa – 5%

Luciano Temoteo dos Santos – 5%

Ma. Aparecida Barbosa Ferreira Gonçalo – 5%

Saulo Almeida de Menezes – 5%

Samara Mendes de Sousa – 5%

Edna Karol Rodrigues de Araujo – 5%

Ana Vaeline Patrício Braga – 5%

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Maria Ivaneide Rocha – 5%