

**Chemical analysis and insecticidal activity of *Ocimum gratissimum* essential oil and its major constituent against *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae)**

**Análise química e atividade inseticida do óleo essencial de *Ocimum gratissimum* e de seu constituinte majoritário contra *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae)**

**Análisis químico y actividad insecticida del aceite esencial de *Ocimum gratissimum* y su principal constituyente contra *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae)**

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**Abstract**

The fall armyworm, *Spodoptera frugiperda*, causes damage at several stages of the maize crop cycle. Due to its resistance to synthetic insecticides and the high costs of pest control, there is an ever-increasing amount of research on alternative or complementary products that have a minor environmental and financial impact on agriculture. Therefore, the aim of this study was to evaluate the chemical composition and insecticidal potential of *Ocimum gratissimum* (african basil) leaves essential oil and the effect of its major component, thymol, on *S. frugiperda* control. Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified p-cymene,  $\gamma$ -terpinene, and thymol compounds as the main constituents of the oil, which presented a yield of 4.75%. Among the 30 identified compounds, thymol (33.2%) was the major constituent, representing 97.8% of the total oil. The efficacy of both the oil and thymol standard (Sigma-Aldrich) was evaluated against *S. frugiperda* using topical acute toxicity and contact surface tests at different concentrations. The oil was more active than thymol standard, with topical acute toxicity of LD<sub>50</sub> at 0.020  $\mu$ l/insect and LC<sub>50</sub> at 0.171  $\mu$ l/cm<sup>2</sup> for contact surface toxicity. The oil proved to be superior to the thymol standard, offering an effective and promising alternative for the control of *S. frugiperda*, which is most likely due to the contribution of other oil components that acted

synergistically. Consequently, this result provides an opportunity for further study and the development of an effective fall armyworm control system.

**Keywords:** Fall armyworm; Botanical insecticide; Chemical constituents; Lamiaceae.

### Resumo

A lagarta-do-cartucho, *Spodoptera frugiperda*, causa danos em vários estágios do ciclo da cultura do milho. Devido à sua resistência aos inseticidas sintéticos e aos altos custos do controle de pragas, há um número cada vez maior de pesquisas sobre produtos alternativos com menor impacto ambiental e financeiro na agricultura. Portanto, o objetivo deste estudo foi avaliar a composição química e o potencial inseticida do óleo essencial de folhas de *Ocimum gratissimum* (manjerição africano) e o efeito de seu principal componente, o timol, no controle de *S. frugiperda*. As análises por Cromatografia Gasosa (CG) e Cromatografia Gasosa-Espectrometria de Massa (CG-EM) identificaram os compostos p-cimeno,  $\gamma$ -terpineno e timol como principais constituintes do óleo, que apresentou rendimento de 4,75%. Entre os 30 compostos identificados, o timol (33,2%) foi o principal constituinte, representando 97,8% do óleo total. A eficácia do óleo e do timol padrão (Sigma-Aldrich) foi avaliada contra *S. frugiperda* usando toxicidade aguda tópica e testes de superfície de contato em diferentes concentrações. O óleo foi mais ativo do que o timol padrão, com toxicidade aguda tópica de LD<sub>50</sub> em 0,020  $\mu$ L/inseto e LC<sub>50</sub> em 0,171  $\mu$ L/cm<sup>2</sup> para toxicidade de superfície de contato. O óleo mostrou-se superior ao padrão timol, oferecendo uma alternativa eficaz e promissora para o controle de *S. frugiperda*, o que provavelmente se deve à contribuição de outros componentes do óleo que atuaram sinergicamente. Consequentemente, este resultado fornece uma oportunidade para estudos adicionais e o desenvolvimento de um sistema de controle eficaz da lagarta do cartucho.

**Palavras-chave:** Lagarta do cartucho; Inseticida botânico; Constituintes químicos; Lamiaceae.

### Resumen

El cartucho de oruga, *Spodoptera frugiperda*, causa daños en varias etapas del ciclo del cultivo del maíz. Debido a su resistencia a los insecticidas sintéticos y los altos costos del control de plagas, existe un número creciente de investigaciones sobre productos alternativos con menor impacto ambiental y financiero en la agricultura. Por tanto, el objetivo de este estudio fue evaluar la composición química y el potencial insecticida del aceite esencial de hojas de *Ocimum gratissimum* (albahaca africana) y el efecto de su componente principal, el

timol, en el control de *S. frugiperda*. Los análisis de cromatografía de gases (CG) y cromatografía de gases-espectrometría de masas (CG-EM) identificaron los compuestos p-cimeno,  $\gamma$ -terpineno y timol como los principales constituyentes del aceite, con un rendimiento del 4,75%. Entre los 30 compuestos identificados, el timol (33,2%) fue el constituyente principal, representando el 97,8% del aceite total. La efectividad del aceite y el timol estándar (Sigma-Aldrich) se evaluó contra *S. frugiperda* usando pruebas de toxicidad tópica aguda y superficies de contacto a diferentes concentraciones. El aceite fue más activo que el timol estándar, con toxicidad tópica aguda de LD<sub>50</sub> a 0.020  $\mu$ L/insecto y LC<sub>50</sub> a 0.171  $\mu$ L/cm<sup>2</sup> para la toxicidad de la superficie de contacto. El aceite demostró ser superior al estándar de timol, ofreciendo una alternativa efectiva y prometedora para el control de *S. frugiperda*, lo que probablemente se deba al aporte de otros componentes del aceite que actuaron sinérgicamente. En consecuencia, este resultado brinda la oportunidad de realizar más estudios y desarrollar un sistema de control de oruga de cartucho eficaz.

**Palabras clave:** Cartucho de oruga; Insecticida botánico; Componentes químicos; Lamiaceae.

## 1. Introduction

Fall armyworm, *Spodoptera frugiperda* (Smith 1797) (Lepidoptera: Noctuidae), is considered a key crop pest of maize because it attacks all stages of plant development and different species of food plants (I. Cruz, 2008; Negrini et al., 2019). Corn, together with soy, is the crop that most demands the use of agrochemicals in the handling of the caterpillar (SINDAG, 2012). This insect comes from the tropics, and the climatic conditions in Brazilian corn-producing regions are favorable to its establishment and development (Campos & Boiça Junior, 2012).

The corn crop (*Zea mays* L.) (Poaceae) has always been notable in Brazil for its importance in human and animal nutrition. However, several factors hamper its production chain and, among the technical aspects, the elimination of insect pests is especially important, as in specific cases when there is no efficient and safe control, it interferes in grain yield (Figueiredo et al., 2006; Michelotto et al., 2013).

Synthetic insecticides are mainly used to control the fall armyworm, and they are effective and quick-acting. However, the emergence of populations resistant to these chemical groups and their toxicity to natural enemies led to the search for biodegradable insecticides that are specific to target insects (Felix et al., 2019; Lima et al., 2013; Niculau et al., 2013).

In this context, essential oils, which have a complex mixture of volatile substances and act synergistically in the control of various insect pests, have been shown as an alternative strategy within Integrated Pest Management. This occurs not only because they do not leave residues in food or the environment, but also because they are generally rapidly degraded, which mitigates environmental contamination (Azevedo et al., 2013; Costa et al., 2017; Isman, 2006; Lima et al., 2013). Other characteristics include their action on mortality, deformations of the different stages of development, repellency and deterrence (Castro et al., 2006; G. S. Cruz et al., 2014; R. K. Lima et al., 2009). Their toxic substances can penetrate the insects through the airways, contact or ingestion. This insecticidal action is due to the diversity of their monoterpenes and sesquiterpenes, which can be identified and quantified through chromatographic analysis coupled with mass spectrometry (Ootani et al., 2013).

Several essential oils with insecticidal activity have been studied. They are suitable for dealing with *S. frugiperda*, which is easy to handle and reproduce in the laboratory.

Research has shown that these oils influence the behavioral and biological parameters of the pest, which attacks various crops. For example, a previous study carried out with *O. gratissimum* demonstrated its action as an insecticide and repellent by fumigation for the control of *Sythophilus zeamais*, a corn crop insect pest, reducing the number of adults and indicating a promising and possibly curative effect of this essential oil (Araújo et al., 2019; Cruz et al., 2016).

A number of biological applications of essential oils of the genus *Ocimum* have been reported, including insecticidal action of their chemical components, which may act in isolation or in association (synergistic or antagonistic form), thus causing a greater or minor reaction. Therefore, the objective of this study was to evaluate the chemical composition and insecticidal activity of *Ocimum gratissimum* (African basil) leaves essential oil and its major component in the control of *Spodoptera frugiperda* through acute topical and surface contact toxicity tests.

## 2. Material and Methods

This study is quantitative, experimental research carried out through sampling, thus producing accurate and safe numerical results (Creswell & Plano-Clark, 2011; Pereira et al., 2018).

## 2.1 Plant material collection and identification

Mature and intact leaves of *O. gratissimum* matrices were collected on May 15 at 8:00 a.m. (rainy season) in the Medicinal Garden of the Seabra Attic Herbarium, located at the Federal University of Maranhão (UFMA), municipality of São Luís - Maranhão, Brazil (2° 33' 13" S 44° 18' 19" W). The specimen was deposited in the UFMA Herbarium SEA under number 5150. The thymol standard (2-isopropyl-5-methyl-phenol) was obtained from Sigma-Aldrich at 99% purity.

## 2.2 Extractions of essential oil and its major compounds

*Ocimum gratissimum* leaves were dried at room temperature ( $26 \pm 3$  °C) for three days, with loss of 75% of water content. After grinding, 25 g were subjected to hydrodistillation using a Clevenger type apparatus (3 h). After drying with anhydrous sodium sulfate, the oil was packed into amber glass ampules at  $5 \pm 2$  °C in a freezer (Teles et al., 2012). The extraction process was carried out at the UFMA Natural Products Laboratory.

The obtained yield was calculated by the dry weight of the plant and the moisture content of the sample, through an infrared and sample moisture analyzer (IV2500, GEHAKA), to measure the loss of water.

## 2.3 Oil composition analysis

The qualitative analysis of the oil was performed by gas chromatography coupled with mass spectrometry (GC-MS), FOCUS equipment (Thermo Electron) DSQ II, helium-entrained gas, equipped with a DB-5 MS capillary column (30 m X 0.25 mm X 0.25 µm). The injection mode was splitless (Split flow 20:1). For each sample, 0.1 µL of oil in hexane (400 ng in the column) was injected. The column temperature was 60 to 240 °C, ranging from 3 °C/min. Ion source: 70 eV (electronic impact), ion source temperature and transfer line 200 °C.

The quantitative analysis was performed under the same conditions by gas chromatography coupled to a flame ionization detector (GC/FID), FOCUS equipment (Thermo Electron), except for the drag gas, which was nitrogen. Retention rates of all volatile constituents were calculated using a homologous series of n-alkanes (C8-C20, Sigma-Aldrich, USA), according to van Den Dool and Kratz (1963). The oils components were identified by

comparing their retention indices and mass spectra (fragmentation standard and molecular mass), with the spectra existing in the libraries and in the GC/MS system literature (Adams & Sparkman, 2007; NIST, 2011).

## **2.4 Insects**

The bioassays were carried out at the Laboratory of Entomology Research, Agrarian Unit, Anhanguera-Uniderp University, Campo Grande, Mato Grosso do Sul. The temperature was  $27 \pm 2$  °C; relative humidity  $70 \pm 5\%$ , and photoperiod of 12 hours. After pupation, the insects were sexed, and eight couples were placed in cages to perform the egg-laying. Eggs were kept in Petri dishes in a climate-controlled room under the same conditions until larval hatching (Panizzi & Parra, 2009).

## **2.5 Acute topical toxicity test**

Tested concentrations were 0.0144; 0.0173; 0.0208; 0.0250; and 0.0312  $\mu\text{L}/\text{insect}$  for the oil and thymol standard, having been determined in the light of data obtained from a previous study (Favero & Conte, 2008). The third instar larvae were used in this experiment and were fed with an artificial diet based on beans and wheat germ. Larvae were treated individually by adding the concentrations to the prothoracic region using a micropipette (1  $\mu\text{L}$ ). After treatment, each larva was kept in Petri dishes (60 mm). Control group contained 10 larvae were treated with the solvent (acetone) and each concentration was replicated three times. Mortality was recorded at 1.0, 6.0, 12.0, 24.0, and 36.0 Hatching After Time (HAT), and dead larvae were removed and counted.

## **2.6 Contact surface toxicity**

Tested concentrations (0.127; 0.152; 0.183; 0.220; and 0.265  $\mu\text{L}\cdot\text{cm}^{-2}$ ) were established as described in Favero & Conte (2008). Also, the larvae were fed with an artificial diet based on beans and wheat germ. Petri dishes (60 mm) were treated with 0.3 mL of the solutions and evaporated after 20 min. After evaporation, the armyworm larvae were placed on the plates, and the plates were closed. The treatment was identical, 10 armyworm larvae for concentration and each concentration with 3 repetitions. Dead individuals were counted after 24 hours.

## 2.7 Data analysis

The tabulated data from both acute and contact toxicity experiments were converted to percentages and then analyzed by ANOVA, after were submitted to PROBIT analysis using the SAS PROC PROBIT program (Sas, 2002) to determine the dose-mortality curve and the sublethal and lethal doses 50% and 90% (LD<sub>50</sub> and DL<sub>90</sub>) (Finney, 1971). The results are presented as the mean ± SE.

The armyworm larvae were found dead after 24 hours, and the dead larvae were removed, and mortality compared to control was calculated using the following equation by Abbott (1925). The LT<sub>50</sub> results of essential oil and thymol are presented as the slope ± SE, LC<sub>50</sub>, hour (at 95% confidence interval), v2 (degree of freedom) and P-value.

$$\text{Corrected mortality (\%)} = \frac{X - Y}{X} \times 100$$

## 3. Results

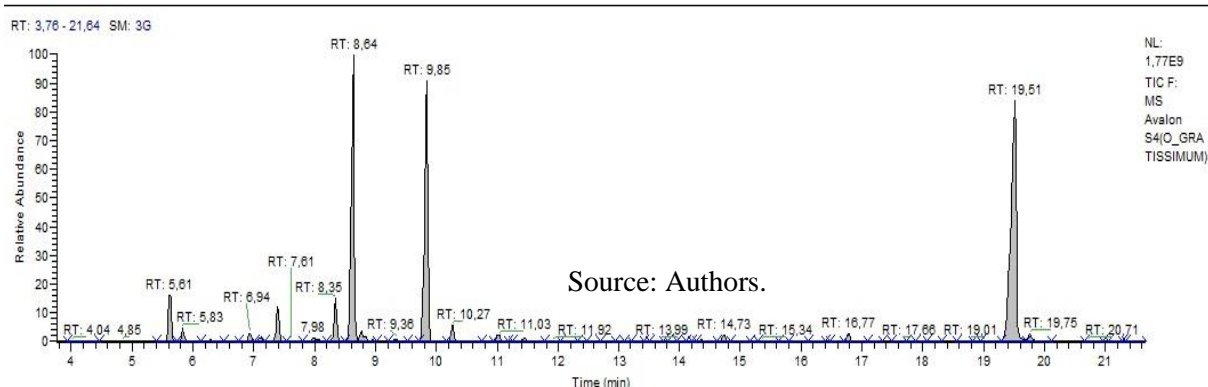
### 3.1 Characterization of essential oil

*Ocimum gratissimum* essential oil produced a liquid yield of 4.75% and, according to the chromatographic analysis, 30 compounds, corresponding to 97.8%, of their total composition (Figure 1 and Table 1) were identified. The compounds consisted of 92.1% of monoterpenes, with predominance of 55.1% of monoterpene hydrocarbons and 37% of oxygenated monoterpenes, as well as 5.7% of sesquiterpenes, distributed in 5.1% of sesquiterpene hydrocarbons and 0.6% of oxygenated sesquiterpenes.

Following the order of elution, the chromatogram shows that the peaks with retention time 8.64, 9.85 and 19.51 were highlighted, the last being the one with the highest intensity. By mass spectrometry and comparison with the spectra from data libraries, it was possible to identify the peaks as being the *p*-cymene,  $\gamma$ -terpinene, and thymol compounds, respectively.



**Figure 1.** Chromatogram of essential oil from *Ocimum gratissimum* leaves.



Source: Authors.

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**Table 1.** Chemical composition of the essential oil of *Ocimum gratissimum* leaves cultivated in the Medicinal Garden of Herbarium Seabra Attic, Federal University of Maranhão, São Luís, Maranhão, May 15, 2017.

Constituents	%	CRI <sup>a</sup>	LRI <sup>b</sup>
$\alpha$ -Thujene	3.5	920	924
$\alpha$ -Pinene	0.8	928	932
Canphene	0.1	944	946
Abinene	0.6	966	969
Myrcene	2.7	981	988
$\alpha$ -Phellandrene	0.4	1002	1002
$\alpha$ -Terpinene	2.8	1013	1014
<i>p</i> -Cimene	22.5	1019	1020
( <i>E</i> )- $\beta$ -Ocimene	0.1	1042	1044
$\gamma$ -Terpinene	21.0	1052	1054
cis-Sabinene hydrate	1.1	1065	1065
Terpinolene	0.5	1083	1086
trans-Sabinene hydrate	0.4	1095	1098
<i>p</i> -1,3,8- Mentatriene	0.1	1107	1108
Borneol	0.2	1165	1165
Terpinen-4-ol	0.5	1174	1174
<i>p</i> -Cimen-8-ol	0.1	1180	1179
$\alpha$ -Terpineol	0.1	1189	1186
Methyl Thymol Ether	0.6	1222	1232
Thymol	33.2	1285	1289
Carvacrol	0.8	1291	1298
$\beta$ -Elemene	0.2	1380	1389
<i>E</i> -Cariofilene	1.6	1409	1417
$\alpha$ -Humulene	0.2	1445	1452
Germacrene D	0.3	1470	1484
$\beta$ -Selinene	2.0	1478	1489
Viridiflorene	0.6	1485	1496
$\beta$ -Curcumene	0.2	1507	1514
Spathulenol	0.3	1568	1577

Caryophyllene Oxide	0.3	1573	1582
<b>Monoterpene hydrocarbons (%)</b>	<b>55.1</b>		
<b>Oxygenated monoterpenes (%)</b>	<b>37.0</b>		
<b>Sesquiterpenes /%</b>	<b>5.1</b>		
<b>Oxygenated sesquiterpenes (%)</b>	<b>0.6</b>		
<b>Total (%)</b>	<b>97.8</b>		

<sup>a</sup> Calculated Retention Index. <sup>b</sup> Library Retention Index. % Percentage of constituents in the sample. Source: Authors.

### 3.2 Acute Topical Toxicity

Leaf essential oil from *O. gratissimum* showed a higher topical acute toxicity with 0.020 (0.017-0.022)  $\mu\text{L}/\text{insect}$  LD<sub>50</sub> (Table 2) when compared to the commercial standard thymol, with LD<sub>50</sub> 0.329 (0.302-0.356)  $\mu\text{L}/\text{insect}$ . By comparison of LD<sub>50</sub> and LC<sub>50</sub> it's possible observe the oil was more active than the major component thymol. The essential oil was 16.5 times more efficient than thymol alone, with a LD<sub>50</sub> of 0.020 and 0.329  $\mu\text{L}/\text{insect}$  (Table 2).

**Table 2.** Acute topical toxicity of the essential oil from the leaves of *Ocimum gratissimum* and its major compound (Thymol) on *Spodoptera frugiperda* (Noctuidae). Temperature: 27 ± 2 °C. Relative humidity: 70 ± 5% and 12-hour photoperiod.

Samples	LD <sub>50</sub> (CI95%) $\mu\text{L}/\text{insect}^{-1}$	LD <sub>90</sub> (CI95%) $\mu\text{L}/\text{insect}^{-1}$	S (± SE)	$\chi^2$	DF	P
Oil	0.020 (0.017-0.022)	0.027 (0.023-0.036)	9.830 ±2.369	0.438	3	0.932
Thymol	0.329 (0.302-0.356)	0.405 (0.372-0.485)	14.210 ±3.213	1.453	3	0.693

N: number of individuals; LD: Lethal Dose;  $\chi^2$ : Chi-square; P: probability; CI (95%): Confidence interval; S: slope; SE: standard error; DF: degree of freedom. Source: Authors.

### 3.3 Contact Surface Toxicity

LC<sub>50</sub> contact surface toxicity of 0.171-0.193  $\mu\text{L}/\text{cm}^2$  (Table 3) was higher when compared to the Thymol, with LC<sub>50</sub> of 0.255 (0.195-0.317)  $\mu\text{L}/\text{cm}^2$ . In the contact surface toxicity test, it was 1.5 times more effective, with LC<sub>50</sub> of 0.171 and 0.255  $\mu\text{L}/\text{cm}^2$  (Table 3), respectively.

**Table 3.** Contact surface toxicity of the *Ocimum gratissimum* leaf essential oil and its major compound (Thymol) on *Spodoptera frugiperda* (Noctuidae). Temperature:  $27 \pm 2$  °C.

Relative humidity:  $70 \pm 5\%$  and photoperiod of 12 hours.

Samples	LC <sub>50</sub> (CI95%) μL/cm <sup>2</sup>	LC <sub>90</sub> (CI95%) μL/cm <sup>2</sup>	S (±SE)	χ <sup>2</sup>	DF	P
Oil	0.171 (0.150-0.193)	0.243 (0.210-0.345)	8.494 ±2.132	1.388	3	0.708
Thymol	0.255 (0.195-0.317)	0.480 (0.374-0.827)	4.667 ±1.103	3.254	3	0.354

N: number of individuals; LC: Lethal Concentration; χ<sup>2</sup>: Chi-square; P: probability; CI (95%): Confidence Interval; S: slope; SE: standard error; DF: degree of freedom. Source: Authors.

#### 4. Discussion

Previous studies have demonstrated quantitative variations in the chemical composition of *O. gratissimum* essential oil (Benelli et al., 2019; Kumar et al., 2019). This may be associated with the genetic difference between *Ocimum* populations, in addition to the stage of development and the plant age, the part of the plant from which the oil was extracted, collection season, analysis conditions and also factors such as precipitation, temperature, duration of daily brightness, soil characteristics, and geographic location (Benelli et al., 2016; Blank, 2013).

For both tests, the oil was more active than when its major component thymol was used alone, a fact observed by the comparisons of LD<sub>50</sub> and LC<sub>50</sub> and their respective toxicity ratios. These results indicate that the mixture of components of the oil acts synergistically with thymol, contributing to greater activity.

The effect of essential oils on insects has been extensively studied, given that they contain combinations of natural and complex volatiles, characterized by strong odor and formed by a diversity of constituents (Bakkali et al., 2008). In the present study, it can be inferred that the synergism of the monoterpenes (92.1%) and sesquiterpenes (5.7%) of *O. gratissimum* essential oil was more effective than commercial thymol for both tests.

Since *p*-cymene and  $\gamma$ -terpinene compounds have greater permeability in the insect cuticle because they are hydrocarbons of low polarity, they have a more hydrophobic character. Therefore, it can be deduced that the insect cuticle permeability potential can increase the toxicity of these two hydrocarbons. On the other hand, thymol, a compound found in greater quantity, belongs to a phenolic hydroxyl group (hydrophilic polar group). It also interacts with the insect cuticle, since the intracellular medium is predominantly aqueous, thus presenting good solubility to the hydrophilic substances.

Experiments performed with *O. gratissimum* under different soil and climatic conditions present changes in the composition of their major components. Franco et al. (2014) verified that the essential oil of *O. gratissimum* leaves collected in the Medicinal Plants Garden at the Federal University of Goiás in February 2001 at 09:00 a.m. (rainy season) presented eugenol (57.8 %),  $\alpha$ -bisabolene (17.2%) and  $\gamma$ -terpinene (13.1%) as the main constituents. In Fortaleza, state of Ceará, Oliveira et al. (2016) verified that the fresh leaves of *O. gratissimum* collected in the Medicinal Plants Garden at the Federal University of Ceará in November 2013 at 12:00 a.m. (dry season), presented eugenol (51.8%) and 1,8-Cineol (23.8%) as major components. In the state of Paraíba, in the town of Bananeiras, the essential oil of *O. gratissimum* leaves obtained from the Federal University of Paraíba was analyzed by Cruz et al. (2016) and presented trans-anethole (34.9%), limonene (15.6%) and eugenol (9.1%) as major components. These data can be justified by the influence of the seasons on the plant. Plants under climate stress can present variability in the metabolite production, which can interfere in the increase or decrease of the biological response (Sampaio et al., 2016). For example, the rainy season affects the essential oil production by Lamiaceae plants, since the structures that accumulate these oils are located on the surface of the leaf (epidermal cells) (Blank et al., 2011).

Among the most recurrent families cited in the literature due to their insecticidal action are Lamiaceae, Verbenaceae, Meliaceae and Piperaceae. Several species belonging to this group have already shown efficiency against several pest insects, such as *Ocimum basilicum* L. (control of *Lymantria dispar* L. 1758) (Lepdoptera: Erebidae) (Popović et al., 2013), *Lippia gracilis* Schauer (control of *Diaphania hyalinata* L., 1767) (Lepdoptera: Crambidae) (Melo et al., 2018), *Azadirachta indica* A. Juss (control of *Brevicoryne brassicae* L., 1758) (Hemiptera:Aphididae) (Carvalho et al., 2008) and *Piper hispidinervum* C. DC. (control of *S. frugiperda* Smith 1797) (Lepdoptera: Owlet moths) (Alves et al., 2014). Thus, it is justifiable to search for other plant species and their essential oils in the control of *S. frugiperda* to minimize the use of chemical control (Fonseca et al. 2012).

*Ocimum gratissimum* essential oil indicated toxicity to *S. frugiperda*, exhibiting a LD<sub>50</sub> of 0.020  $\mu$ L/insect. Another study has also demonstrated similar results, although the concentrations were different, as in the work by Cruz et al. (2016) which investigated the essential oil of the species *Eucalyptus staigeriana* F., *O. gratissimum*, *Foeniculum vulgare* Mill. in the control of *S. frugiperda*. However, only the essential oil of *O. gratissimum*, whose main component was trans-anethole (34.9%), used through topical contact and with LD<sub>50</sub> 1.52 mg/gram insect, demonstrated toxicity for *S. frugiperda* as well as changes in the biological

and reproductive parameters. It is of note that the essential oils obtained from our work showed greater toxicity than those presented by Cruz et al. (2016).

*Ocimum gratissimum* was toxic to the *S. frugiperda* caterpillar, with thymol, which is a monoterpenoid, aromatic phenol, biosynthesized in plants from  $\gamma$ -terpinene and *p*-cymene as a major component (Franco et al., 2014). Thymol is present in 60% of essential oils from native species in northeastern Brazil (Fontenelle et al., 2007). This compound is found in the essential oil of species of the genera *Thymus* and *Origanum*, Lamiaceae, such as *T. vulgaris* and *O. vulgare* (Hudaib et al., 2002). It can also be detected in species of the genus *Lippia*, Verbenaceae, such as *L. gracilis* and *L. sidoides* (Franco et al., 2014).

Essential oils from species of the genus *Ocimum* have shown satisfactory results in relation to their insecticidal effect against insect pests. Ogendo et al. (2008) observed that *O. gratissimum* essential oil rich in (Z) - $\beta$ -ocimene and eugenol caused 99% and 100% mortality, respectively, against *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae), *Oryzaephilus surinamensis* L. (Coleoptera: Silvanidae) and *Callosobruchus chinensis* L. (Coleoptera: Chrysomelidae), using the concentration of 1  $\mu$ L/L of this oil. Ilboudo et al. (2010) verified the insecticidal activity and persistence of essential oils extracted from *Ocimum americanum* L. and other Lamiaceae species, such as *Hyptis spicigera* Lam., *Hyptis suaveolens* L. and *Lippia multiflora* Moldenke on *Callosobruchus maculatus* F. (Coleoptera: Bruchidae) in cowpea (*Vigna unguiculata* L. Walp) seeds. These authors demonstrated that the essential oil of *O. americanum* showed insecticidal activity, killing all the insects in a concentration lower than 0.5  $\mu$ L/L. Silva Lima et al. (2018) studied the effect of the essential oil of *O. gratissimum* with chemo-thymol in the control of the bovine tick, *Rhynchophalus microplus* (Acari: Ixodidae), verifying its acaricidal activity with the lethal concentration LC<sub>50</sub> 0.84 mg/mL.

The synergism that occurs among the compounds present in the essential oils of plants can result in a greater bioactivity than that observed for the isolated compounds. These effects are common in terpenes, hydrophobic compounds that present synergistic actions in relation to other compounds, solubilizing them and facilitating their passage through the membranes (Rattan, 2010). It also allows the use of smaller dosages of the mixture, thus reducing costs with pest management and environmental risks (Ribeiro et al., 2002).

These effects suggest several mechanisms of action of the oil components, among them the inhibition of acetylcholinesterase activity (AChE), gamma-aminobutyric acid receptors (GABA), and the octopaminergic system interfering with neuromodulation and insect hormones (Ingkaninan et al., 2003; Isman, 2006; Sigel & Steinmann, 2012; Zhou et al., 2008). The rapid action of some oils against insects is indicative of neurotoxicity, thus

suggesting that the action of *O. gratissimum* essential oil may be associated with a neurotoxic response.

Studies have shown the relationship between the chemical structure and the biological activity of the compounds present in the essential oils, since the higher the hydrophobicity is, the greater the penetration in the insect's integument (Rattan, 2010). The essential oils insecticidal properties depends on these factors to achieve pest insects susceptibility to synthetic compounds (Mairesse et al., 2007). Resistance to pyrethroids and organophosphates is common among *S. frugiperda* populations (Poletti & Alves, 2013). Due to its activity against *S. frugiperda*, the essential oil extracted in this study presents the possibility of controlling this fall armyworm resistance.

## 5. Final Considerations

According to results obtained, the tests indicated that *O. gratissimum* essential oil showed a topical and contact toxic effect on *S. frugiperda* caterpillars and was more active than the commercial thymol standard. Therefore, the use of essential oils is a promising option to reduce the use of synthetic insecticides against this pest.

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