

# ***Ocimum basilicum* essential oil combined with deltamethrin to improve the management of *Spodoptera frugiperda***

## **Óleo essencial de *Ocimum basilicum* associado à deltametrina no manejo de *Spodoptera frugiperda***

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### **ABSTRACT**

For an important and expensive crop such as corn, the resistance of *Spodoptera frugiperda* J.E. Smith to various pesticides has led to research throughout the world for a potential insecticide from a natural source. For the management of pest resistance, natural compounds associated with synthetic insecticides can be a promising tool because they can reduce the application of the synthetics molecules while maintaining their effectiveness and promoting the control of the pests. Linalool is a potential insecticide that is easily obtained because it is found in high concentrations in the essential oil of *Ocimum basilicum* L. Therefore, the present study aimed to evaluate the toxicity of this essential oil and its combination with deltamethrin to control *S. frugiperda*. Through dose response assays, the acute toxicities (LD<sub>50</sub>) of the essential oil and deltamethrin were estimated. Additionally, the combination of these materials was also assessed, attaining a reduction of 80% of the LD<sub>50</sub> of deltamethrin while obtaining the same result as when the pyrethroid was administered alone. From these results, it is expected that the combination of natural compounds and synthetic insecticides will be a promising practice, helping to manage resistance while reducing the environmental impact of toxic compounds.

**Index terms:** Natural insecticides; linalool; fall armyworm; pyrethroid; synergism.

### **RESUMO**

Para uma cultura importante e expressiva como o milho, a resistência de *Spodoptera frugiperda* J.E. Smith a vários inseticidas atraiu a atenção no mundo para pesquisar o potencial inseticida de compostos naturais. Para o manejo da resistência, os compostos naturais associados a inseticidas sintéticos podem ser uma ferramenta promissória por reduzirem a aplicação das moléculas sintéticas para que não percam sua eficácia, além de promover o controle das pragas. O linalol é um terpenoide considerado como inseticida potencial, pode ser facilmente obtido naturalmente uma vez que é encontrado em alta concentração no óleo essencial de *Ocimum basilicum*. Portanto, o presente estudo teve como objetivo avaliar a toxicidade deste óleo essencial e sua combinação com deltametrina no controle de *Spodoptera frugiperda*. Através do ensaio de dose-resposta, foi estimada a toxicidade aguda (DL50) do óleo essencial e da deltametrina. Adicionalmente, foi também avaliada a combinação entre ambos, alcançando uma redução de 80% da DL<sub>50</sub> de deltametrina para se obter o mesmo resultado quando o piretroide foi administrado sozinho. A partir dos nossos resultados, espera-se que uma combinação de uso de compostos naturais e inseticidas sintéticos possa ser uma prática promissora, auxiliando no manejo da resistência de pragas e principalmente reduzindo os impactos ambientais de compostos tóxicos.

**Termos para indexação:** Inseticidas naturais; linalol; lagarta-do-cartucho; piretroides; sinergismo.

## **INTRODUCTION**

Among the different species of pests, *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae) is the main pest occurring on corn plantations in Brazil, causing losses of up to 100% in production if not managed correctly (Michelotto et al., 2011). The effective chemical control of this pest has faced a number of difficulties, among them, the rapid development of resistance to different groups of

insecticides, the constant need for rotation of insecticide mechanisms of action, as well as the application of specific insecticides for the different stages of the pest (Castle et al., 2010).

This situation has become more aggravated as the resistance of *S. frugiperda* to pyrethroids, organophosphates, carbamates, neonicotinoids and growth regulators has been reported (Yu, 2008). For example,

acetylcholinesterase (AChE), a key enzyme of the central nervous system of insects and a target of organophosphates and carbamates, has become insensitive to these molecules (Wang et al., 2004). Consequently, the emerging development of products from the chemical industry that have different mechanisms of action and a higher broad spectrum has been instigated (Langat et al., 2011), so that other molecules do not lose their effectiveness.

Insecticides from natural sources can serve as tools to solve these problems, while reducing the impact of conventional pesticides on beneficial insects and human health during the production of food. Among the compounds that are synthesized by the secondary metabolism of plants, terpenoids are a rich source of bioactive molecules that exhibit toxicity to various pests (Ali et al., 2010; Lima et al., 2013; Lima et al., 2009; Silva et al., 2010). Some studies have shown that these compounds act as inhibitors of AChE, impair oviposition (Alexenizer; Dorn, 2007; Cavalcante; Moreira; Vasconcelos, 2006) and cellular respiration (Yu, 2008) and act as synergists of other bioactive agents (Fazolin et al., 2016).

Linalool is a terpenoid that jointly operates with other compounds in the cholinergic system of insects (Lopez; Pascual-Villalobos, 2010) and possibly acts indirectly as a modulator of acetylcholinesterase (Ryan; Byrne, 1988; Shaaya; Rafaeli, 2007). As one of the major natural sources of linalool, more than 80%, on average, of the essential oil of *Ocimum basilicum* (Lineu; Lamiaceae) varieties (Blank et al., 2007) contains this terpenoid. Regarding its spectrum of toxicity, linalool showed potential in controlling *S. frugiperda* by causing a repellent effect, acute toxicity, non-preference and a knockdown effect (Labinas et al., 2002; Praveena; Sanjayan, 2011).

Recently, researchers have emphasized the importance of the synergism between products, for example, the association between natural and synthetic compounds (Radhika; Sahayaraj, 2014), mainly aiming to reduce the amount of a pesticide necessary to result in the same toxic effect (Casida, 1970; Brindley; Selim, 1984; Raffa; Priester, 1985). Furthermore, insecticides used in combination can enhance their toxicity compared to their individual use (Khann et al., 2013). A good example of this combination is a mixture of pyrethroid, safrole and rotenone (terpenoids), known to inhibit the cytochrome P450, which is the main route of metabolism for pesticides (Fazolin et al., 2016).

Terpenoids and pyrethroids have different chemical structures and mechanisms of action, but they cause similar toxicity in pests, such as rapid nervous breakdown, and they are less toxic to mammals and other beneficial

organisms (Kariuki et al., 2014). However, currently, there are few studies that have proven the compatibility and efficacy of this combination. The present work aimed to evaluate *O. basilicum* essential oil toxicity alone and in combination with deltamethrin to improve the management of *S. frugiperda*.

## MATERIAL AND METHODS

### Fall armyworm collection and rearing

Initially, the larvae of *S. frugiperda* were collected in maize plants during the beginning of the cultivation of corn in 2016 at the "Gloria Experimental Farm" (18°57'S and 48°12'W). The plants were at the third stage of vegetative development, and the larvae were collected inside the cartridge of the corn. The larvae were reared *in vitro* at the Laboratory of Entomology of the Federal University of Uberlândia (UFU). Insects were reared on an artificial diet according to the methodology adapted from Burton and Perkins (1972), Greene, Leppla and Dickerson (1976) and Kasten, Precetti and Parra (1978). Due to the defensive behaviour (cannibalism) of *S. frugiperda*, each larva was individualized in disposable 50-mL plastic cups containing an artificial diet. With this individualization, the cups were placed in a room at 25±2 °C with 60±10% relative humidity and 12 hours of light. Every two days, the artificial diet was changed until the larvae had completed development and moved to the pupal stage. During the pupal stage, they were placed in Petri dishes lined with filter paper and kept in cages. After emergence, the adults were maintained in cylindrical cages (150 x 200 mm), fed honey + beer yeast (1:1), and placed inside a cage of cotton that was moistened daily. Eggs that were laid on the filter paper were removed daily, maintained in a Petri dish until hatching, and then immediately provided the same artificial diet as previously described. The laboratory conditions were similar for all stages of insect rearing.

### Production of *Ocimum basilicum* L. and essential oil

The variety of *O. basilicum*, chemotype linalool, was obtained from accession PI 197442, originating from the Germplasm Bank of the North Central Regional PI Station, Iowa State University, United States of America. Seeds were obtained from the Program of Breeding of Aromatic Plants of the Federal University of Sergipe and cultivated during the spring of 2015 at the Gloria Experimental Farm/UFU. In full bloom, fresh leaves were harvested for essential oil extraction using a hydrodistillation Clevenger-type apparatus (Blank et al., 2007).

## Chemical characterization of essential oil

Chemical analyses of the essential oil were performed using a gas chromatograph coupled with a mass spectrometer (Shimadzu GC-2010 + QP-5000) and equipped with a DB-5 fused silica capillary column (30 m x 0.25 mm x 0.25  $\mu\text{m}$ ). The operation mode was as follows: helium as the carrier gas at 1.7 mL  $\text{min}^{-1}$ , temperature of 240 °C for the injector, temperature of 230 °C for the detector, and a temperature programme from 60 to 240 °C with a 3 °C increase every minute. Half of the flow was split, and the flow rate was 1 mL  $\text{min}^{-1}$ . The identification of compounds was performed by comparing their mass spectra with system databases and literature (McLafferty; Stauffer, 1989) and by determining the Kovats retention indices and comparing them with the literature (Adams, 2007).

The quantification of the compounds was performed using a gas chromatograph coupled with a flame ionization detector (Shimadzu GC-2010/FID) and a DB5 capillary column. The carrier gas was helium with a flow rate of 1.0 mL  $\text{min}^{-1}$  and a split ratio of 1/20, the injector temperature was set to 240 °C, the detector temperature was set to 230 °C, and the temperature was ramped from 60 °C to 165 °C at 4 °C  $\text{min}^{-1}$  and from 165 °C to 240 °C at 10 °C  $\text{min}^{-1}$ .

## Toxicological evaluations

### Acute toxicity test

Dose-response assays were performed with the essential oil from the *O. basilicum* chemotype linalool, the technical product deltamethrin (99.6% Pestanal, Sigma-Aldrich) and the commercial product of deltamethrin (Decis 25 EC, Bayer Crop science). Third instar larvae of *S. frugiperda* were maintained in six-well cell plates, reared on an artificial diet and maintained in a climatic chamber (25 $\pm$ 1 °C; 65 $\pm$ 10% RH and 12 hours of light). Each concentration was tested with four replicates and 24 larvae. The determination of the larval stage was performed according to Parra and Carvalho (1984). Acute toxicity tests were performed according to the adapted methodology of OECD-OCDE (1998).

For deltamethrin and the essential oil, the solutions for determining the LD<sub>50</sub> were prepared in acetone. In the case of the essential oil, a range of dilutions were prepared at concentrations (v/v) ranging from 100% (pure) to 1%. For deltamethrin, dilutions were prepared ranging from 1000 to 0.0001 ng a.i.  $\mu\text{L}^{-1}$ . The solutions of the commercial product were prepared in an aqueous solution from 5  $\mu\text{g}$  a.i.  $\mu\text{L}^{-1}$  to 0.125  $\mu\text{g}$  a.i.  $\mu\text{L}^{-1}$ . Intoxication of the larvae was performed by means of a topical application

of 1  $\mu\text{L}$  of the respective solution using a microsyringe. The evaluations were performed every 24 and 48 hours after the treatments, and the number of dead larvae were counted. A negative control treatment was also applied that included the application of only acetone.

### Assessment of the combinations of deltamethrin and essential oil

This assessment aimed to study the combination of deltamethrin (a.i.) and essential oil (e.o.), taking as a reference the LD<sub>50</sub> values previously obtained. The pairwise comparison was performed using ten random mixtures: (1) LD<sub>50</sub> a.i.+ LD<sub>50</sub> e.o.; (2) 50% LD<sub>50</sub> a.i.+ 50% e.o.; (3) 75% LD<sub>50</sub> a.i.+ 25% e.o.; (4) LD<sub>50</sub> a.i.+1% e.o.; (5) LD<sub>50</sub> a.i.+ 5% e.o.; (6) LD<sub>50</sub> a.i.+ 10% e.o.; (7) 20% LD<sub>50</sub> a.i.+ LD<sub>50</sub> e.o.; (8) 20% LD<sub>50</sub> a.i.+ 1% e.o.; (9) 20% LD<sub>50</sub> a.i.+ 5% e.o.; and (10) 20% LD<sub>50</sub> a.i.+ 10% e.o. All procedures during the bioassays were carried out as previously described. The mortality rate was determined daily until the death of all individuals.

### Assessment of the combinations of the commercial product and essential oil

The interaction of the commercial product and essential oil against *S. frugiperda* was also investigated. As previously described, pairwise comparisons were made using solutions prepared over a range of possible combinations, as follows: (1) 12.5  $\mu\text{g}$  a.i.  $\mu\text{L}^{-1}$  + 50% e.o.; (2) 12.5  $\mu\text{g}$  a.i.  $\mu\text{L}^{-1}$  + 25% e.o.; (3) 5  $\mu\text{g}$  a.i.  $\mu\text{L}^{-1}$  + 50% e.o.; (4) 5  $\mu\text{g}$  a.i.  $\mu\text{L}^{-1}$  + 25% e.o.; (5) 2.5  $\mu\text{g}$  a.i.  $\mu\text{L}^{-1}$  + 50% e.o.; (6) 2.5  $\mu\text{g}$  a.i.  $\mu\text{L}^{-1}$  + 25% e.o.; (7) 0.25  $\mu\text{g}$  a.i.  $\mu\text{L}^{-1}$  + 50% e.o.; (8) 0.25  $\mu\text{g}$  a.i.  $\mu\text{L}^{-1}$  + 20% e.o.; (9) 0.25  $\mu\text{g}$  a.i.  $\mu\text{L}^{-1}$  + 10% e.o.; (10) 0.25  $\mu\text{g}$  a.i.  $\mu\text{L}^{-1}$  + 5% e.o.; (11) 0.25  $\mu\text{g}$  a.i.  $\mu\text{L}^{-1}$  + 1% e.o.; (12) 0.125  $\mu\text{g}$  a.i.  $\mu\text{L}^{-1}$  + 10% e.o. All procedures were performed as previously described. The mortality rate was determined daily until the death of all individuals.

### Data analysis

All data were analysed using R software (2016). For the acute toxicity assay, the mortality recorded was analysed using the package “drc” (Ritz and Streibig, 2005). From the fitted model, the LD<sub>50</sub> values were determined for the essential oil and the technical and commercial products, in addition to the confidence interval, chi-square and degrees of freedom. The data obtained from the combinations of the products were submitted to the Shapiro-Wilk test for a normal distribution, Levene’s test of the homogeneity of variance, and Tukey’s F-test of additivity at a 0.01 level of significance using SPSS 20

(SPSS, 2011). When relevant, an F-test was completed through an analysis of variance and comparison of means via the Scott-Knott test at a 0.05 level of significance.

## RESULTS AND DISCUSSION

### Chemical composition of *O. basilicum* essential oil

Nineteen compounds in the *O. basilicum* essential oil were identified (Table 1 and Figure 1). The three major compounds observed were linalool, 1,8-cineole and geraniol, amounting to 95% of the essential oil content. Some other minor components were observed such as  $\alpha$ -(E)-bergamotene and epi- $\alpha$ -cadinol (1.46 and 1.06%). The specific density of the oil was 0.85 g cm<sup>-3</sup>, and the content and yield were 2.34% and 13.57 g per plant, respectively.

In this study, the chemical composition of the essential oil was similar to results described by Blank et al. (2007), who reported a content of up to 80% linalool. However, Duman et al. (2010) found that *O. basilicum* essential oil originating from Turkey contained only 54.4% linalool. In the same study *Coriandrum sativum*

var. microcarpum was found to be a rich source of linalool, containing 90.6% of the compound. On the other hand, Govindarajan et al. (2013) found that the major chemical components identified in *O. basilicum* essential oil originating from India were linalool (52.42%), methyl eugenol (18.74%) and 1,8-cineole (5.61%).

### Acute toxicity to *S. frugiperda*

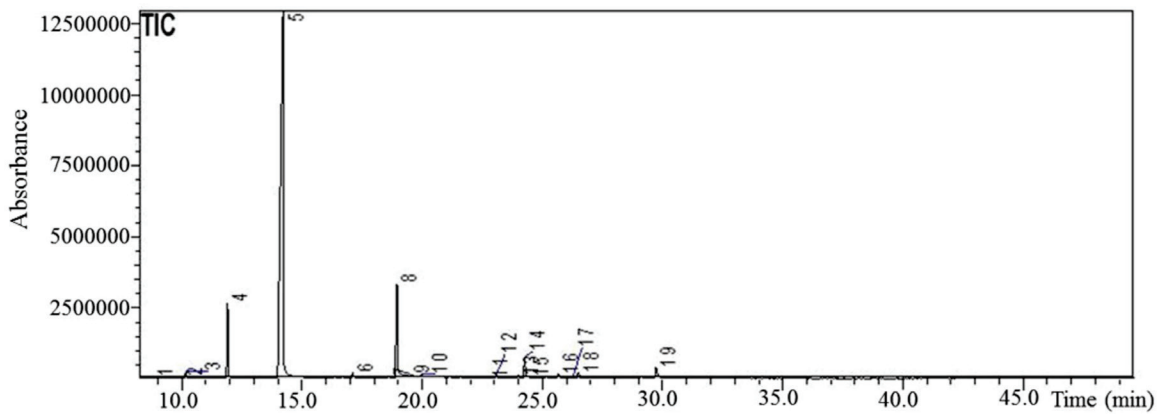
After topical application of the essential oil, deltamethrin and commercial product, a rise in mortality of *S. frugiperda* was observed (Figures 2, 3 and 4). The LD<sub>50</sub> values, including the fitted parameters, are shown in Table 2. No mortality was recorded for the control treatment that used only acetone.

Specifically, for the high concentration of the essential oil, extreme agitation and hyperactivity of the caterpillars was observed, followed by the loss of motor coordination, a reduction in feeding and death. Similar behaviour was observed for the application of deltamethrin and the commercial product on larvae, with paralysis, starvation and death observed within 48 h.

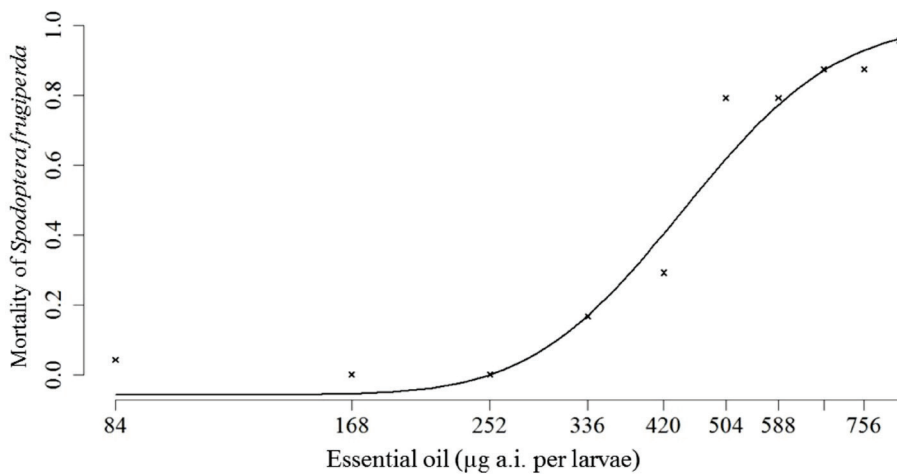
**Table 1:** Chemical composition of *Ocimum basilicum* L. determined by gas chromatography.

| Peak | RT <sup>1</sup> | ICR <sup>2</sup> | IRL <sup>3</sup> | Compound                  | %Area | %GC-FID |
|------|-----------------|------------------|------------------|---------------------------|-------|---------|
| 1    | 8.809           | 923              | 932              | $\alpha$ -pinene          | 0.01  | 0.15    |
| 2    | 10.029          | 963              | 969              | sabinene                  | 0.11  | 0.12    |
| 3    | 10.156          | 967              | 974              | $\beta$ -pinene           | 0.53  | 0.49    |
| 4    | 11.895          | 1021             | 1026             | 1,8-cineole               | 6.02  | 5.00    |
| 5    | 14.214          | 1091             | 1095             | linalool                  | 77.34 | 79.29   |
| 6    | 17.095          | 1181             | 1186             | $\alpha$ -terpineol       | 0.48  | 0.41    |
| 7    | 18.587          | 1229             | 1235             | neral                     | 0.12  | 0.11    |
| 8    | 18.950          | 1241             | 1249             | geraniol                  | 9.86  | 9.05    |
| 9    | 19.447          | 1258             | 1264             | geranial                  | 0.16  | 0.87    |
| 10   | 19.982          | 1275             | 1287             | bornyl acetate            | 0.24  | 0.53    |
| 11   | 22.700          | 1369             | 1359             | neryl acetate             | 0.04  | 0.10    |
| 12   | 23.091          | 1382             | 1389             | $\beta$ -elemene          | 0.30  | 0.30    |
| 13   | 24.000          | 1415             | 1417             | caryophyllene             | 0.20  | 0.18    |
| 14   | 24.260          | 1424             | 1432             | $\alpha$ -(E)-bergamotene | 1.91  | 1.46    |
| 15   | 24.413          | 1430             | 1437             | $\alpha$ -guaiene         | 0.12  | 0.10    |
| 16   | 25.660          | 1476             | 1484             | germacrene D              | 0.43  | 0.36    |
| 17   | 26.272          | 1498             | 1509             | $\alpha$ -bulnesene       | 0.12  | 0.10    |
| 18   | 26.495          | 1507             | 1513             | $\gamma$ -cadinene        | 0.58  | 0.33    |
| 19   | 29.729          | 1636             | 1638             | epi- $\alpha$ -cadinol    | 1.43  | 1.06    |

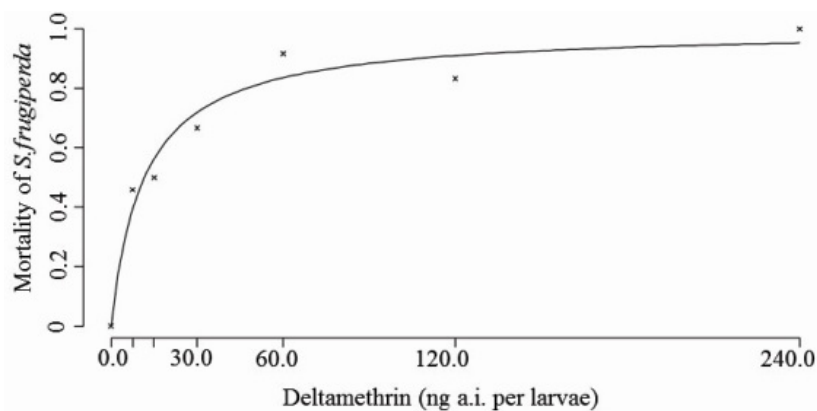
<sup>1</sup>Retention Time; <sup>2</sup>Index of Calculated Retention; <sup>3</sup>Index of Literature Retention.



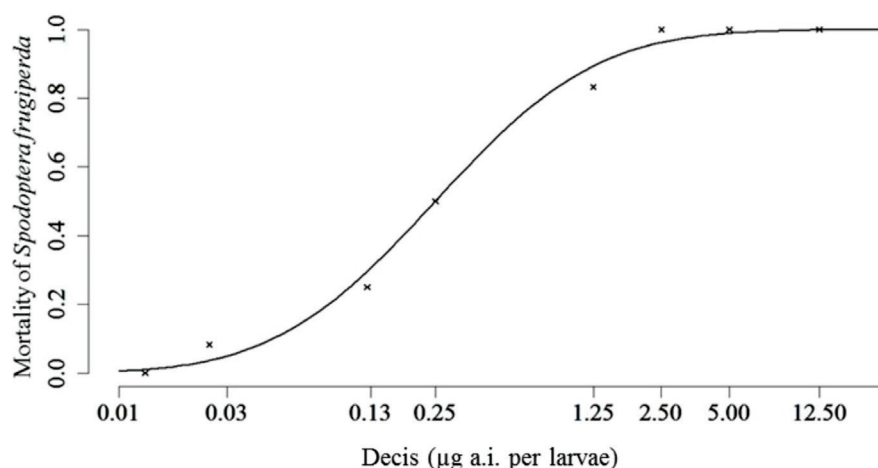
**Figure 1:** Major compounds in *O. basilicum* essential oil obtained by gas chromatography and mass spectrometry. The compounds 1,8-cineole, linalool and geraniol are represented by peaks 4, 5 and 8, respectively.



**Figure 2:** Mortality of third instar larvae of *S. frugiperda* (48 h) after intoxication with different doses of *O. basilicum* essential oil.



**Figure 3:** Mortality of the third instar larvae of *S. frugiperda* (48 h) after intoxication with different doses of deltamethrin.



**Figure 4:** Mortality of the third instar larvae of *S. frugiperda* (48 h) after intoxication with different doses of Decis 25 EC.

**Table 2:** Summary of the parameters obtained during the acute toxicity assays of *O. basilicum* essential oil, deltamethrin and the commercial product against *S. frugiperda*.

| Time  |        | 95% C.I. <sup>b</sup> | D.F. <sup>c</sup> | $\chi^2$ <sup>d</sup> |
|---|--------|-----------------------|-------------------|-----------------------|
| Essential oil (LD <sub>50</sub> <sup>a</sup> µg a.i.) |        |                       |                   |                       |
| 24 h  | 490.00 | 453.40-526.04         | 41                | 51.57                 |
| 48 h  | 480.00 | 447.51-512.48         | 25                | 34.07                 |
| Deltamethrin (LD <sub>50</sub> ng a.i.)               |        |                       |                   |                       |
| 24 h  | 19.25  | 8.96-29.54            | 22                | 30.448                |
| 48 h  | 17.26  | 7.81-27.11            | 21                | 23.138                |
| Commercial product (LD <sub>50</sub> µg a.i.)         |        |                       |                   |                       |
| 24 h  | 0.25   | 0.173-0.359           | 35                | 4.08                  |
| 48 h  | 0.25   | 0.173-0.359           | 34                | 4.08                  |

<sup>a</sup>Lethal dose; <sup>b</sup>Confidence interval; <sup>c</sup>Degrees of freedom; <sup>d</sup>Chi-square.

Our findings are supported by Pavela et al. (2014), who showed that bioactive terpenoids are neurotoxic. Different levels of toxicity and mortality of *S. frugiperda* of 30, 90, 84 and 64% occurred using 3 µg a.i. mg<sup>-1</sup> insect with geraniol, linalool, carvone and citral, respectively (Niculau et al., 2013).

Specifically, linalool toxicity to the genus *Spodoptera* was established previously as 85.5 µg LD<sub>50</sub> per larva of *S. litura*, but this compound is less toxic to other pests such as *Helicoverpa armigera* (Hübner, 1805) (Lepidoptera: Noctuidae) and *Chilo partellus* (Swinhoe, 1885) (Lepidoptera: Crambidae) on the order of 431.5 µg and 462.4 µg per larva, respectively (Koul et al., 2013). Additionally, the same authors found an LD<sub>50</sub> value of 126.6 µg for 1,8-cineole against *S. litura*.

In our study, the rich composition of the essential oil appeared to exert a positive impact against *S. frugiperda*. For example, similar associations between linalool and 1,8-cineole were highly toxic to species of the same genus (Koul et al., 2013). In this case, alcohols and plant phenols were more active in combination than as isolated compounds.

Carballo and Rubio (2012) tested the essential oils of four aromatic species on the feeding habits of populations of *S. frugiperda* and *Heliothis virescens* (Lepidoptera: Noctuidae). The *Coriandrum sativum* essential oil, with 76% linalool, inhibited the feeding behaviour of caterpillars and the development of new generations, showing that the presence of more bioactive compounds in the essential oil possibly potentiated this effect.

El-Aziz, Omer and Sabra (2007) noted that the association of the constituents of the *Ocimum americanum* L. (Lamiaceae) essential oil caused high mortality in caterpillars, reducing the development of pupae and consequently lowering the fertility and viability of the *Agrotis ipsilon* (Hufnagel, 1767) (Lepidoptera: Noctuidae) population. Popovic et al. (2013) also observed a synergistic effect of essential oil components of another variety of *O. basilicum* on the feeding habits of *Lymantria dispar* (Linnaeus, 1758) (Lepidoptera: Lymantriidae). In this case, a repellent effect was observed with a reduction in defoliation with only 0.5% essential oil containing more than 90% linalool, as well as 1,8-cineol, limonene and anetole.

The toxicity of terpenoids was also reported for other pests. Wang, Liab and Leia (2009) found that only linalool was more toxic to *Sitophilus zeamais* (Motschulsky, 1855) (Coleoptera: Curculionidae), with an LD<sub>50</sub> of 13.90 µg per adult. In the cholinergic synapses of insects, acetylcholinesterase (AChE) has a key role in regulating the transmission of nerve impulses and catalysing the hydrolysis of acetylcholine. Therefore, Praveena and Sanjayan (2011), while studying the interaction between AChE and linalool in the focal pests *S. litura* and *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae), showed that oxygenated monoterpene formed a stable intermolecular complex with the enzyme of the cholinergic system, effectively inhibiting its interaction with acetylcholine. These results show promise and are similar to the present study, contributing to knowledge of the insecticidal properties of terpenoids.

However, one disadvantage was found by Lopez and Pascual-Villalobos (2010); high concentrations of linalool are required for potent AChE inhibition because at low concentrations, the compound behaves as a weak inhibitor compared to other terpenoids, for example, 1,8-cineole. Similar results were found using *Piper hispidinervum* C.DC. essential oil against *S. frugiperda*, requiring higher doses of this oil to cause mortality (Lima et al., 2009).

This was also observed in the present study, where we found that the LD<sub>50</sub> of the essential oil with a high concentration of linalool was very high compared to that of deltamethrin. Thus, toxicity testing with this oil may still produce better results compared to other pests that are possibly more sensitive to the concentrations of the essential oil and that were not the target of this study.

### Essential oil combined with deltamethrin

The combination of the essential oil and deltamethrin showed high toxicity in an *in vitro* test with

larvae (Table 3). The effects observed in larvae were similar to the application of essential oil: extreme agitation, hyperactivity and death. From this outcome, it is expected that a reduction of approximately 80% of deltamethrin is possible when in combination with the essential oil (LD<sub>50</sub>). This means that the dosage of deltamethrin can be reduced from 19.25 ng µL<sup>-1</sup> to 3.85 ng µL<sup>-1</sup>, when adding 480 µg µL<sup>-1</sup> essential oil.

The combination of the essential oil and the commercial product also showed a promising result (Table 4). For example, with just 1% commercial product and more than 20% essential oil in comparison to the maximum rate of both products, the mortality obtained was greater than 95%.

Relevant combinations of natural and synthetic products have already been mentioned in many studies. Abassy, Abdelgaleil and Rabie (2009) determined an LD<sub>50</sub> of 2.48 µg e.o. µL<sup>-1</sup> of *Majorana hortensis* L. (Lamiaceae) to larvae of *S. litura*. From these authors, a positive combination of these compounds with profenofos allowed a reduction of more than twice the dose of the pesticide to control of the caterpillar.

Similar to the present study, Fazolin et al. (2016) noted significant synergistic effects when combining *Piper aduncum* essential oil with pyrethroids for use against *S. frugiperda*. The LD<sub>50</sub> values of alpha-cypermethrin, fenpropathrin, gamma-cyhalothrin and beta-cypermethrin were reduced to ½ and/or ¼ by the presence of the essential oil, causing significant toxicity to larvae. Safrole was the major component of this essential oil (82%), and it is believed that it acts as a cytochrome P450 inhibitor in insects, excluding the main route of pesticide metabolism.

Kariuki et al. (2014) reported that the association between products has contributed significantly to an increase in the effectiveness of insecticides, as well as serving as a tool to improve the management of resistance. There is a consensus among the authors that the use of combinations of products might have more rapid effects on the target organisms compared to the synthetic formulations employed in isolation (Srivastava et al., 2011).

War et al. (2014) assessed the efficacy of combinations of neem oil with endosulfan on the feeding and enzymatic activity of *H. armigera*. The antifeedant activity caused by 0.01% endosulfan combined with 1% neem oil (in a ratio of 1:1) was 85.34%, significantly greater than effects of the individual products. Study of the detoxification enzyme indicated a significant reduction in glutathione-S-transferase activity.

**Table 3:** Mortality of *S. frugiperda* due to the combined use of *O. basilicum* essential oil and deltamethrin (as a technical grade compound).

| Deltamethrin         |                            | Essential oil        |                                      | Mortality (%) |         |
|----------------------|----------------------------|----------------------|--------------------------------------|---------------|---------|
| Dose (%)             | ng a.i. $\mu\text{L}^{-1}$ | Dose (%)             | $\mu\text{g e.o. } \mu\text{L}^{-1}$ | 24 h          | 48 h    |
| 20% LD <sub>50</sub> | 3.85                       | 1%                   | 8.40                                 | 50.00b        | 66.60b  |
| 20% LD <sub>50</sub> | 3.85                       | 5%                   | 42.00                                | 8.30d         | 8.30d   |
| 20% LD <sub>50</sub> | 3.85                       | 10%                  | 84.00                                | 50.00b        | 66.60b  |
| 20% LD <sub>50</sub> | 3.85                       | LD <sub>50</sub>     | 480.00                               | 100.00a       | 100.00a |
| 50% LD <sub>50</sub> | 9.62                       | 50% LD <sub>50</sub> | 245.00                               | 100.00a       | 100.00a |
| 75% LD <sub>50</sub> | 14.43                      | 25% LD <sub>50</sub> | 122.50                               | 79.10a        | 83.30a  |
| LD <sub>50</sub>     | 19.25                      | 1%                   | 8.40                                 | 75.00b        | 79.10a  |
| LD <sub>50</sub>     | 19.25                      | 5%                   | 42.00                                | 37.50c        | 41.60c  |
| LD <sub>50</sub>     | 19.25                      | 10%                  | 84.00                                | 37.50c        | 41.60c  |
| LD <sub>50</sub>     | 19.25                      | LD <sub>50</sub>     | 480.00                               | 100.00a       | 100.00a |
| Control (Acetone)    |                            |                      |                                      | 0             | 0       |
| F                    |                            |                      |                                      | 21.170        | 18.922  |
| VC (%)               |                            |                      |                                      | 23.80         | 25.32   |

The means followed by lowercase letters differ based on the Scott-Knott test at 5% probability.

**Table 4:** Mortality of *S. frugiperda* due to the combined use of *O. basilicum* essential oil and the commercial product (Decis 25 EC).

| Commercial product |                                      | Essential oil |                                 | Mortality (%) |         |
|--------------------|--------------------------------------|---------------|---------------------------------|---------------|---------|
| Dose (%)           | $\mu\text{g a.i. } \mu\text{L}^{-1}$ | Dose (%)      | $\mu\text{g } \mu\text{L}^{-1}$ | 24 h          | 48 h    |
| 50.00              | 12.50                                | 50.00         | 420.00                          | 100.00a       | 100.00a |
| 50.00              | 12.50                                | 25.00         | 210.00                          | 100.00a       | 100.00a |
| 20.00              | 5.00                                 | 50.00         | 420.00                          | 100.00a       | 100.00a |
| 20.00              | 5.00                                 | 25.00         | 210.00                          | 100.00a       | 100.00a |
| 10.00              | 2.50                                 | 50.00         | 420.00                          | 100.00a       | 100.00a |
| 10.00              | 2.50                                 | 25.00         | 210.00                          | 100.00a       | 100.00a |
| 1.00               | 0.25                                 | 50.00         | 420.00                          | 100.00a       | 100.00a |
| 1.00               | 0.25                                 | 20.00         | 168.00                          | 95.80a        | 95.00a  |
| 1.00               | 0.25                                 | 10.00         | 84.00                           | 84.00b        | 83.50b  |
| 1.00               | 0.25                                 | 5.00          | 42.00                           | 54.10b        | 54.00b  |
| 1.00               | 0.25                                 | 1.00          | 8.40                            | 37.50b        | 35.00b  |
| 0.50               | 0.125                                | 10.00         | 84.00                           | 34.50b        | 34.00b  |
| Control (Acetone)  |                                      |               |                                 | 0             | 0       |
| F                  |                                      |               |                                 | 16.653        | 16.653  |
| VC (%)             |                                      |               |                                 | 15.55         | 15.54   |

The means followed by lowercase letters differ based on the Scott-Knott test at 5% probability.



All these results are promising and indicate that more studies should be conducted in order to support the use of combinations of natural and synthetic compounds to combat pests. Similar to our results, a combination of terpenoids and pyrethroids is possible, and we support this new tool for pest management.

Consequently, field tests have also become crucial to provide further clarification of the time and residual effect of these combinations on the environment as part of integrated pest management.

## CONCLUSIONS

The results of this study showed that the tested essential oil has significant toxicity towards *S. frugiperda*, making it a promising sustainable tool for pest management. The efficacy of the combination of this compound with low doses of deltamethrin also reflects its possible use in the resistance management of *S. frugiperda* to this insecticide.

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