

SYNERGISTIC EFFECTS OF BINARY MIXTURES OF LINALOOL WITH PYRETHROIDS AGAINST FALL ARMYWORM

EFEITOS SINÉRGICOS DE MISTURAS BINÁRIAS DE LINALOL COM PIRETROIDES CONTRA LAGARTA-DO-CARTUCHO

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ABSTRACT: The present work aimed to determine the toxicity of linalool and evaluate the lethal and toxic effects of linalool associated with pyrethroids in binary mixtures to fall armyworm (*Spodoptera frugiperda*). The insects used in the experiment were obtained from stock breeding initiated from larvae collected from conventional corn plants, grown in an experimental area, in the city of Uberlândia, Minas Gerais. Also, it was obtained essential oil from a variety of *Ocimum basilicum*, with a high content of linalool (80%), found naturally, as a measure of comparison of different linalool (97.5%) assays. Dose-response bioassays with 3rd instar larvae were performed to determine lethal dose for 50% mortality (LD₅₀) of linalool. Toxicity tests were also performed with *O. basilicum* essential oil and with pyrethroid insecticides: deltamethrin and its commercial product (Decis 25 EC, Bayer®). After this, combinations between different doses of these products were made and applied on 3rd instar larvae of *Spodoptera frugiperda* (Smith). Linalool presented high toxicity to *S. frugiperda* (LD₅₀ = 0.177 µL a.i. µL⁻¹). It was observed neurotoxic effects after the linalool application since the insects presented an aspect of confusion, followed by extreme agitation and finally death. All binary mixtures caused mortality higher than the products applied alone (deltamethrin and linalool) used at 100% LD₅₀, except to 75% LD₅₀ deltamethrin added to 25% LD₅₀ linalool, whose mortality did not differ the products alone, in 24 hours. It was obtained over 90% larval mortality when linalool was combined with 25% LD₅₀ of deltamethrin, in 24 and 48 hours after application, and over 80% of mortality when linalool was combined with 25% LD₅₀ of Decis, only in 48 hours after application. We conclude that linalool is a potential insecticidal and can be associated with pyrethroids to control of *S. frugiperda*. Further studies are required in order to evaluate the synergistic combinations against field populations of *S. frugiperda*.

KEYWORDS: Deltamethrin. Insecticidal activity. *Spodoptera frugiperda*. Synergism. Terpenoids.

INTRODUCTION

Spodoptera frugiperda (Smith, 1797) (Lepidoptera: Noctuidae) is the main insect pest of the corn crop in Brazil, causing losses up to 100% in production if control measures are not realized (MURÚA et al., 2015). Because it is a polyphagous insect and has high reproductive capacity in several crops, the main management tactics adopted lately to reduce population are chemical control by using synthetic insecticides or biological control for conventional hybrids as well as the use of genetically modified plants (MALONE; GATEHOUSE; BARRATT, 2008).

In this context, the indiscriminate and intensive use of synthetic insecticides caused a selection of insect pest populations resistant to

different groups of insecticides, further compromising the use of chemical control (OMOTO et al., 2015; SANTOS-AMAYA et al., 2017). As an example, acetylcholinesterase (AChE), a key enzyme of the insect cholinergic system and target of organophosphorus and carbamates insecticides, has become insensitive to these molecules (WANG et al., 200). Consequently, this has increased product development by the chemical industry to provide new molecules that effectively integrate insect pest management and make agricultural production more sustainable (LANGAT et al., 2011).

Researchers have tested natural or plant-derived products efficacy (BAGAVAN et al., 2009; SILVA et al., 2016), as well as associated molecules in the same formulation, searching safer alternatives

for resistance management to insecticides (ISMAN; MIRESMAILLI; MACHIAL, 2011; RADHIKA; SAHAYARAJ, 2014).

Recent studies have focused on a combination of molecules with different mechanisms of action, wide spectrum of action, selectivity for non-target organisms, and the possibility of higher efficacy combined with synthetic products so that molecules do not lose quickly their effectiveness (LANGAT et al., 2011). It is known that associations between different products can promote greater toxicity to insects, since a compound can potentiate the action of another active ingredient by weakening the insect detoxification system, acting on the inhibition of P450 enzymes and esterase's (FAZOLIN et al., 2016).

In addition to the plant-insect interaction mechanism, linalool is a terpenoid that has proven to be effective in the control of sucking insects and defoliant *in vitro* (FARAONE, HILLIER, CUTLER, 2015), mainly affecting the transmission of nerve impulses by inhibiting the acetylcholinesterase enzyme (LOPEZ; VILLALOBOS, 2010). This compound, produced by secondary plant metabolism, can be found in high concentrations in the essential oils of some varieties of *Aniba rosaeodora* Ducke (Lauraceae) and *Ocimum basilicum* L. (Lamiaceae).

Effects observed during toxicological tests and attributed to these oils are due to the action of several essential oil compounds, which cannot be assigned exclusively to linalool. There are doubts about the toxicological effects of linalool exclusive application on *S. frugiperda*, as the possible synergistic effects over this pest mortality when linalool and synthetic insecticides were combined. Terpenoids and pyrethroids insecticides have different chemical structures and mechanisms of action, but they cause similar toxicity to pests, such as rapid nervous breakdown (KARIUKI et al., 2014). However, it is necessary more studies about the compatibility and efficacy of this combination to enable its use at crop protection against insect-pest. In addition, the synergistic effect may lead to the reduction of pesticide application which would be less harmful to the environment.

If this is the case, a safer, easier, and more sustainable tool to assist in the management of pest resistance to insecticides may be feasible. Therefore, the present work aimed to determine the toxicity of linalool and evaluate the lethal and toxic effects of linalool associated with pyrethroids in binary mixtures to fall armyworm (*Spodoptera frugiperda*).

MATERIAL AND METHODS

Obtaining and rearing caterpillars

In order to obtain the caterpillars, an area of 6,000 m² was prepared at the Experimental Farm "Capim Branco", belonging to the Federal University of Uberlândia, (Uberlândia, Minas Gerais State, Brazil) for corn cultivation. The farm is situated at an altitude of 842 meters, with geographical coordinates 18° 53' 23.46" S of latitude and 48° 20' 27.46" W of longitude, flat topography, and Aw climate type (Tropical humid with winter dry).

Conventional corn hybrid BM709 (Helix Sementes Ltda, Patos de Minas, Minas Gerais State, Brazil) was sown on March 14, during the second crop of 2018, in crop lines spaced 0.5 m and population density of 65.000 plants ha⁻¹. Fertilization, located in the culture line, with NPK formulation 4-14-8 with a dose of 350 kg ha⁻¹, according to the culture requirement. When reaching the phenological stage of V7, the crop received cover fertilization of NPK formulation 20-05-20, at a dose of 350 kg ha⁻¹.

Spodoptera frugiperda caterpillar gathering occurred after the beginning of the crop infestation, in the first phenological stages. For this, highly infested plants were randomly selected and had the cartridge (inner leaves not yet fully opened) removed, containing the insects. Then, the caterpillars were identified to compose a reared population at the laboratory, being maintained with a natural diet, containing leaves of the same cultivated hybrid.

Due to the defensive behavior (cannibalism) of *S. frugiperda*, each larva was individualized in disposable plastic pots of 300 mL containing 50 cm² of fresh corn leaf, as a natural diet. After the individualization, the pots were conditioned in an air-conditioned room at 25±2°C, 60±10% relative humidity, and 12-hour photo phase. Until the complete development of the caterpillar and change to the pupa stage, the natural diet was replaced daily. During this stage, these were placed in Petri dishes lined with filter paper and kept inside screened cages.

After adult emergence, the moths were kept in cylindrical PVC cages (150 x 200 mm), lined with filter paper and fed with honey and beer yeast (1: 1), as well as a cotton swab, moistened daily. The egg mass deposited on the filter paper was removed daily and wrapped in Petri dishes until the larvae hatch, and the same natural diet was immediately provided. In all stages of insect

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breeding, the climatic conditions were identical to those described previously.

***Ocimum basilicum* L. essential oil obtaining**

The essential oil was extracted from leaves and flowers of *O. basilicum* ("Maria Bonita" variety), held in the experimental area of the Federal University of Uberlândia. The material originated from access PO 197442, from the Germplasm Bank North Central Regional PI Station, Iowa State University, USA, and provided by the Aromatic Plant Breeding Program of the Federal University of Sergipe. This variety presented mean values over 80% of linalool in the chemical composition (SILVA et al., 2017) (Table 1), which was used in the present study as a comparison measure in toxicological evaluations.

Then, to confirm the quantity of each compound, it was realized a new chemical analyzes of the essential oil, being performed on a gas chromatograph coupled with a mass spectrometer (Shimadzu GC-2010 + QP-5000), provided with a fused silica capillary column DB-5 (30 m x 0.25 mm x 0.25 μ M), in according to methodology used by Silva et al. (2017). The operation mode was: helium as carrier gas at 1.7 ml min⁻¹, temperatures of 240 °C of injector, a detector of 230 °C, and the program of temperature of 60 to 240 °C, 3 °C increase every minute, 1/2 being split and flow 1ml min⁻¹. The identification of compounds was performed by comparison of their mass spectra with the system databases and literature (MCLAFFERTY; STAUFFER, 1989) and determined whether the Kovats retention indices, comparing them with the literature (ADAMS, 2007).

The quantification of the compounds was performed on a gas chromatograph coupled with a flame ionization detector (Shimadzu GC-2010/ FID) and DB5 capillary column. The carrier gas was helium with a flow of 1.0 ml min⁻¹ and split ratio of 1/20, the injector to 240 °C, detector to 230 °C and the program of temperature was 60 °C to 165 °C, with the addition of 4 °C min⁻¹ from 165 °C to 240 °C with an increase of 10 °C min⁻¹.

Toxicological evaluations

Dose-response bioassays were performed with linalool (Quinarí - Casa das Essências, Ponta Grossa, Rio Grande do Sul State, Brazil, with 97.5% purity), and the lethal dose 50 (LD₅₀) for *S. frugiperda* was determined. The tests were carried out in six-well cell culture plates maintained in an air-conditioned chamber (25 ± 1 °C, 65 ± 10% RH, and 12-hour photo phase). In each well, the natural

diet and a third instar larva of *S. frugiperda* were conditioned. The determination of the stages of development of larvae was performed according to Parra; Carvalho (1984). The acute toxicity tests were performed according to the methodology proposed by the "Organization for Economic Cooperation and Development" (OECD/OCDE, 2020), that establish methods for laboratory tests to assess the impact of pesticides on insects.

Solutions for determination of LD₅₀ were prepared in acetone. A dilution range with the product was prepared with concentrations (v/v) ranging from 100% (pure) to 1%, with each caterpillar being topically applied 1 μ L, using a microsyringe (0.5-5 μ L volume, Hamilton), which allows a precision volume of 0.5 μ L. For each concentration evaluated, 24 third instar larvae were used.

Subsequently, the cell culture plates containing the insects were maintained in similar conditions as described above. The evaluations occurred every 24 hours after the application, counting the number of dead larvae. A negative control treatment was also performed, only with the acetone application. The insects that received the different concentrations of linalool were observed for 1 hour, to characterize the behavioral effects caused after the applications.

The toxicity tests with *O. basilicum* essential oil, as a natural source of linalool, and with pyrethroid insecticides were also performed: deltamethrin (purity 99.5%, Pestanal®) and its commercial product (Decis 25 EC, Bayer®), in according to methodology used by Silva et al. (2017). For this, a dilution range of each of the products was prepared in acetone, covering the following concentrations: 0.1 to 1 μ L⁻¹ for essential oil, 0.1 to 0.1 x 10⁻⁴ μ g μ L⁻¹ of deltamethrin and from 25 to 0.25 g L⁻¹ of Decis. Again, each of the 24 caterpillars received, per each concentration the topical application of 1 μ L of a respective insecticide concentration or the essential oil and maintained as previously described. Dead larvae were counted every 24 hours after the application.

Synergistic effect between linalool and pyrethroids

After determining the lethal dose of linalool and pyrethroids, the following LD₅₀ doses: 25% LD₅₀, 50% LD₅₀, and 75% LD₅₀ of each product were established.

Again, third instar caterpillars received the topical application of 1 μ L of the following treatments: LD₅₀ deltamethrin + LD₅₀ linalool; LD₅₀ deltamethrin + 50% LD₅₀ linalool; 50% LD₅₀

deltamethrin + 50% LD₅₀ of linalool; 50% LD₅₀ deltamethrin + LD₅₀ linalool; 75% LD₅₀ deltamethrin + 25% LD₅₀ linalool; 25% LD₅₀ deltamethrin + 75% LD₅₀ linalool. For the commercial product, the following combinations were made: LD₅₀ Decis + LD₅₀ linalool; 75% LD₅₀ Decis + 25% LD₅₀ linalool; 50% LD₅₀ Decis + 50% LD₅₀ linalool; 25% LD₅₀ Decis + 75% LD₅₀ linalool; 50% LD₅₀ Decis + LD₅₀ linalool. All experimental conditions were identical to those described previously. The mortality data were obtained at 24 and 48 hours for each treatment.

Data analysis

The mortality data of each of the assays were submitted to the analysis of the dose-response type, using the tool "Probit" of SPSS Software (2011). Based on the mathematical model adjusted for the observed data, the LD₅₀ values of the products were determined, as well as the value of the confidence interval, chi-square, and degrees of freedom. The difference between median lethal concentrations or dose of insecticide was considered statistically significant when 95% confidence

intervals were non-overlapping. On the other hand, the data obtained from the mortality of insecticides combinations were submitted to the Shapiro Wilk test of normality distribution of residues, Levene homogeneity of variances, and Tukey's block additivity test, at 0.01 significance. When pertinent, the F test was performed by analysis of variance and the means compared by the Scott-Knott Test, at 0.05 significance.

RESULTS

Acute toxicity

In Table 1, the compounds of *O. basilicum* L. essential oil used as a comparison measure for linalool. The essential oil content found was 2% of *O. basilicum* biomass used. The gas chromatography-mass spectrometry analyses of *O. basilicum* essential oil revealed the presence of 19 compounds and the main components were linalool, 1,8-cineole and geraniol. The chemical composition was similar to results presented by Silva et al. (2017).

Table 1. Chemical composition of *Ocimum basilicum* L. essential oil obtained by gas chromatography and mass spectrometer.

Peak	Retention time	CRI ¹	LRI ²	Compound	%Area	%GC-FI ³
1	8.809	923	932	α-pinene	0.01	0.14
2	10.029	963	969	sabinene	0.11	0.17
3	10.156	967	974	β-pinene	0.53	0.47
4	11.895	1021	1026	1,8-cineol	6.23	5.13
5	14.214	1091	1095	linalool	79.55	79.59
6	17.095	1181	1186	α-terpineol	0.49	0.46
7	18.587	1229	1235	neral	0.18	0.15
8	18.950	1241	1249	geraniol	9.72	9.22
9	19.447	1258	1264	geranial	0.17	0.89
10	19.982	1275	1287	bornila acetat	0.25	0.58
11	22.700	1369	1359	neril acetat	0.02	0.16
12	23.091	1382	1389	β-elemene	0.30	0.33
13	24.000	1415	1417	(E)-caryophyllene	0.23	0.16
14	24.260	1424	1432	α-(E)-bergamotene	1.94	1.47
15	24.413	1430	1437	α-guaien	0.17	0.19
16	25.660	1476	1484	D germacrene	0.49	0.35
17	26.272	1498	1509	α-bulnesene	0.15	0.14
18	26.495	1507	1513	γ-cadinene	0.51	0.33
19	29.729	1636	1638	epi-a-cadinol	1.48	1.12

¹CRI: Calculated retention index; ²LRI: Literature retention index; ³GC-FI: Flame ionization gas chromatography.

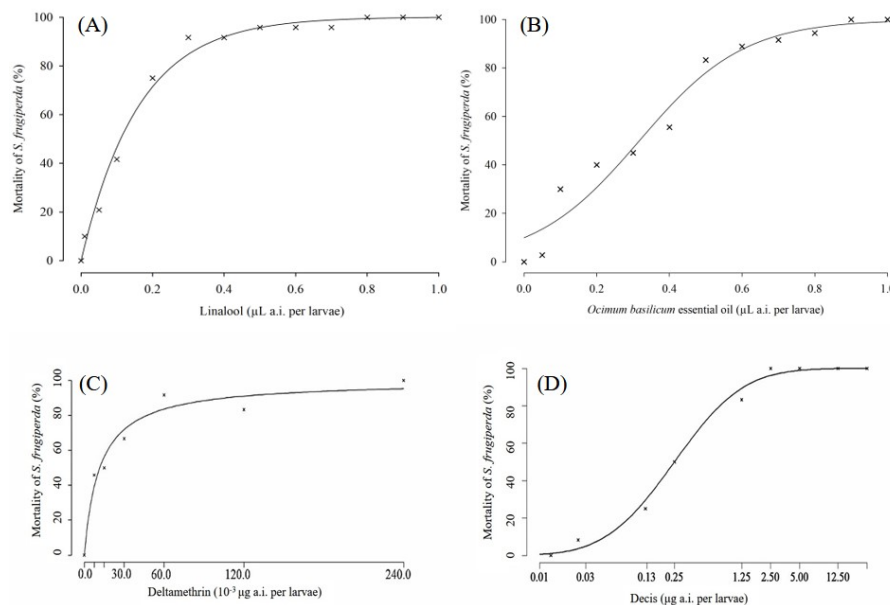
The LD₅₀ values of the products and the parameters obtained by the toxicity tests are available in Table 2. The mathematical models with the best fit for the linalool, essential oil, and insecticides can be visualized in Figure 1. In

according to the Figure 1, the mortality of larvae increases as the dose increases for all products, but total mortality of larvae was not reach at the same concentration for all products.

Table 2. Summary of the parameters obtained by the acute toxicity test of linalool, *Ocimum basilicum* essential oil, deltamethrin, and Decis against larvae of *Spodoptera frugiperda*.

	Time	LD ₅₀ ^a	CI 95% ^b	DF ^c	χ^2 ^d
Linalool		$\mu\text{L } \mu\text{L}^{-1}$			
	24h	0.190	0.149 – 0.232	10	9.94
	48h	0.177	0.132 – 0.215	9	6.70
<i>O. basilicum</i> essential oil		$\mu\text{L } \mu\text{L}^{-1}$			
	24h	0.401	0.244 – 0.529	8	34.42
	48h	0.398	0.206 – 0.428	8	42.81
Deltamethrin*		$10^{-3} \mu\text{g } \mu\text{L}^{-1}$			
	24h	19.250	8.960 – 29.540	22	30.44
	48h	17.260	7.810 – 27.110	21	23.13
Decis		$\mu\text{g } \mu\text{L}^{-1}$			
	24h	0.249	0.173 – 0.359	7	23.657
	48h	0.250	0.173 – 0.359	7	23.657

^aLD: Lethal dose; ^bCI: Confidence interval; ^cDF: Degrees of freedom; ^d χ^2 : Chi-square; *Data obtained from Silva et al. (2017);

**Figure 1.** Mortality of 3rd instar larvae of *Spodoptera frugiperda* after 48h of acute toxicity with different doses of linalool (A), *Ocimum basilicum* essential oil (B), deltamethrin (C) and Decis (D).

After the application of linalool, it was observed extreme insect agitation, loss of motor coordination, loss of spatial orientation, extravasation of hemolymph, loss of feeding capacity, and death. It was also observed a high penetration capacity of the product with fast absorption by the cuticle of the insects. It was also observed that the higher the product dose, the more pronounced were the behavioral effects. Finally, the death of several individuals occurred a few moments after the application.

Regarding the essential oil, after its application, the observed behavioral effects were the

same described previously, but in a more lenient way. After the pyrethroids application, the behavioral effects observed in insects were more subtle in relation to linalool, with little or no movement of the caterpillars, loss of motor coordination, and more prolonged death of the individuals, with maximum mortality occurring up to 48 hours after the application.

Lethal effect of product mixtures against *S. frugiperda* larvae

All binary mixtures caused mortality higher than the products applied alone (deltamethrin and

linalool) used at 100% LD₅₀, except to 75% LD₅₀ of deltamethrin added to 25% LD₅₀ linalool, whose mortality did not differ the products alone, in 24 hours (Table 3). At highest dose of linalool, over 90% larval mortality occurs when linalool was

combined with 25% LD₅₀ deltamethrin, in 24 and 48 hours after application (Table 3), and over 80% of mortality when linalool was combined with 25% LD₅₀ Decis, in 48h after application (Table 4).

Table 3. Mortality of larvae of *Spodoptera frugiperda* after topical application of linalool (μL of active ingredient per μL) combined with deltamethrin ($10^{-3}\mu\text{g}$ of active ingredient per μL).

Deltamethrin		Linalool		Mortality (%)	
Dose	$10^{-3}\mu\text{g a.i. } \mu\text{L}^{-1}$	Dose	$\mu\text{L a.i. } \mu\text{L}^{-1}$	24h	48h
^a LD ₅₀	19.25	LD ₅₀	0.177	87.25a*	100.00a
50% LD ₅₀	9.62	LD ₅₀	0.177	95.75a	95.75a
LD ₅₀	19.25	50% LD ₅₀	0.088	91.50a	95.75a
25% LD ₅₀	4.81	75% LD ₅₀	0.132	91.50a	91.50a
50% LD ₅₀	9.62	50% LD ₅₀	0.088	78.75b	78.75a
75% LD ₅₀	14.43	25% LD ₅₀	0.044	58.00c	70.00b
LD ₅₀	19.25	0	0	53.75c	58.00c
0	0	LD ₅₀	0.177	45.75c	58.00c
Control (Acetone)				0	0
F ^b				15.30	23.23
CV (%) ^c				23.10	17.31

* Means followed by lowercase letters in the column differ by the Scott-Knott test at 5% of probability; ^a LD₅₀: Lethal dose for 50% mortality at 95% confidence interval; ^bF: F value calculated; ^cCV: Coefficient of variation.

Table 4. Mortality of larvae of *Spodoptera frugiperda* after topical application of linalool (μL of active ingredient per μL) combined with Decis® (25CE) (μg of active ingredient per μL).

Decis		Linalool		Mortality (%)	
Dose	$\mu\text{g a.i. } \mu\text{L}^{-1}$	Dose	$\mu\text{L a.i. } \mu\text{L}^{-1}$	24h	48h
^a LD ₅₀	0.25	LD ₅₀	0.177	100.00a*	100.00a
50% LD ₅₀	0.125	LD ₅₀	0.177	83.33a	83.33a
25 % LD ₅₀	0.062	75% LD ₅₀	0.132	77.00a	83.00a
50% LD ₅₀	0.125	50% LD ₅₀	0.088	72.00a	72.00a
75 % LD ₅₀	0.187	25% LD ₅₀	0.044	72.00a	77.00a
0	0	LD ₅₀	0.177	44.33b	49.66b
LD ₅₀	0.25	0	0	44.33b	44.33b
Control (Acetone)				0	0
F ^b				29.32	25.39
CV (%) ^c				12.76	16.24

* Means followed by lowercase letters in the column differ by the Scott-Knott test at 5% of probability; ^a LD₅₀: Lethal dose for 50% mortality at 95% confidence interval; ^bF: F calculated value; ^cCV: Coefficient of variation.

DISCUSSION

Products toxicity

The obtained results confirmed the insecticidal potential of linalool. Its LD₅₀ was lower than essential oil LD₅₀, being half of it. The lower toxicity of the essential oil was already expected because, in its chemical composition, linalool was present in a lower concentration (79.29%).

Previous studies concluded that the bioactive compounds present in the essential oils of aromatic species can interact synergistically and provide higher toxicity to the pests, concerning the exclusive application of some compounds

(AKHTAR et al., 2012; AFSHAR et al., 2017). Differently in the present work, the exclusive application of linalool caused higher toxicity than the essential oil of *O. basilicum* application. Koul et al. (2013) also showed higher toxicity of linalool to caterpillars, obtaining LD₅₀ of 85.5 μg per larva of *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae).

The observed effects after the linalool application are possibly related to the neurotoxicity of this compound since the insects presented an aspect of confusion, followed by extreme agitation and death. Pavela (2014) also confirmed the neurotoxic effects of this terpenoid. Previous studies have shown that linalool forms a stable

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intermolecular complex with the enzyme AChE, effectively inhibiting its interaction with acetylcholine (PRAVEENA; SANJAYAN, 2011), confirming the insecticidal potential of this compound.

As for the pyrethroids, the obtained results were remarkably close to the values obtained by Silva et al. (2017) like high toxicity, followed by caterpillar mortality. This underscores the effectiveness of this chemical group and the continuity of its application in the productive systems since appropriate measures are adopted to reduce the resistance of pests to insecticides.

On the other hand, as observed, LD₅₀ linalool and essential oil values were higher than the values obtained for the pyrethroids. This fact could lead to questioning the real effectiveness of the former in relation to the latter. This happens because the pyrethroids present a different route of action in relation to linalool, presenting more intracellular action, specifically in the channels of Na⁺ ions, in the cells of the nervous system.

The terpenoids act in the cholinergic system, in the region of the synaptic clefts, in an intercellular form, inhibiting neurotransmitters and receptors. According to Lopez; Villalobos (2010), a higher concentration of linalool would be required for it to be considered a potent inhibitor of AChE enzyme in relation to other insecticides that act on the same route. Lima et al. (2009) also reported higher doses of the terpenoid to control *S. frugiperda*.

It is also believed that lepidopteran pests have characteristics that make their control somewhat difficult. These characteristics would be related to a greater aggressiveness of their young forms in the competition for food, with a higher capacity of exploration and search for other hosts (polyphagous). Besides that, a greater capacity of detoxification and metabolization of xenobiotics (insecticides), being this, which make them more resistant to insecticides with different mechanisms of action, in relation to other insects.

All these characteristics indicate the urgent need to adopt more integrated management practices in agricultural systems, such as the constant rotation of products with different mechanisms of action and the application in the young stages of the corn fall armyworm, in propitiating the reduction of their population density, and consequently, their possible damages to the culture.

Lethal effect of product mixtures against *S. frugiperda* larvae

The results of synergist effect of binary combinations of linalool and pyrethroids were similar to others toxicological studies. Fazolin et al. (2016) obtained significant control of *S. frugiperda* in the combination of pyrethroids and terpenoids. In this study, the alpha-cypermethrin, fenpropathrin, gamma-cyhalothrin, and beta-cypermethrin LD₅₀ were reduced to ½ and/or ¼ in the presence of terpenoids, causing significant acute toxicity to larvae.

Abbassy et al. (2009) tested the synergistic effect of γ -terpinene and terpinen-4-ol with profenofos insecticide against *Spodoptera littoralis* (Boisd.) (Lepidoptera, Noctuidae). The results indicated that the isolated compounds enhanced the effectiveness of the insecticides against the tested insect.

Kariuki et al. (2014) reported that the association between products contributes significantly to increase the effectiveness of insecticides, and this tool is aimed at improving the management of resistance to insecticides. Srivastava et al. (2011) also emphasized that such combinations may cause faster effects on target organisms than synthetic formulations applied alone, possibly due to the greater complexity to metabolize different compounds combined in insect organisms.

The results of synergist effect of binary combinations of linalool and pyrethroids were very promising as more sustainable option in the pest management resistance to insecticides. In addition, the synergistic effect may lead to the reduction of the application rates of these insecticides which would be less harmful to the environment. The synergistic effect of this essential oil with insecticides could help to decrease the negative effects of synthetic chemicals such as residues in products, development of insect resistance, food contamination, and environmental pollution (ABBASSY et al., 2009).

According to the results found in the present work, the lethal doses of essential oil and linalool were higher than the doses of the synthetics insecticides, that is, to cause the same mortality of *Spodoptera frugiperda* caterpillars, greater quantities of natural products were needed in relation to the synthetics insecticides. As the content of the essential oil of *Ocimum basilicum* is extremely low (2%), obtaining large quantities of the oil becomes awfully expensive. Thus, at present, it is believed that the most viable option for the applicability of natural products in field pest management would be in combination with

synthetic insecticides, requiring lesser amounts of natural products to make their use feasible.

CONCLUSION

Linalool is highly toxic against *Spodoptera frugiperda* larvae (LD₅₀ of 0.177 $\mu\text{L } \mu\text{L}^{-1}$) and cause neurotoxic effects, which culminate in its death.

All binary product mixtures produced synergistic lethal effects under laboratory conditions.

Further studies are required in order to evaluate the synergistic combinations against field populations of *S. frugiperda*.

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RESUMO: O objetivo deste trabalho foi determinar a toxicidade do linalol e avaliar os efeitos tóxicos e letais do linalol associado a piretroides em misturas binárias para lagarta do cartucho do milho (*Spodoptera frugiperda*). Os insetos utilizados no experimento foram obtidos de criação estoque iniciada a partir de larvas coletadas em plantas de milho convencional, cultivado em área experimental, no município de Uberlândia, Minas Gerais. Também foi obtido óleo essencial de uma variedade de *Ocimum basilicum*, com alto teor de linalol (80%), encontrado naturalmente, como medida de comparação para ensaios com linalol (97.5%). Os bioensaios do tipo dose-resposta com larvas de 3º instar foram realizados para determinar a dose letal do linalol para 50% de mortalidade da população (DL₅₀). Também foram realizados testes de toxicidade com óleo essencial de *Ocimum basilicum* e com inseticidas piretroides: deltametrina e seu produto comercial (Decis 25 EC, Bayer®). Em seguida, foram realizadas combinações entre diferentes doses desses produtos e aplicadas em larvas de 3º instar de *Spodoptera frugiperda* (Smith). De acordo com os resultados, observou-se que o linalol apresentou alta toxicidade para *S. frugiperda* (DL₅₀ = 0,177 $\mu\text{L a. i. } \mu\text{L}^{-1}$). Foram observados efeitos neurotóxicos após a aplicação do linalol, uma vez que os insetos apresentaram um aspecto de confusão, seguido de extrema agitação e, por fim, morte. Todas as combinações binárias causaram mortalidade maior que os produtos aplicados isoladamente (deltametrina e linalol) utilizando-se 100% da DL₅₀, exceto para 75% DL₅₀ de deltametrina somada a 25% DL₅₀ de linalol, cuja mortalidade não diferiu dos produtos isolados, em 24 horas após a aplicação. Foi obtida mais de 90% de mortalidade de larvas quando se combinou linalol com 25% da DL₅₀ de deltametrina, em 24 e 48 horas após a aplicação, e mais de 80% de mortalidade quando se combinou linalol com 25% da DL₅₀ do produto comercial, somente 48 horas após a aplicação. Concluímos que o linalol é um potencial inseticida e pode ser associado a piretroides no controle de *S. frugiperda*. Mais estudos são necessários em vista de avaliar as combinações sinérgicas contra populações de campo de *S. frugiperda*.

PALAVRAS-CHAVES: Atividade inseticida. Deltametrina. Sinergismo. *Spodoptera frugiperda*. Terpenoides.

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