

Toxicity of *Cymbopogon flexuosus* essential oil and citral for *Spodoptera frugiperda*

Toxicidade do óleo essencial de *Cymbopogon flexuosus* e do citral para *Spodoptera frugiperda*

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ABSTRACT

Fall armyworm (FAW) (*Spodoptera frugiperda*) is a polyphagous insect responsible for damage to several crops. Synthetic chemical insecticides and genetically modified plants are the most commonly used methods for FAW control. However, the selection of resistant populations has been reported in several studies, justifying the search for new molecules to be used in the control of *S. frugiperda*. The aim of the present study was to evaluate the toxicity of lemongrass (*Cymbopogon flexuosus*) essential oil (LEO) and its major component (citral) in relation to FAW. Additionally, the anticholinesterase activity of LEO and citral was evaluated using acetylcholinesterase (AChE) from *Electrophorus electricus*. The LEO was toxic to FAW when added to an artificial diet ($LC_{50} = 1.35 \text{ mg mL}^{-1}$) at the highest concentrations tested, and the median lethal time (LT_{50}) was 18.85 h. Major components of LEO were identified by gas chromatography-mass spectrometry, and citral, the most abundant component, was used in FAW bioassays. The insecticidal activity of citral was statistically similar to that of LEO, demonstrating that citral was responsible for the insecticidal activity of LEO. Inhibition of AChE was measured, and the mean inhibitory concentration (IC_{50}) values for LEO and citral were 650- and 405-fold higher, respectively, than that verified for the positive control (methomyl insecticide), suggesting selectivity for non-target organisms. Based on these results, citral and *C. flexuosus* have the potential to be applied in the development of new products for the control of *S. frugiperda*.

Index terms: Lemongrass; (2Z)-3,7-dimethylocta-2,6-dienal; monoterpenes; botanical insecticide; fall armyworm.

RESUMO

A lagarta do cartucho (LCM) (*Spodoptera frugiperda*) é um inseto polífago que causa danos em várias culturas. Inseticidas químicos sintéticos e plantas geneticamente modificadas são os métodos mais comumente empregados para o seu controle. Entretanto, existem muitos relatos da seleção de populações resistentes, o que justifica a busca por novas moléculas para o controle de *S. frugiperda*. O objetivo desse trabalho foi avaliar a toxicidade do óleo essencial de capim limão (*Cymbopogon flexuosus*) (OECL) e seu componente majoritário, citral, para LCM. Adicionalmente, a atividade anticolinesterase do OECL e do citral foram avaliadas usando a acetilcolinesterase (AChE) de *Electrophorus electricus*. OECL foi tóxico para LCM, quando incorporado em dieta artificial ($CL_{50} = 1,35 \text{ mg mL}^{-1}$), nas mais altas concentrações testadas, o tempo letal mediano (TL_{50}) foi de 18,85 h. Os componentes majoritários do OECL foram identificados por cromatografia gasosa acoplada a espectrometria de massas. Citral, o composto mais abundante, foi empregado em bioensaios com LCM. A atividade inseticida do citral foi estatisticamente similar àquela do OECL, demonstrando que o citral é responsável pela atividade inseticida do OECL. A inibição da AChE foi mensurada, sendo os valores de concentração inibitória média (CI_{50}) encontrados para o OECL e citral, 650 e 405 vezes maiores, respectivamente, do que o detectado para o controle positivo, o inseticida metomil, sugerindo seletividade para organismo não-alvo. Os resultados encontrados tornam o citral e *C. flexuosus* promissores para serem empregados no desenvolvimento de novos produtos para o controle de *S. frugiperda*.

Termos para indexação: Lemongrass; (2Z)-3,7-dimethylocta-2,6-dienal; monoterpenos; inseticida botânico; lagarta do cartucho.

INTRODUCTION

The fall armyworm (FAW) *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) is considered one of the primary pests occurring in the most important agricultural commodities in the Americas. The FAW is a polyphagous insect that reproduces throughout the

phenological stages of several crops, both in productive and surrounding areas, because of intensive cultivation (Casmuz et al., 2010; Murúa et al., 2015).

The high population density and economic damages caused by *S. frugiperda* lead to great dependence on the use of synthetic chemical insecticides and genetically modified plants for control. However, the

indiscriminate and intensive use of these technologies, associated with the high reproductive rate and dispersion of this insect, has led to the selection of resistant populations (Farias et al., 2016; Omoto et al., 2016; Santos-Amaya et al., 2017; Yu; McCord, 2007), justifying the constant search for molecules that may be used to control this insect.

In this context, secondary plant metabolites are identified as a promising tool in the control of insect pests. Substances from plants are often less toxic to non-target organisms, such as natural enemies, pollinators and humans (Isman, 2017; Pavela; Benelli, 2016). Among secondary plant metabolites, essential oils have received increasing attention for their insecticidal activity and sublethal effects (Krinski; Foerster, 2016; Regnault-Roger, 2013; Silva et al., 2017b; Walia et al., 2017). Furthermore, essential oils are compounds that may reduce the potential for resistance to pests because of their complex chemical composition that may direct their performance at different sites and at different stages of development of the target insect (Akhtar et al., 2012).

Species from the genus *Cymbopogon* of the Poaceae family are widely known for producing lemongrass essential oil (LEO) and for their insecticidal properties. Among the species from this genus, *Cymbopogon flexuosus* (Steud.) Wats. (Poaceae) is one of the most widely grown plants producing essential oil in tropical and subtropical regions of India, Indonesia, Madagascar, and countries in Africa and South America (Ganjewala; Luthra, 2010). Toxicity of this plant is observed against stored grain pests (Caballero-Gallardo; Olivero-Verbel; Stashenko, 2012), insect-borne diseases (Tawatsin et al., 2001; Tennyson et al., 2013; Vera et al., 2014), and oilseed pests (Hernández-Lambraño, Caballero-Gallardo; Olivero-Verbel, 2014).

Note that LEO is considered as a minimum risk insecticide according to the United States Environmental Protection Agency (EPA, 2015); however, to the best of our knowledge, LEO insecticidal activity against *S. frugiperda* has not been reported to date. Thus, the aim of the present study was to chemically characterize the *C. flexuosus* essential oil and evaluate the toxicity of LEO and that of the major constituent to *S. frugiperda*. Additionally, the potential inhibitory effects of the essential oil and citral were evaluated *in vitro* on the acetylcholinesterase (AChE) enzyme from electric eel (*Electrophorus electricus*), which can provide information on the selectivity of these metabolites to non-target organisms.

MATERIAL AND METHODS

Insects

Spodoptera frugiperda were reared from caterpillars and pupae obtained from maize in an experimental field of the Federal University of Lavras, Minas Gerais, Brazil. To perform the bioassays, *S. frugiperda* caterpillars were used at 48 h of age from the second reproduction of laboratory specimens fed artificial diet (Parra, 2001). Adults were fed 10% honey aqueous solution. All insects were kept in an acclimatized room at 25 ± 2 °C, $70 \pm 10\%$ RH and with a 12 h photoperiod.

Essential oil

The tillers of *C. flexuosus* were collected from the Horto of Medicinal Plants at Federal Lavras University. Plants of *C. flexuosus* were cultivated in soil with organic fertilizer (cow dung) at a dose of 3.0 kg m⁻² irrigated periodically with plant spacing of 45 cm (21°14'43" S, 44°59'59" W). The plants were identified on the basis of morphological features and deposited in the herbarium of the Department of Biology at Federal Lavras University (UFLA). The fresh plant leaves were collected at 20 cm from the soil at seven months of age. The plant material (1000 g) was steam-distilled for 90 min in a Marconi MA480 essential oil distiller (Piracicaba, São Paulo, Brazil). The LEO was separated by decantation for 20 min and stored in a freezer at -10 °C until chemical analyses and biological tests.

GC-MS analysis of *C. flexuosus* essential oil

The quantitative analysis of *C. flexuosus* essential oil was performed using an Agilent® 7890A chromatograph operated with the HP GC ChemStation data processing system ver. A.01.14 and equipped with a CombiPAL Autosampler System (CTC Analytic AG, Switzerland) and flame ionization detector (GC-FID). Samples were prepared by diluting the essential oil with ethyl acetate (10 mL L⁻¹). Volume injection was 1.0 µL in split mode at a 50:1 injection ratio. An HP-5ms fused silica capillary column (30 m length x 250 µm internal diameter x 0.25 µm film thickness) (California, USA) was used. Helium gas was used as the carrier gas with flow of 1.0 mL min⁻¹. The injector and detector temperatures were maintained at 240 °C. The initial oven temperature was 60 °C maintained for 1 min, followed by a temperature ramp of 3 °C min⁻¹ up to 240 °C, followed by a ramp of 10 °C min⁻¹ up to 250 °C, with the isothermal condition maintained for 1 min. The concentrations of the constituents were expressed by the

average relative percentage of area of the chromatographic peaks \pm the standard deviation of three analyzed samples.

Qualitative analyzes were performed on an Agilent® 7890A chromatograph coupled to an Agilent® MSD 5975C mass selective detector (Agilent Technologies, California, USA) operated by electronic impact ionization at 70 eV in scan mode at a speed of 1.0 scan s⁻¹, with a mass acquisition interval of 40-400 m/z. The operating conditions were the same as those used in GC-DIC analyses.

Mass spectra from the database of NIST/EPA/NHI (National Institute of Standards and Technology - NIST, 2008) were compared to identify the chemical constituents of samples. Additionally, the retention indices from the Adams (2007) literature were compared with retention indices calculated based on the equation of Dool and Kratz (1963) relative to coinjection of a standard solution of n-alkanes (C₈-C₂₀; Sigma-Aldrich®, St. Louis, USA) and coinjection with citral (neral and geranial mixture with 95% purity; Sigma-Aldrich®).

Time-concentration-mortality responses for FAW fed diet containing LEO

LEO at the range of concentrations from 0.5 to 4.0 mg mL⁻¹ diet was leached in aqueous 0.01 g mL⁻¹ Tween® 80 (Polysorbate 80; Sigma-Aldrich®) (20 mL) and incorporated into artificial diet (200 mL) at 40 °C. To ensure that the aqueous solution of Tween® 80 was homogenized with the essential oil, 10 drops of food coloring agent (Arcolor®) were added using a Pasteur pipette. Dietary pieces (1 cm diameter x 1.5 cm height, weight = 9 \pm 0.35 g) were transferred to glass tubes (8 cm x 2.5 cm) in which a caterpillar at 48 h of age was introduced, previously fed artificial diet without the addition of essential oil. A completely randomized design was used composed of LEO leached at different concentrations and the two controls: diet with water and food coloring agent added and diet with Tween® 80 aqueous solution and dye added, totaling 14 treatments. The experimental plot consisted of one caterpillar maintained individually, and 60 replicates were used per treatment. Insect survival was evaluated every 24 h for 11 days. Survival data after 72 h were used to calculate the median lethal concentration (LC₅₀).

Sublethal effects of LEO for FAW

The LEO [0.675 mg mL⁻¹ diet (LC₂₀) and 1.35 mg mL⁻¹ diet (LC₅₀)] was incorporated into the artificial diet as described in the subitem above. The control treatments were diet with water and food coloring agent added and diet with aqueous solution Tween® 80 and dye added. Each treatment consisted of 120 replicates with one caterpillar per

replicate; the experimental plot consisted of one individual caterpillar. Insect development was evaluated daily until the pupal stage, with records of the larval survival every 24 h for 264 h, the larval phase duration and the pupal weight.

Toxicity to citral for FAW

The reference citral (neral and geranial mixture with 95% purity; Sigma-Aldrich®) was added to the artificial diet of FAW at concentrations of (0.521, 1.042 and 1.58 mg mL⁻¹ diet), as previously described.

The bioassay was performed in a completely randomized design consisting of eight treatments: the estimated values for the LC₂₀ (0.675 mg mL⁻¹), LC₅₀ (1.35 mg mL⁻¹) and LC₉₀ (2.053 mg mL⁻¹) of the LEO; the estimated values for the LC₂₀ (1.58 mg mL⁻¹), LC₅₀ (1.042 mg mL⁻¹) and LC₉₀ (0.521 mg mL⁻¹) of citral, and two controls, diet with water and food coloring agent added and diet with Tween® 80 aqueous solution and dye added.

Citral concentrations were calculated using the values obtained in the quantitative chromatographic analysis of the essential oil, using the formula $LC = [(c \times p)/100]$, where LC = expected lethal concentration of citral, c = lethal concentration of the essential oil and p = percentage of citral in the constitution of the LEO.

Each treatment consisted of 60 replicates, each represented by a 48-h-old caterpillar maintained in a glass tube (8 cm x 2.5 cm) containing an artificial diet of the same size (1 cm diameter x 1.5 cm height).

Inhibition of enzymatic activity of the AChE

The inhibitory activity of AChE was determined according to the methodology described by Aazza, Lyoussi and Miguel (2011) with modifications. In this respect, 4.25 μ L of Tris-HCl buffer (0.1 M, pH = 8) and 25 μ L of the test constituent (LEO or citral) were dissolved in ethanol at different concentrations from 0.01 to 2.24 mg mL⁻¹. Subsequently, 25 μ L of AChE enzyme (0.22 U/mL) (Type-VI-S, EC 3.1.1.7, Sigma-Aldrich®) was added and homogenized. After incubation at 37 °C for 15 min, 75 μ L of 15 mM acetylcholine iodide substrate (Sigma-Aldrich®) and 475 μ L of 3 mM 5,5'-dithiobis-(2-nitrobenzoic acid) (Sigma -Aldrich®) were added, and the resulting solution was incubated at room temperature for 30 min. The same procedure was performed for the positive control, a commercial product based on methomyl (Lannate®, DuPont™), and for the negative control (ethanol). The solutions were transferred to microplates, and the absorbance was measured at 405 nm in a TECAN Infinite® M200 PRO microplate reader.

Statistical analyses

The survival data of insects over time were subjected to survival analysis by applying the Weibull model through the Survival package (Therneau, 2015) of the R software® (R Core Team, 2018). After selecting the most appropriate mathematical model through residue analysis, the contrast analysis was performed to verify the similarity among the used treatments to form congeners, with the median lethal time (LT_{50}) calculated for each formed group. To verify the data fitting to the model, the Kolmogorov-Smirnov test was applied.

To determine the concentration-mortality response, the data were subjected to Logit analysis using the drc package (Ritz; Streibig, 2016) of the R software® (R Core Team, 2018). Data on the duration of the larval period and weight of pupae were subjected to the Shapiro-Wilk test, using the Mvnormteste package (Jarek, 2012) to verify the normality. Subsequently, data were subjected to ANOVA and to the Scott-Knott test of the Laercio package (Silva, 2010).

RESULTS AND DISCUSSION

GC-MS analysis of *C. flexuosus* essential oil

The LEO was composed of 21 constituents, corresponding to 90.57% of the chemical composition. The major constituents of LEO were neral/Z-citral (32.59%) and geranial/E-citral (44.65%). These isomeric monoterpenes totaled 77.25% of this essential oil, whereas the other constituents summed to 13.32% of total oil content (Table 1).

The major constituents generally found in *C. flexuosus* essential oil are the geranial and neral aldehydes, which together form citral and can vary in content according to numerous factors. The recorded content of citral (77.25%) in the present study was relatively low in relation to the content described in other studies (Adukwu et al., 2012; Ahmad; Viljoen, 2015; Silva et al., 2015). By contrast, the citral content reported in this study was higher than that in the studies of Anaruma et al. (2010) and Vera et al. (2014).

Table 1: Chemical composition of the essential oil of *Cymbopogon flexuosus* leaves.

	Constituent	RT	RI*	Area %
1	5-Hepten-2-one, 6-methyl-	7.820	985	1.58
2	Myrcene	7.971	990	0.45
3	<i>Trans</i> -linalool oxyde	11.711	1088	0.14
4	Linalool	12.171	1100	2.79
5	Exo-isocitral	14.127	1144	0.42
6	Photocitral A	14.345	1149	0.38
7	Citronellal	14.493	1152	0.29
8	Z-isocitral	15.003	1164	0.98
9	<i>E</i> -isocitral	15.815	1182	1.28
10	Estragole	16.513	1198	0.35
11	n-decanal	16.843	1205	0.42
12	Neral	18.523	1242	32.59
13	Geraniol	19.061	1254	0.93
14	Geranial	19.897	1273	44.65
15	Nerolic acid	22.168	1323	0.54
16	Ni <i>m/z</i> = 168	22.850	1339	2.31
17	Geranic acid	23.939	1363	0.18
18	Ni <i>m/z</i> = 169	24.479	1375	3.41
19	Geranyl acetate	24.882	1384	1.92
20	Ar-curcumene	29.165	1483	0.46
21	Sandacopimara-8(14), 15-diene	47.356	1966	0.22

RT = retention time; *RI = retention index calculated in relation to the n-alkane series (C8-C20) on HP-5 MS column in the elution order; Ni = non-identified constituent.

The differences in the quantitative chemical composition of *C. flexuosus* observed in the present study can be explained by the variation in genotype, geographic origin, environmental factors, plant development stage, harvest season, fertilization type and the method of obtaining the essential oil (Gobbo-Neto; Lopes, 2007; Bakkali et al., 2008). Note that plants from the same species but from different origins can express different patterns of metabolites.

Time-concentration-mortality responses for FAW fed diets containing LEO

The *C. flexuosus* essential oil presented insecticidal activity for *S. frugiperda* caterpillars. The survival analysis of *S. frugiperda* caterpillars fed a diet containing LEO produced four congener groups ($\chi^2 = 321$; $df = 13$; $p \leq 0.01$) for which the data were fitted to the Weibull distribution ($D = 0.054409$, $p\text{-value} = 0.4092$). The caterpillars fed diets containing the essential oil at the highest concentrations (2.25, 2.5 and 4.0 mg mL⁻¹ diet) formed the first group with the LT_{50} of only 18.85 h, and 100% of the insects were dead after 240 h. The second group consisted of treatments at intermediate concentrations (1.5, 1.75 and 2.0 mg mL⁻¹ diet), with the LT_{50} of 106.5 h and accumulated survival at the end of the trial evaluation period of only 28%. The lowest concentrations (0.5 to 1.4 mg mL⁻¹) formed the third

group, which presented an LT_{50} greater than 264 h and a cumulative survival rate of 68%. The two controls, diet with water and food coloring added and diet with aqueous 0.01 g mL⁻¹ Tween[®] 80 and dye added, formed the fourth group, with an LT_{50} greater than 264 h and a cumulative survival of 90% at the end of the test (Figure 1).

After 72 h of feeding by the caterpillars on the artificial diet containing the LEO, the estimated concentration in causing the death of 50% of the population (LC_{50}) was 1.33 ± 0.05 mg mL⁻¹ diet. The LC_{90} was estimated at 2.81 ± 0.26 mg mL⁻¹ diet, and the estimated LC_{20} was 0.84 ± 0.06 mg mL⁻¹ diet ($\chi^2 = 395.68$; $df = 351$; $p = 0.1088$).

The LEO was toxic to FAW, corroborating results in other studies in which the insecticidal activity of essential oil for *S. frugiperda* from other plant species of the genus *Cymbopogon* was verified, such as *Cymbopogon winterianus* (Silva et al., 2016; Silva et al., 2017a) and *Cymbopogon citratus* (Knaak et al., 2013). Specifically, for *C. flexuosus*, this report is the first of insecticidal activity against *S. frugiperda*, although this species is known for insecticidal activity on other lepidopteran species (Hernández-Lambrano; Caballero-Gallardo; Olivero-Verbel, 2014).

In the present study, fast insecticidal activity was also verified for the highest concentrations of LEO (LT_{50} of only 18.85 h). This result was reinforced by analyzing

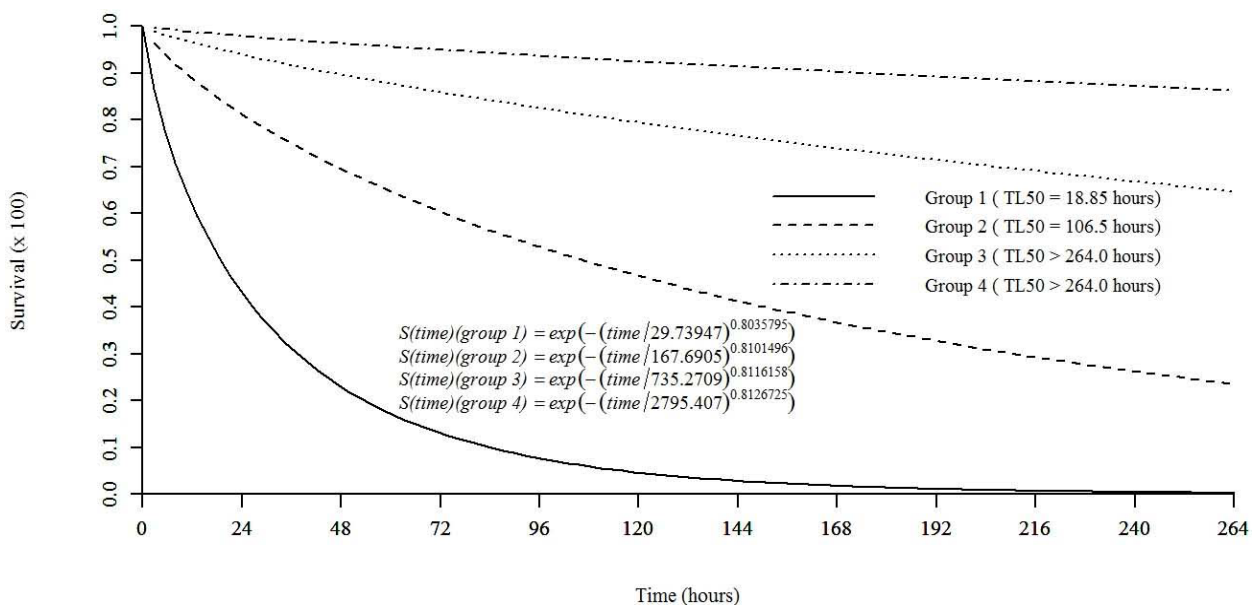


Figure 1: Survival curves for *Spodoptera frugiperda* caterpillars fed artificial diet containing different concentrations of *Cymbopogon flexuosus* essential oil. Where: $S(t) = \exp(-(\text{time}/\delta)^\alpha)$; δ = skewness parameter; α = scale parameter. With: Group 1 = diet with *C. flexuosus* essential oil added (2.25, 2.5 and 4.0 mg mL⁻¹ diet); Group 2 = diet with *C. flexuosus* essential oil added (1.5, 1.75 and 2.0 mg mL⁻¹ diet); Group 3 = diet with *C. flexuosus* essential oil added (0.5, 1.0, 1.1, 1.2, 1.3 and 1.4 mg mL⁻¹ diet); Group 4 = diet with water and dye added and diet with aqueous 0.01 g mL⁻¹ Tween[®] 80 added.

the risk function of the survival curve in which the (alpha) scale parameter was less than 1 (0.67) and therefore decreasing, which was evidence of significant mortality of caterpillars in the first hours of evaluation, suggesting the interaction of the constituents with sites of action in the nervous system of the caterpillars.

In this context, the toxicity of LEO is consistent with other studies that evaluated the insecticidal potential of *Cymbopogon* species on Lepidoptera. Labinas and Crocomo (2002) verified insecticidal activity of the essential oil of *C. winterianus* at the concentration of 5.0 mg mL⁻¹ for *S. frugiperda* neonate caterpillars, conferring 85% mortality. Kolani et al. (2016) observed a strong antifeedant effect on third instar caterpillars of the essential oil of *Cymbopogon schcoenanthus* (LC₅₀ 52.39 mg mL⁻¹) and inhibition of adult emergence of *Plutella xylostella* based on a feeding method. Murcia-Meseguer et al. (2018) applied 1 µL of *C. winterianus* at the concentration of 500 mg mL⁻¹ to third instar caterpillars of *Spodoptera exigua* and verified a mortality of 42%, in addition to developmental interruptions and reduction in pupal size.

The values of LC₅₀ found in the present study demonstrate the potential of *C. flexuosus* for the control of *S. frugiperda*, because the LC₅₀ value (1.33 ± 0.05 mg

mL⁻¹ diet) is comparable to that of other natural products that are considered promising for use in the control of FAW, such as secondary metabolites from stem bark of *Duguetia lanceolata* A.St.-Hil. (Annonaceae) (LC₅₀ 946.5 µg mL⁻¹ diet) (Alves et al., 2016).

Sublethal effects of LEO for FAW

The treatment consisting of artificial diet with added LEO (1.35 mg mL⁻¹ diet) caused 65.83% mortality after 264 h of evaluation with a median lethal time (LT₅₀) of 102.6 h. For the concentration of 0.675 mg mL⁻¹ essential oil, the accumulated mortality was 29.17%, and the LT₅₀ was greater than 264 h. The control diets with water and food coloring agent added and with aqueous 0.01 g mL⁻¹ Tween® 80 and dye added did not differ significantly from one another, with accumulated survival of 94% (χ² = 141.66; df = 3; p ≤ 0.05). The data were fitted to the Weibull distribution (D = 0.031315, p-value = 0.9729) (Figure 2).

In addition to causing mortality, LEO increased the duration of the larval stage of *S. frugiperda*. For caterpillars that were fed an artificial diet containing essential oil at concentrations equivalent to the LC₅₀ and LC₂₀, an average increase in larval stage duration of up to 23% was observed

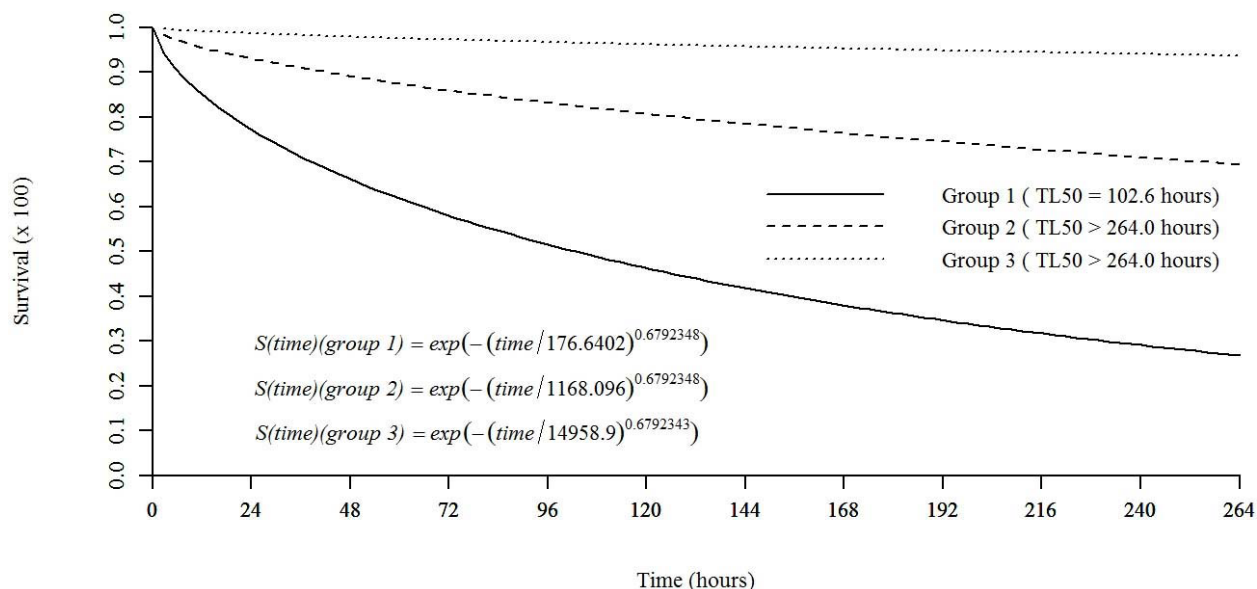


Figure 2: Survival curve for *Spodoptera frugiperda* caterpillars fed artificial diet containing different concentrations of the *Cymbopogon flexuosus* essential oil. Where: $S(t) = \exp(-(time/\delta)^\alpha)$; δ = skewness parameter; α = scale parameter. With: Group 1 = diet with *C. flexuosus* essential oil added at the concentration equivalent to the LC₅₀ (1.35 mg mL⁻¹ diet); Group 2 = diet with *C. flexuosus* essential oil added at the concentration equivalent to the LC₂₀ (0.675 mg mL⁻¹ diet); Group 3 = control diets with water and food coloring agent added and with aqueous 0.01 g mL⁻¹ Tween® 80 and dye added.

in relation to that of the controls. However, for pupae weights, no significant difference was detected among treatments (Table 2).

This effect was also observed in other studies in which the action of botanical insecticides was evaluated on lepidopteran pests (Ansante et al., 2015; Cruz et al., 2016; Hummelbrunner; Isman, 2001; Kaleeswaran et al., 2018). With regard to integrated pest management, the increase in the larval period observed with sublethal doses of insecticidal plants may be an important strategy when associated with biological control, for example.

The increase in FAW larval phase duration did not lead to a reduction in pupal weight. This finding, combined with the rapid mortality, suggested a neurotoxic effect of the essential oil. The requirement for caterpillars to feed for a longer period may be associated with the higher energy expenditure required by an insect to metabolize the toxic compounds in the essential oil.

Toxicity to citral for FAW

Citral, the major constituent of the *C. flexuosus* essential oil, when evaluated at concentrations equivalent to those estimated in the quantitative chromatographic analysis of the essential oil, showed insecticidal activity for *S. frugiperda* caterpillars that was statistically equal to that of the *C. flexuosus* essential oil. Based on survival analysis, four congener groups were formed. The first group was formed by treatments corresponding to the LC_{90} of the LEO (2.053 mg mL⁻¹) and the LC_{90} of citral (1.58 mg mL⁻¹) with an LT_{50} of 44.5 h and a cumulative mortality of 93%. Similarly, the treatments with the LC_{50} of the LEO (1.35 mg mL⁻¹) and the LC_{50} of citral (1.042 mg mL⁻¹) formed the second group, with an LT_{50} of 120.5 h and accumulated

mortality of 71%. The third group consisted of treatments with concentrations equivalent to the LC_{20} values of the LEO (0.675 mg mL⁻¹) and citral (0.521 mg mL⁻¹), with an LT_{50} greater than 264 h and accumulated survival of 69.9%. The two controls, diet with water and food coloring agent added and diet with aqueous solution Tween® 80 added, formed the fourth group with a cumulative survival of 98.33% (Figure 3).

Essential oils generally show greater insecticidal activity than that of any of the components in their chemical constitution. However, this activity may be linked to synergistic or antagonistic interactions among the structural components of an essential oil in which more than one active constituent is responsible for bioactivity (Akhtar et al., 2012; Heshmati Afshar et al., 2017; Jiang et al., 2009).

However, in the present study, the insecticidal activity of the LEO was statistically equal to that of its major constituent (citral). Based on this result, the toxicity of this essential oil was due to the action of citral. This result of the current study is similar to that found by Tak, Jovel and Isman (2016) when evaluating the insecticidal activity of *Cymbopogon citratus* Stapf. (Poaceae) essential oil and its major constituents citral and limonene for *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae). These authors verified that the citral toxicity was similar to that of the essential oil and that the insecticidal activity was attributed to this constituent. Similar results were also observed when the toxicity of *Lippia alba* (Mill) N. E. Brown essential oil was evaluated for *S. frugiperda* caterpillars, and the insecticidal property of this plant was also attributed to citral (Niculau et al., 2013).

Table 2: Duration of the larval stage (average \pm standard error) of *Spodoptera frugiperda* when 48-h-old larvae were fed an artificial diet containing *Cymbopogon flexuosus* essential oil.

Treatment	Larval period (days)	Pupa weight (mg) ^{ns}
Water + Dye	16.90 \pm 0.16 a	243.83 \pm 2.25
Water + Dye + 1% Tween® 80	17.32 \pm 0.14 a	243.12 \pm 2.75
<i>C. flexuosus</i> – 0.675 mg mL ⁻¹ diet	18.36 \pm 0.37 b	242.17 \pm 3.32
<i>C. flexuosus</i> – 1.35 mg mL ⁻¹ diet	20.85 \pm 0.72 c	241.81 \pm 4.25
p \leq 0.05	0.0000	0.968
F	24.472	0.0855
df	3	3

Averages followed by the same letter in the column do not differ by the Scott-Knott test ($p \leq 0.05\%$).

^{ns}Values were not significantly different.

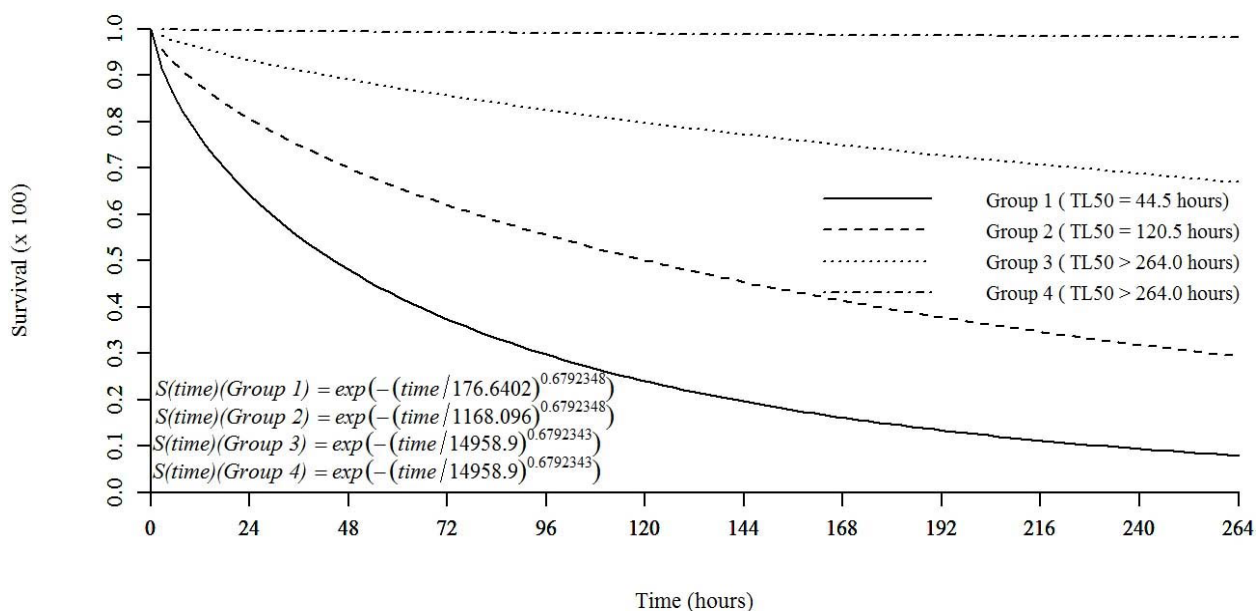


Figure 3: Survival of *Spodoptera frugiperda* caterpillars after exposure to artificial diet containing *Cymbopogon flexuosus* essential oil and its pure major constituent (citral) at different concentrations. Where: $S(t) = \exp(-(\text{time}/\delta)^\alpha)$; δ = skewness parameter; α = scale parameter. Group 1: LC_{90} (2.053 mg mL⁻¹) of *C. flexuosus* essential oil and LC_{90} (1.58 mg mL⁻¹) of citral. Group 2: LC_{50} (1.35 mg mL⁻¹) of *C. flexuosus* essential oil and LC_{50} (1.042 mg mL⁻¹) of citral. Group 3: LC_{20} (0.675 mg mL⁻¹) of *C. flexuosus* essential oil and LC_{20} (0.521 mg mL⁻¹) of citral. Group 4: Controls, diet with water and food coloring agent added and diet with aqueous 0.01 g mL⁻¹ Tween® 80 and dye added.

Although some research has been conducted, the mode of action of citral in insects is not well elucidated. Citral can reversibly inhibit purified AChE from the brain of *Galleria mellonella* L. (Lepidoptera: Pyralidae) (Keane; Ryan, 1999) and also promotes a similar neurophysiological effect on the neuromodulator, octopamine, in cockroaches (Price; Berry, 2006). However, to date, no assays have been performed that evaluate the inhibition of AChE purified from the brain of *S. frugiperda* by citral. Although the amino acid sequences in the AChEs of insects are well conserved, evolutionarily intraspecific differences in the amino acid residues are verified (Pang et al., 2009). Thus, because of the rapid mortality observed in the present study, only that citral acts on the nervous system of *S. frugiperda* can be suggested.

Inhibition of enzymatic activity of the AChE

When AChE was used from electric eel (*E. electricus*), which is commonly used as a standard for seeking substances that inhibit AChE in the nervous system of humans, particularly for the treatment of neurodegenerative diseases (Bozkurt et al., 2017; Mesquita et al., 2018) and pesticide monitoring (Assis et al., 2012;

Ahmed et al., 2012), citral and LEO caused 50% inhibition of enzyme activity at concentrations 405 and 654-fold higher, respectively, than those required to cause the same inhibition value as the positive control, methomyl commercial insecticide (Table 3). These values suggest low toxicity for non-target organisms.

This finding did not exclude the possibility that the mode of action of citral in *S. frugiperda* occurred through the inhibition of AChE, as verified in a study in which purified AChE was used from the brain of another lepidopteran species (Keane; Ryan, 1999). This AChE was employed because mammalian AChE differs from that found in insects by an amino acid residue, known as the insect-specific cysteine residue (Jankowska et al., 2018). Few studies compare the inhibition of AChE in vertebrates with that in insects; however, differences are detected (Picollo et al., 2008; Jankowska et al., 2018).

Field and semi-field studies must be conducted to assess whether the same pattern of results obtained in laboratory studies is maintained in the field, both for *S. frugiperda* and for other insects and food crops. This report is the first showing the use of LEO to control *S. frugiperda*.

Table 3: Percent inhibition of the acetylcholinesterase enzyme for *C. flexuosus* essential oil and citral.

Concentration (mg.mL ⁻¹)	% Inhibition ± SD		% Inhibition ± SD
	<i>C. flexuosus</i>	Citral	Control (methomyl)
0.01	3.88 ± 2.14	3.70 ± 0.51	73.64 ± 0.23
0.02	5.81 ± 0.91	4.11 ± 0.99	79.86 ± 0.12
0.04	6.41 ± 1.11	7.05 ± 0.28	90.09 ± 0.25
0.07	10.48 ± 1.57	8.55 ± 0.24	98.35 ± 0.18
0.14	18.71 ± 0.05	16.08 ± 0.86	99.89 ± 0.37
0.28	25.59 ± 0.67	31.06 ± 0.52	99.99 ± 0.09
0.56	40.22 ± 0.15	51.57 ± 0.77	99.99 ± 0.07
1.12	61.30 ± 0.21	64.40 ± 0.36	99.99 ± 0.05
2.24	74.36 ± 0.37	75.57 ± 0.56	99.99 ± 0.05

SD: Standard deviation.

CONCLUSIONS

The *C. flexuosus* essential oil caused high mortality for *S. frugiperda* caterpillars. To the best of our knowledge, this study is the first to investigate the insecticidal activity of *C. flexuosus* essential oil for *S. frugiperda*. When the major constituent (citral) was evaluated for toxicity to *S. frugiperda*, citral was affirmed as the monoterpene responsible for the insecticidal activity. The high IC₅₀ values required to cause inhibition of AChE indicated selectivity for non-target organisms. Thus, because of the rapid mortality observed in the present study, citral possibly acted on the nervous system of *S. frugiperda*. Based on the results of this study, citral and *C. flexuosus* have promise for use in the development of new products for the control of *S. frugiperda*.

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