

Bioactivity of 1-octacosanol from *Senna crotalarioides* (Fabaceae: Caesalpinioideae) to control *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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Abstract

Spodoptera frugiperda J. E. Smith (Lepidoptera: Noctuidae) (fall armyworm) is a pest native to the Americas that affects a variety of crops. Its control is based on chemical insecticides. However, this practice has been associated with changes in the susceptibility of pests to various insecticides. The use of plant products represents an eco-friendly alternative. The objective of this work was to evaluate the larvicidal activity of the chloroform extract of *Senna crotalarioides* (Kunth) H.S. Irwin & Barneby (Fabaceae) to control *S. frugiperda*. The chloroform extract of *S. crotalarioides* caused significant larval mortality, and reduced pupal weight and adult emergence. The analysis by gas chromatography coupled with mass spectrometry (GC-MS) revealed the presence of 22 compounds in the chloroform extract of *S. crotalarioides* leaves, with the straight-chain aliphatic fatty alcohol 1-octacosanol as the main component. This study revealed that the leaves of *S. crotalarioides* synthesize long chain alcohols, which increased the mortality of *S. frugiperda* in its larval stage, including the pupal stage. The extract also caused a decrease in the *S. frugiperda* pupal weight. The potential use of the chloroform extract obtained from *S. crotalarioides* and its principal chemical constituent is proposed as a promising alternative to control *S. frugiperda*.

Key Words: botanical; fall armyworm; insecticide; management

Resumen

Spodoptera frugiperda J. E. Smith (Lepidoptera: Noctuidae) es una plaga nativa del continente Americano que afecta a muchos cultivos. Para su control, se emplean insecticidas químicos sintéticos. Sin embargo, esta práctica se ha asociado con la generación de resistencia del insecto a estos productos. El uso de productos botánicos representa una alternativa eco amigable. El objetivo de este trabajo fue evaluar la actividad larvicida del extracto clorofórmico de *Senna crotalarioides* (Kunth) H.S. Irwin & Barneby (Fabaceae) para controlar *S. frugiperda*. El extracto ocasionó mortalidad significativa de la larva. También se observó reducción de peso pupal y de emergencia de adultos. El análisis mediante cromatografía de gases acoplada a espectrometría de masas (GC-MS) reveló la presencia de 22 compuestos en el extracto clorofórmico de las hojas de *S. crotalarioides*, siendo el alcohol alifático de cadena lineal 1-octacosanol el componente mayoritario. Este estudio reveló que las hojas de *S. crotalarioides* sintetizan alcoholes de cadenas largas, los cuales ocasionan un incremento en la mortalidad en el estado larval e incluso en el estado pupal de *S. frugiperda*. También el extracto indujo la disminución del peso pupal. El uso potencial del extracto clorofórmico obtenido de *S. crotalarioides* así como de su compuesto mayoritario para controlar *S. frugiperda* se plantea como alternativas promisorias.

Palabras Clave: botánico; gusano cogollero de maíz; insecticida; manejo

The genus *Spodoptera* (Lepidoptera: Noctuidae) includes some of the most important insect pests that cause significant yield reductions and economic losses in the American and African continents (Aragón et al. 2011; Igyuve et al. 2018). *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae), commonly known as “gusano cogollero del maíz” (Spanish), fall armyworm, corn leafworm, and southern grass-

worm, is a highly polyphagous pest that affects more than 180 crops, among which the following stand out for their importance in Western Hemisphere countries: *Arachis hypogaea* L. (peanut) (Fabaceae), *Glycine max* L. Merrill (soybean) (Fabaceae), *Gossypium hirsutum* L. (upland cotton) (Malvaceae), *Linum usitatissimum* L. (linseed) (Linaceae), *Medicago sativa* L. (alfalfa) (Fabaceae), *Oryza sativa* L. (Asian

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rice) (Poaceae), *Phaseolus vulgaris* L. (common bean) (Fabaceae), *Saccharum officinarum* L. (sugar cane) (Poaceae), *Solanum lycopersicon* L. (tomato) (Solanaceae), *Solanum tuberosum* L. (potato) (Solanaceae), *Sorghum bicolor* L. Moench (sorghum) (Poaceae), and *Zea mays* L. (maize) (Poaceae) (Hernández-Mendoza et al. 2008; Casmuz et al. 2010). In the case of maize, the larvae of *S. frugiperda* cause damage at all growth stages, including senescence (Rodríguez-del-Bosque et al. 2011). The presence of this indigenous insect in the Americas also has been reported in African cornfields (Goergen et al. 2016). Many yr ago, the control of *Spodoptera* species had been based on the use of conventional synthetic insecticides (approximately 3,000 tons of active ingredient per yr) (Blanco et al. 2014). However, the intense and non-rational use of these products has been associated with a strong selection pressure on insects, genetic variability (Pérez-Zubiri et al. 2016), and the development of insecticide resistance (León-García et al. 2012). This phenomenon limits the success of pest control in many countries. In addition, there is evidence of human intoxication due to exposure to the insecticides used in the management of *Spodoptera* pests (Barrientos-Gutiérrez et al. 2013). Alternative strategies have been proposed to control *S. frugiperda*, including the use of genetically modified crops (Aguirre et al. 2016), natural enemies (Nuñez-Valdez et al. 2008; Ordóñez-García et al. 2015), semiochemicals, and other natural product-based approaches (Guerrero et al. 2014).

The genus *Senna* Mill. (Fabaceae: Caesalpinioideae) comprises more than 350 species (<http://www.theplantlist.org>) of herbs, shrubs, woody climbers, and tree species, unarmed or armed, distributed in a wide range of zones, with different climates and latitudes (Marazzi et al. 2006). Some species of the genus *Senna* are used as foods, ornamental plants, or with medicinal purposes (Mazzio & Soliman 2010), while others have become invasive species (Richardson & Rejmánek 2011; Singhurst et al. 2013), or are considered as noxious woody weed (Parolin 2005). Previous phytochemical studies have allowed the identification of a variety of compounds found in *Senna* spp., including alkaloids (Moo-Puc et al. 2007), anthraquinones (Branco et al. 2011), cardiac glycosides (Essiett & Bassey 2013), glucosides (sennosides) (Monkheang et al. 2011), naphthopyrones (Graham et al. 2004), phenols (Viegas Junior et al. 2013), saponins (Oluwole et al. 2016), triterpenes, (Luximon-Ramma et al. 2002), among others. The metabolic content of *Senna* species has been associated with a variety of biological properties, including insecticidal activity (Yagi et al. 2013; de Souza Tavares et al. 2014; Vasudev et al. 2015). *Senna crotalarioides* (*Cassia crotalarioides* Kunth [Fabaceae]) is an arborous species distributed in various states of Mexico (Estrada et al. 2004). Considering the wide range of secondary metabolites in the genus *Senna* and their insecticidal activity, the objective of this study was to evaluate the insecticidal and insecticidal activities of the chloroform extract of *S. crotalarioides* on the polyphagous lepidopteran *S. frugiperda*.

Materials and Methods

All reagents used in this study were of analytical grade and commercially available. Agar, brewer's yeast, L-ascorbic acid, and neomycin sulfate were purchased from Fisher Scientific (Thermo-Fisher Scientific, Waltham, Massachusetts, USA); acetone, ethanol, formaldehyde, methyl *p*-hydroxybenzoate, 1-octacosanol were from Sigma-Aldrich (St. Louis, Missouri, USA).

PLANT MATERIAL

The aerial parts (leaves, stems, buds, pods, and seeds) of *S. crotalarioides* were collected in Sep 2017, from 10 random specimens in

the locality of Comadres (22.616666°N, 100.400000°W, 1640 masl), a municipality of Guadalcázar (state of San Luis Potosí, Mexico). The specimens of the plant were authenticated, based on macro- and microscopic features of the plant: texture, shape, apex and leaf margin, pods, seeds, stems, and floral structure, by the Biólogo José García-Pérez, Instituto de Investigación en Zonas Desérticas, Universidad Autónoma de San Luis Potosí, San Luis Potosí, state of San Luis Potosí, Mexico. A voucher herbarium is preserved in the collection of the Isidro Palacios Plant Herbarium at the Universidad Autónoma de San Luis Potosí with the code number SPLM43012. The leaves were separated and dried in the shade at 27 ± 2 °C, for 15 d. Subsequently, the dry plant material was milled in a Thomas Model 4 Wiley™ mill to 1.0 mm particle size (Thomas Scientific, Swedesboro, New Jersey, USA). The ground material was placed in a 1 L flask with 500 mL of chloroform, and extracted under reflux conditions at 50 °C. Then, the supernatant was filtered under vacuum through a Büchner funnel (Corning Inc., Corning, New York, USA), and the solvent evaporated until dry under reduced pressure using a BUCHI R-210 rotary evaporator (Büchi, Flawil, Switzerland) to obtain the crude extract.

SPODOPTERA FRUGIPERDA TEST INSECTS AND DIET

The experiments were carried out in the Laboratorio de Compuestos Naturales Insecticidas, Universidad Autónoma de Querétaro, Querétaro, state of Querétaro, Mexico. Larvae of *S. frugiperda* obtained from the University of Querétaro were used throughout the study. The insects had been reared in the laboratory since 2012. Periodically, the population is replaced to avoid inbreeding. The artificial diet used to establish a laboratory population consisted of a mixture prepared with 30 g common bean grains and 90 g maize grains (ground in a Thomas Model 4 Wiley™ mill to 1.0 mm particle size), 20 g brewer's yeast, 10 g vitamin mix Lepidoptera # 722 (calcium pantothenate, crystalline biotin, folic acid, niacin, pyridoxine HCl, riboflavin, sucrose, thiamine HCl, vitamin B12, 1% mannitol) (Bio Serv, Flemington, New Jersey, USA), 17 mL of a 10% w/v ethanol solution of L-ascorbic acid, 2.5 mL formaldehyde, 1.7 g methyl *p*-hydroxybenzoate, and 0.6 g neomycin sulfate, mixed in 800 mL of boiled agar solution (12.5 g per L). Second instar *S. frugiperda* were reared according to the proposed methodology (Capataz-Tafur et al. 2007; Quintana-López et al. 2016) with minimal modifications. Under a laminar flow hood, in 25 mL disposable plastic containers with a lid (Envases Primo Cuevas, Ecatepec de Morelos, state of Mexico, Mexico), 1 larva of the second instar of *S. frugiperda* was deposited, the larva was fed with artificial diet (2–3 g cube), and replaced by a new one each wk until the larva completed its pupal stage. Larvae were maintained at 27 ± 2 °C, $70 \pm 5\%$ relative humidity (RH), and a 14:10 h (L:D) photoperiod, in a climatic chamber with a timer, and revised every third d. When molted to pupae after 24 h, the insects were collected and transferred to another plastic container. The plastic containers were closed with the lid to avoid contamination, and to prevent larvae from escaping until adult emergence. Twenty pupae were placed in each container.

INSECTICIDAL ACTIVITY

Insect culturing and bioassays were run in the same experimental conditions, in a room kept at 27 ± 2 °C, $70 \pm 5\%$ RH, and a 14:10 h (L:D) photoperiod. Preliminary screening was performed by testing 5 logarithmic concentrations ranging from 0.5 to 5,000 ppm. For the final bioassay, the concentrations evaluated were 5,000, 4,000, 2,000, 1,000, and 500 ppm. Polyvinylpyrrolidone was used as co-solvent of distilled water to prepare all chloroform extracts of *S. crotalarioides*. The extracts were mixed with the larval diet ingredients during prepara-

ration. The extract was added when the temperature of the agar solution cooled to hand hot temperature (about 45 °C). Control larval diet was prepared by adding the same volume of distilled water and polyvinylpyrrolidone to the artificial diet. A 2 to 3 g cube of artificial larval diet was placed in each container. The artificial diet was replaced by a new one each wk. Bioassays were carried out using 20 second instars for each concentration and for the control, divided into 5 experimental units with 4 larvae each, selected randomly, distributed in 20 plastic containers with a larva each. The containers were covered with plastic lids and stacked close to each other. The larvae were maintained inside the same plastic containers at 27 ± 2 °C, $70 \pm 5\%$ RH, and a 14:10 h (L:D) photoperiod until reaching the pupal stage. The pupae were weighed 24 h after pupation (mg), and then each pupa was moved to another plastic container ($3 \times 3 \times 3.8$ cm) to allow the development of adults. The insecticidal and insectistatic parameters evaluated were mortality (%) of larval and pupal stages and cumulated, and duration from larva to adult (d). The median lethal concentration (LC_{50}) of the larval population of *S. frugiperda* was calculated by using data from the total larval period.

GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS

Samples of the *S. crotalarioides* extract were dissolved in distilled water. The gas chromatography-mass spectrometry analysis was performed using an Agilent 5973 inert Gas Chromatograph/Mass Spectrometer (Agilent Technologies Inc., Santa Clara, California, USA) equipped with an Agilent HP-5MS fused-silica capillary column (length 30 m; inner diameter 0.250 mm; film thickness 0.25 µm), coated with 5% phenyl-methylsiloxane, at 250 °C. Pure helium was used as a carrier gas with a flow rate of 1 mL per min. Split ratio was 2:1. The column temperature was initially 50 °C (for 3 min) and was gradually increased to 240 °C, at 3 °C min^{-1} ; this temperature was held for 2 min. The injector temperature was 250 °C and 1 L of samples was injected twice. The spectra were collected at 71 eV ionization voltages, and the analyzed mass range was 15 to 600 m per z. The identification of the components was confirmed by comparison of the retention indices with those of authentic compounds using the Kovats index (Kovats 1958), based on n-alkanes C6 to C26, and by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral database. The relative percentage of the individual components in the crude extract from leaves of *S. crotalarioides* was expressed as percentage based on the peak areas obtained.

STATISTICAL ANALYSIS

Data are expressed as the means \pm standard errors of the mean for 4 replicates. Results were excluded from analysis if the mortality rate

in the control samples was above 20%. In addition, if the percentage of larvae killed during each time interval in the control samples ranged between 5 and 20%, the mortality of treated samples was corrected using Abbott's formula (Abbott 1925).

$$\text{Mortality} = [(x - y) / y] \times 100$$

where x = percentage mortality in the treated sample, and y = percentage mortality in the control.

The SYSTAT (vers. 9) analysis program (SYSTAT Software Inc., San Jose, California, USA) (Stein et al. 1997) was used to fit treatment concentration–response, and for calculating the LC_{50} , 95% lower and upper fiducial limits, and chi-square values by Probit analysis. Accumulated mortality at each concentration was expressed as the sum of the percentage of larval mortality plus the percentage of pupal mortality. The differences in the mean values were evaluated by analysis of variance (ANOVA). The Tukey test was used for all pair-wise multiple comparisons of groups.

ChemBioDraw Ultra 13 molecule editor (PerkinElmer, Waltham, Massachusetts, USA) was used for drawing chemical structures.

Results

INSECTICIDAL AND JUVENOMIMETIC ACTIVITIES OF *SENNA CROTALARIOIDES*

The chloroform extract of *S. crotalarioides* caused an increase in *S. frugiperda* mortality ($P < 0.001$) during the development of the insects (Table 1). Comparison of the mean mortality at each concentration with the 0 ppm concentration (control) showed a concentration-dependent effect (Fig. 1).

Higher rates of mortality were obtained when higher concentrations of the chloroform extract were used. From 1,000 ppm and above, the chloroform extract of *S. crotalarioides* significantly affected *S. frugiperda* larvae, hindering their pupation. At a concentration of 2,000 ppm of chloroform extract, 45% of the larvae completed the larval stage, but only 30% were able to pupate and develop into adults. The chloroform extract of *S. crotalarioides* at 4,000 ppm caused 100% accumulated mortality. At the pupal stage, a concentration-dependent effect could not be observed.

The exposure to the chloroform extract of *S. crotalarioides* extends the duration of the larval stage of *S. frugiperda*, including the prepupal period. In particular, larvae exposed to 2,000, 4,000, and 5,000 ppm of chloroform extracts of *S. crotalarioides* took longer to reach the pupal stage compared to the control insects (Table 2). In the pupal stage, the most marked effects were observed when the larvae were exposed to

Table 1. Insecticidal activities of the chloroform extract of *Senna crotalarioides* leaves to control *Spodoptera frugiperda*.

Concentration (ppm)	Mortality (%)		
	Larvae	Pupae	Cumulative
5,000	90.0 \pm 6.9 A	10.0 \pm 6.9 A	100.0 \pm 0.0 A
4,000	80.0 \pm 9.2 A	20.0 \pm 9.2 A	100.0 \pm 0.0 A
2,000	70.0 \pm 10.5 AB	15.0 \pm 8.2 A	85.0 \pm 8.2 A
1,000	55.0 \pm 11.4 AB	15.0 \pm 8.2 A	70.0 \pm 10.5 A
500	35.0 \pm 10.9 BC	0.0 \pm 0.0 A	35.0 \pm 10.9 B
Control	10.0 \pm 6.9 C	0.0 \pm 0.0 A	10.0 \pm 6.9B
LC_{50} [LFL-UFL]	1001.1 [463.2–1410.1 ppm]*		872.7 [689.8–1027.4 ppm]*

Results are the mean value based on 4 determinations \pm standard error of the mean. Means within a column not labeled by the same letter are different.

* LC_{50} values and 95% fiducial limits [in brackets] were determined through Probit analyses of the percent of dead larvae results corrected using Abbott's formula.

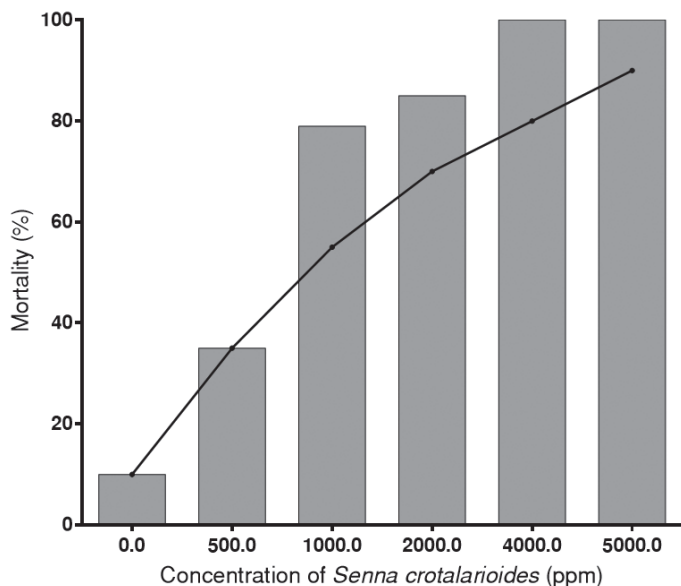


Fig. 1. Effect of *Spodoptera frugiperda* larval treatment fed at the second instar on artificial diet treated with different concentrations of chloroform extract of *Senna crotalarioides*.

4,000 and 5,000 ppm of the extract, observing statistically significant differences when comparing the duration of the stages in relation to the control. On the other hand, exposure of larvae to the chloroform extract at concentrations higher than 1,000 ppm reduced the body weight of the pupae.

GAS CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY ANALYSIS OF THE CHLOROFORM EXTRACT OF *SENNA CROTALARIOIDES*

A total of 22 compounds were identified in the chloroform extract of *S. crotalarioides* leaves using gas chromatography-mass spectrometry, by comparing their retention indices and mass spectra fragmentation patterns with the reference spectra of the National Institute of Standards and Technology library. Among these compounds were fatty acids, terpenes, aldehydes, esters, and primary aliphatic alcohols, which constitute 99.2% of the chemical components present in the extract. The retention times, peak areas (%), and retention indexes of these compounds are presented in Table 3.

Table 2. Insectistatic activities of the chloroform extract of *Senna crotalarioides* leaves to control *Spodoptera frugiperda*.

Concentration (ppm)	Duration of stage (d)		Pupal weight (mg)
	Larva	Pupa	
5,000	56.5 ± 4.5 A	ND	98.0 ± 3.0 C
4,000	50.0 ± 1.2 A	ND	105.8 ± 7.7 C
2,000	31.3 ± 2.4 B	13.7 ± 0.3 A	140.2 ± 10.8 BC
1,000	29.4 ± 0.7 B	11.0 ± 0.4 B	164.5 ± 11.9 B
500	23.7 ± 1.1 C	9.8 ± 0.2 C	207.5 ± 11.2 A
Control	21.8 ± 0.7 C	9.6 ± 0.2 C	229.8 ± 4.6 A

Results are the mean value based on 4 determinations ± standard error of the mean. Groups with the same letter are not significantly different at $P < 0.001$; ND = No data because no adult emerged.

INSECTICIDAL AND JUVENOMIMETIC ACTIVITIES OF 1-OCTACOSANOL

The 1-octacosanol caused an increase in *S. frugiperda* mortality ($P < 0.001$) during the development of the insects (Table 4). Higher rates of mortality were obtained when higher 1-octacosanol concentrations were used. From 1,000 ppm and above, 1-octacosanol significantly affected *S. frugiperda* larvae, hindering their pupation. At a concentration of 1,000 ppm of 1-octacosanol, 35% of the larvae completed the larval stage, but only 15% were able to pupate and develop into adults. At the pupal stage, a dose-dependent effect could not be observed, starting with the lowest concentration tested.

The exposure to 1-octacosanol extends the duration of the larval stage of *S. frugiperda*. In particular, larvae exposed to 400, 600, and 1,000 ppm of 1-octacosanol took longer to reach the pupal stage compared to the control insects of the corresponding treatment (Table 5). In the pupal stage, the most marked effects were observed when the larvae were exposed to 1,000 ppm 1-octacosanol, observing statistically significant differences when comparing the duration of the stage in relation to the control ($P < 0.001$). On the other hand, the exposure of the larvae to 1-octacosanol at concentrations higher than 600 ppm reduced the body weight of the pupae.

Discussion

The insecticidal effect of various *Senna* species has been evaluated to control coleopterans that infest stored grains, and that produce important economic losses and affect their quality and safety. The n-hexane extract of the pods of *Senna italica* Mill. (Fabaceae) caused 100% mortality in adults of *Callosobruchus analis* F. (Coleoptera: Chrysomelidae) (Yagi et al. 2013). The leaf extract from *Senna obtusifolia* (L.) H.S. Irwin & Barneby (Fabaceae) showed repellency activity of class II (between 20.1–40%) to control adults of *Sitophilus zeamais* (Motschulsky) (Coleoptera: Dryophthoridae) (de Souza Tavares et al. 2014). For its part, the ethyl acetate extract of the seeds (EtOAc) and secondary metabolites of *Senna tora* (L.) Roxb. (Fabaceae) (= *Cassia tora*) showed an LT_{50} (h) of 1.080 for EtOAc, 1.743 for compound 1, and 1.687 for compound 2, as well as 20% and 35% oviposition deterrence activity at 200 and 300 $\mu\text{g mL}^{-1}$ with EtOAc, 60%, 75%, and 75% with compound 1, and 60%, 70%, and 70% with compound 2 at 100, 200, and 300 $\mu\text{g mL}^{-1}$, respectively. The antifeedant effects were determined for EtOAc showing less than 5% at 100, 200, and 300 $\mu\text{g mL}^{-1}$, but with compound 1, this activity was 40%, 65%, and 80%, and 55%, 70%, and 75% with compound 2 at the same concentrations to control *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae) (Mbatchou et al. 2018). On the other hand, certain *Senna* spp. contain proteinaceous inhibitors that deregulate proteolytic processes in insects (Vasudev et al. 2015), which induces a larval food stress that can affect the growth rate, development time, body weight, survival (Fürstenberg-Hägg et al. 2013), fecundity, and percentage of larvae hatching (Vasudev et al. 2015). The results of these studies have demonstrated that this genus synthesizes metabolites with potential insecticidal activity. In this work, the insecticidal activity of *S. crotalarioides* was evaluated to control the fall armyworm, considering that Mexico is the fifth largest maize producer, and *S. frugiperda* is the main pest that attacks this crop (Blanco et al. 2014), limiting the yield and productivity (Martínez-Jaime et al. 2018). A preliminary study indicated that *S. crotalarioides* displayed larvicidal activity to *S. frugiperda* (Quintana-López et al. 2016). The chloroform extract of the leaves of *S. crotalarioides* exerts its toxicity at the early instar stage of *S. frugiperda*. The most important toxic action was observed on larvae. The early instar affected is important because *Spodoptera* larvae died in the larval and prepupal period, particularly between the first and second instars (Montezano et al. 2014). This is important considering that the larval

Table 3. Chemical composition of the chloroform extract of *Senna crotalarioides* leaves.

No	Retention time	Name of compound	Total %	Kovats index (KI)*	
				Experimental	Literature
1	13.523	Neophytadiene	1.551	1,774	1,827 ¹
2	13.637	Myristic acid	0.360	1,788	1,778 ²
3	13.961	3,7,11,15-tetramethyl-2-hexadecen-1-ol	0.418	2,045	2,114 ³
4	15.587	Palmitic acid	5.281	1,987	1,955.4 ²
5	16.759	Phytol	0.507	2,086	2,099.1 ²
6	17.087	(9Z,12Z)-Octadecadienoic acid	0.140	2,202	2,150.5 ²
7	17.125	(9E)-Octadecenoic acid	0.075	2,194	2,223 ⁴
8	17.167	α -Linolenic acid, trimethylsilyl ester	0.369	2,210	2,222 ⁵
9	17.333	Stearic acid, trimethylsilyl ester	0.694	2,186	2,245 ⁵
10	20.865	Heptacosane	0.279	2,705	2,700 ⁴
11	21.874	Squalene	0.815	2,914	2,814 ⁴
12	22.256	Tetratetracontane	0.589	4,395	4,400 ⁶
13	22.575	1-Hexacosanol	1.638	2,890	2,935 ⁵
14	23.296	Octacosanal	5.209	2,993	3,016 ⁷
15	24.013	1-Octacosanol	63.245	3,089	3,133 ⁴
16	24.194	α -tocopherol	0.533	3,226	3,131 ⁹
17	24.724	Trimethylsilyl octacosanoate	0.475	3,180	3,229 ¹⁰
18	24.848	Triacontanal	0.857	3,192	3,180 ⁸
19	24.848	Stigmasterol	1.045	2,797	3,269 ⁴
20	25.706	1-Triacontanol	9.472	3,287	3,330 ⁴
21	26.225	β -Sitosterol,	4.549	2,789	3,332 ¹
22	26.871	Stigmast-5-ene, 3 β -(trimethylsiloxy)-, (24S)-	1.095	2,789	3,348 ¹¹

Results are mean of 3 replicates calculated from gas chromatography-mass spectrometry areas; ¹Kovats (1958), ¹Bicchi et al. (1997), ²Babushok et al. (2011), ³Li et al. (1998), ⁴Khannoon et al. (2011), ⁵Ivanov et al. (2018), ⁶Naemi et al. (2014), ⁷Mizuno et al. (2018), ⁸Radulovic et al. (2014), ⁹Tokuda et al. (1988), ¹⁰Harris et al. (2012), ¹¹Ishidorov et al. (2008).

Table 4. Insecticidal activities of 1-octacosanol to control *Spodoptera frugiperda*.

Concentration (ppm)	Mortality (%)		
	Larvae	Pupae	Cumulative mortality
1,000	65.0 \pm 10.9 A	20.0 \pm 9.2 A	85.0 \pm 8.2 A
600	40.0 \pm 11.2 AB	25.0 \pm 9.9 A	65.0 \pm 10.9 AB
400	35.0 \pm 10.9 AB	15.0 \pm 8.2 A	50.0 \pm 11.5 ABC
120	25.0 \pm 9.9 AB	15.0 \pm 8.2 A	40.0 \pm 11. BC
80	20.0 \pm 9.9 B	15.0 \pm 8.2 A	35.0 \pm 10.9 BC
Control	10.0 \pm 6.9 B	10.0 \pm 6.9 A	20.0 \pm 9.2 C
LC ₅₀ [UFL-LFL]	832.2 [732.3–973.9 ppm]*		434.4 [362.0–508.2 ppm]*

Results are the mean value based on 4 determinations \pm standard error of the mean. Means within a column not labeled by the same letter are different.

*LC₅₀ values and 95% fiducial limits [in brackets] were determined through Probit analyses of the percent of dead larvae results corrected using Abbott's formula.

Table 5. Insectistatic activities of 1-octacosanol against *Spodoptera frugiperda*.

Concentration (ppm)	Duration stage (d)		Pupal weight (mg)
	Larval	Pupal	
1,000	37.0 \pm 1.2 A	17.5 \pm 0.50 A	170.3 \pm 10.0 C
600	37.9 \pm 1.63 A	15.0 \pm 0.63 AB	190.6 \pm 7.2 BC
400	31.3 \pm 0.70 B	14.1 \pm 0.55 AB	210.5 \pm 8.3 AB
120	28.6 \pm 0.83 BC	14.8 \pm 0.68 AB	221.0 \pm 6.4 A
80	27.8 \pm 0.40 C	13.5 \pm 0.33 AB	219.0 \pm 5.4 A
Control	23.5 \pm 0.54 D	12.2 \pm 0.78 B	225.9 \pm 5.8 A

Results are the mean value based on 4 determinations \pm standard error of the mean. Different letters indicate significant differences between groups ; $p < 0.001$.

stage of *S. frugiperda* is the stage that causes the greatest damage to crops (Tavares et al. 2013). On the other hand, the exposure to the chloroform extract of the leaves of *S. crotalarioides* extends the duration of the larval stage. This response has been described as a compensatory action for the larvae to recover when feeding on a low-quality host and still be able to pupate and achieve a greater weight (Silva et al. 2017).

Analysis by gas chromatography-mass spectrometry allowed the identification of various chemical compounds within the chloroform extract of the leaves of *S. crotalarioides*. Major chemical constituents were 1-octacosanol ($C_{28}H_{58}O$) (63.245%, Rt 23.296 min), a primary 28 carbon atom saturated alcohol (Fig. 2), followed by 1-triacontanol ($C_{30}H_{62}O$) (9.472%, Rt 25.706 min), palmitic acid ($C_{16}H_{32}O_2$) (5.281%, Rt 15.587 min), and octacosanal (5.209%, Rt 23.296 min). Other identified components appeared at a proportion of less than 5%. The lowest percentage content of peak area (0.075%) was for (9E)-octadecenoic acid (Rt 17.125 min). Some components identified by gas chromatography-mass spectrometry in *S. crotalarioides* chloroform extract, such as phytol, tetracontane, squalene, α -tocopherol, triacontanol, octadecadienoic acid, and stigmasterol, also have been identified in the species *S. italica* and *Senna* spp. (Gololo et al. 2016; Silva et al. 2016; Madkour et al. 2017).

Exposure to 1-octacosanol, the major component of the chloroform extract of *S. crotalarioides*, increased mortality of the *S. frugiperda* in the larval stage, including the pupal stage. Also, this C28-chain alcohol caused a decrease in the body weight of *S. frugiperda* pupae. Although there are no data on the insecticidal activity of 1-octacosanol, previous studies indicate that some long-chain alcohols deploy antifeedant activities (Ganassi et al. 2016; Aznar-Fernández et al. 2018), besides ovicide and larvicide activities (Sinniah 1983). The second most abundant compound in the extract (triacontanol) is a plant growth regulator that partially reverses the jasmonic acid-induced proteinase inhibition (Ramanarayan & Swamy 2004). On the other hand, it has been suggested that fatty acids, such as palmitic acid, possess insecticidal activity and inhibit the growth of the related species *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) (Frag et al. 2011). Finally, the C28-aldehyde, octacosanal, suppresses aggressiveness in some insects (Mizuno et al. 2018). The obtained results suggest that the major compounds identified in the chloroform extract of *S. crotalarioides* contribute significantly to the larvicidal and pupicidal activities.

The chloroform extract of *S. crotalarioides* caused significant larval mortality and reduction of the pupal weight and adult emergence in *S. frugiperda*. Chromatographic analysis using gas chromatography-mass spectrometry revealed that the leaves of *S. crotalarioides* synthesize long chain alkanes that increase the mortality of the *S. frugiperda* larval stage, including the pupal stage. The insecticidal and insectistatic evaluation of 1-octacosanol, as the major component of *S. crotalarioides* chloroform extract, is presented for the first time. These results can serve as a starting point for the development of botanical insecticides based on *S. crotalarioides* leaf extracts to be used in integrated pest management, and to reduce the use of synthetic pesticides and their negative effects on the environment.

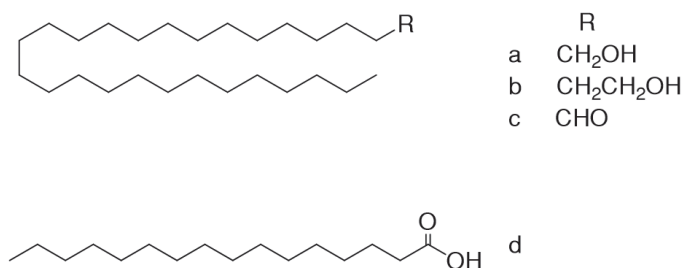


Fig. 2. Structure of the major compounds identified in the chloroform extract of *Senna crotalarioides*: (a) 1-octacosanol, (b) triacontanol, (c) octacosanal, and (d) palmitic acid.

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All mandatory laboratory health and safety procedures were followed at all times during the experiment. The handling of the insects was conducted following the "Recommendations concerning insect handling and insect allergies," <https://www.biology.lu.se/internal/employment/work-environment/insect-handling>.

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