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Activity of Chloroformic Extract from *Salvia connivens* (Lamiales: Lamiaceae) and Its Principal Compounds against *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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Abstract: *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) is one of the most damaging pests in maize crops. In order to manage it, synthetic insecticides such as diamides, neonicotinoids, and pyrethroids are used, but they present a risk for humans and the environment. Investigations of safer alternatives include the use of natural extracts. Thus, this research evaluated the effects of chloroform extract ($CHCl_3Sc$) (5000, 4000, 2000, 1000, and 500 ppm) on aerial parts of *Salvia connivens* and of nonanal and pyrocatechol (1000, 600, 400, and 80 ppm) on *S. frugiperda* mortality, duration of the larva and pupae phases, and pupae weight after 24 h. The second instars of *S. frugiperda* larvae were fed an artificial diet incorporating the extract and compounds. The $CHCl_3Sc$ had insecticidal activity against *S. frugiperda*, showing an LC50 of 1504 ppm. Insectistatic activity began at 1000 ppm, increasing pupal and larval duration in 7.6 and 1.4 days, respectively. Pyrocatechol and nonanal were found in this extract. The first did not have any significant difference in larval or pupal mortalities. On the other hand, insectistatic activity was shown at 500 ppm, increasing the larval duration by 1.7 days compared with the control. In the case of nonanal, the insecticide activity was LC50 of 200 ppm, and insectistatic activity started at 80 ppm, increasing larval duration by 3.2 days compared with the control and reducing pupal weight by 3.4%. The results show that chloroformic extract had insecticidal and insectistatic activities against *S. frugiperda;* nonanal was an aldehyde compound present in this extract, which confers insecticidal and insectistatic activities against this pest.

Keywords: fall armyworm; botanical compounds; nonanal; pyrocatechol; chloroformic extract

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1. Introduction

Fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), is a polyphagous insect pest that causes economic losses and affects approximately 80 different crops [1]. This pest is reported in America, Africa, and Asia [2,3]. For the control of this plague, synthetic insecticides, such as chlorantraniliprole, malathion, and imidacloprid (which belong to

the diamide, organophosphorus, and neonicotinoid chemical groups, respectively [4]), are used but cause environmental damage [5], health hazards, and death [6]. Other concerns related to insecticides include insect resistance and the death of non-target organisms [7]; furthermore, neonicotinoids are related to the reduction in bee populations [8].

One of the alternatives for managing *S. frugiperda* is the use of plant-based (or botanical) products with insecticidal activity, such as essential oils and organic extracts [9]. The genus *Salvia* is the most abundant in the Lamiaceae family, reporting approximately 900 species. *Salvia* plants have been used for medicine, food, cosmetics, and pharmaceuticals [10]. Furthermore, some plant-derived products from *Salvia* species are studied for their potential use as pest management; examples of this include *Salvia hispanica* essential oil against *Spodoptera exigua* (Lepidoptera: Noctuidae) [11]; essential oils from *S. dorisiana*, *S. dolomitica*, and *S. somalensis* against *Aedes albopictus* (Diptera: Culicidae) larvae [12]; and *S. officinalis* essential oil against *Tribolium castaneum* (Coleoptera: Tenebrionidae) adults and *Spodoptera littoralis* larvae [13,14]. *S. sclarea* aqueous extract has shown insecticidal activity against *Trialeurodes vaporiarorum* (Hemiptera: Aleyrodidae) adults [15]; a methanolic extract of *S. leriifolia* mixed with wood vinegar was used against *Lasioderma serricorne* (Coleoptera: Anobiidae) [16]; and an ethanolic extract of *S. sclarea* was tested against *Culex pipiens* (Diptera: Culicidae) larvae [17].

The aim of this study was to evaluate the effect of a chloroformic extract from aerial parts of *S. connivens* and its major components against *S. frugiperda*.

2. Materials and Methods

2.1. Plant Material

Aerial parts (leaves and stems) of *S. connivens* (Figure 1) were collected from the locality Guadalcázar, San Luis Potosí, México (22°39′50.3″ N, 100°24′59.5″ W). Authentication was made by José García-Pérez at the Isidro Palacios Herbarium of the San Luis Potosí Autonomous University (Voucher SLPM 43013).

Figure 1. *Salvia connivens*.

2.2. Preparation of Extracts of Salvia connivens

Dried and powdered aerial parts (200 g leaves and stems) from *S. connivens* were extracted with 1 L of technical grade chloroform (Meyer, Tlahuac, México City, México) under reflux for 4 h. The extract was filtered, and the solvent was removed under reduced pressure using an RV10 basic rotatory evaporator (IKA, Wilmington, NC, USA). The yield of the extract was 5.13% [18].

2.3. Insect Rearing

Larvae of *S. frugiperda* were reared at the Insecticidal Natural Compounds Laboratory of the Autonomous University of Queretaro, State of Queretaro, México. The insects were divided into two groups. The control group was fed an artificial diet according to [19]. Briefly, the diet was prepared by incorporating 90 g of maize grains, 30 g of bean grains, 0.6 g of neomycin sulfate, 1.7 g of sorbic acid (Meyer, Tlahuac, México City, México), 1.7 g of

methyl p-hydroxybenzoate (Sigma-Aldrich, Toluca, State of México, México), 10 g of agar (Meyer, Tlahuac, México City, México), 2.5 mL of formaldehyde (Meyer, Tlahuac, México City, México), 17 mL of ethanol (Meyer, Tlahuac, México City, México), 20 g of brewer's yeast (Meyer, Tlahuac, México City, México), and 800 mL of distilled water (Ecopura, Queretaro, México). The experimental groups were fed the control group diet mixed with different concentrations of chloroformic extract from the aerial parts of *S. connivens*, polyvinylpyrrolidone (Sigma-Aldrich, Toluca, State of México, México), and distilled water. The extract was added to the diet when the temperature was 40 °C. The diet was replaced weekly until the pupal stage. Larvae were maintained in a climatic chamber at 27 ± 2 °C, 70 ± 5% relative humidity (RH), and a 14:10 h light–dark photoperiod.

2.4. Bioassays of $CHCl_3Sc$ on Spodoptera frugiperda

Preliminary evaluation of $CHCl_3Sc$ was carried out at five different logarithmic concentrations (ranging from 0.5 to 5000 ppm) and a negative control (diet only). Test extracts were mixed with the diet and evaluated in groups of 20 L2 larvae of *S. frugiperda*. Based on the results, the concentration-dependent insecticidal and insectistatic activities were measured. For the final bioassay, the concentrations evaluated were 5000, 4000, 2000, 1000, 500, and 0 ppm [20].

One larva of the second instar of *S. frugiperda* was deposited in a 25 mL disposable plastic container with a lid (Envases Primo Cuevas, Ecatepec de Morelos, State of México, México) and fed 3 g of the artificial diet. Pupae were weighed 24 h after formation and then left until the adults emerged. The following parameters were assessed: larval mortality, pupal mortality, larval duration, pupal duration, and weight of pupae at 24 h [21].

2.5. Determination of the Major Compounds of $CHCl_3Sc$

Silica-gel (60) column chromatography (Sigma-Aldrich, Toluca, State of México, México) was used to separate the $CHCl_3Sc$. Hexane (Avantor, PA, USA) was used as the stationary phase, and ethyl acetate was used as the mobile phase. In order to increase polarity, the ethyl acetate (Sigma-Aldrich, Toluca, State of México, México) ratio was changed from 5% to 50%. The fractions were analyzed in thin layer chromatography; similar fractions were mixed. The resulting precipitate was filtered in a Buchner funnel followed by hexane (Sigma-Aldrich, St. Louis, MO, USA) rinsing and clarification with activated carbon (Meyer, Tlahuac, México City, México). Furthermore, the sample was analyzed at the Excellent Analytical Measurement Center in México, part of the Agilent Technologies corporation. The analysis was conducted in an Agilent Infinity 1290 liquid chromatography system coupled with Agilent Q-TOF 6530 high-resolution mass spectrometry (Santa Clara, CA, USA). The conditions of the Agilent Zorbax RRHD (2.1 × 50 mm, 1.8 µm) silica column were 30 °C, 0.4 mL min^{-1} flow rate, and 15 µL injection volume. For the mobile phase, the elution gradients were 90% water (Ecopura, Queretaro, México) and 10% acetonitrile (Sigma-Aldrich, Toluca, State of México, México) for 10 min, then 5% water and 95% acetonitrile for 12 min, and 5% water and 95% acetonitrile for 15 min. In all cases, 1% formic acid (Sigma-Aldrich, Toluca, State of México, México) was added to the water and acetonitrile.

For mass spectrometry (Q-TOF 6530), positive polarity electrospray ionization with a high voltage power supply (ESI) was used. Other equipment parameters were gas temperature, 300 °C; drying gas flow rate, 7 L min^{-1}; nebulizing-gas pressure, 40 psi; jet stream gas flow rate, 11 L min^{-1}; temperature, 325 °C; capillary voltage, 3500 V; and fragmentor voltage, 175 V. A mass analyzer (Q-TOF) was used at a mass-to-charge ratio of 30–700 m z^{-1} and an acquisition rate of 1 spectra per second. Component identification was conducted with "Mass Hunter" qualitative analysis software and "Metlin metabolite" accurate mass spectral library [22].

2.6. Bioassay of $CHCl_3Sc$ Major Compounds on *Spodoptera frugiperda*

Preliminarily, five logarithmic concentrations were evaluated (1000, 100, 10, 1, and 0.1 ppm), along with a negative control (diet only), to estimate the biological activity of the major compounds from $CHCl_3Sc$, taking as reference the highest activity and the first response without activity. Nonanal and pyrocatechol were the main compounds, and different concentrations of each (1000, 600, 400, 120, and 80 ppm) were mixed with the artificial diet [21,22], as well as food-grade polyvinylpyrrolidone (PVP) (Sigma-Aldrich, Toluca, State of México, México) in a 2:1 weight/volume ratio (PVP:main compound) to obtain an emulsion.

The diet was mixed with each concentration, and a 3 g cube was deposited inside a 25 mL disposable plastic container with a lid containing one L2 larva. Five larvae were used as the experimental unit for each treatment, with four repetitions. The conditions and measured variables were the same as previously described.

2.7. Statistical Analysis

Data were tested for homoscedasticity and normality. The non-parametric Kruskal–Wallis analysis of variance (ANOVA) and means comparison (Steel–Dwass) tests were used when normality could not be corrected using transformations. Larvae, pupae, and adult mortality were transformed to Bliss degrees. Analysis of variance and Tukey test ($p = 0.05$) were used to determine differences between the control and the treatments. Lethal concentration fifty (LC_{50}) was calculated for each treatment by Probit analysis. Data analyses were conducted using SYSTAT 9 software (SPSS Inc., Chicago, IL, USA) [23].

3. Results

3.1. Effect of *S. connivens* Extract on *S. frugiperda*

Larval mortality followed a dose-response curve ($p < 0.0001$), with a mortality of 55, 70, 80, and 90% at 1000, 2000, 4000, and 5000 ppm, respectively, and LC_{50} was 1504.03 ppm. There was a positive correlation ($p < 0.05$) between the larval mortality and the increase in the concentration of $CHCl_3Sc$. There were differences between treatments in pupal mortality (Table 1). There was a statistically significantly ($p < 0.01$) longer larval duration at 7.6, 9.5, 28.2, and 34.7 days at concentrations of 1000, 2000, 4000, and 5000 ppm, respectively, compared with the control (21.8 days). The period of pupal phase was significantly higher at 1.6 and 4.1 days at 1000 and 2000 ppm compared with the control (9.6 days). The results (Table 1) showed a positive correlation between larval ($p < 0.05$) and pupal ($p < 0.05$) phase duration and increased concentrations of $CHCl_3Sc$. Moreover, pupal weight was negatively correlated ($p < 0.05$) with the concentration of $CHCl_3Sc$. Decreases in pupal weights by 39, 54, and 57.4% were observed at $CHCl_3Sc$ concentrations of 2000, 4000, and 5000 ppm, respectively, compared with the control (229.8 mg).

Table 1. Insecticide and insectistatic activities from chloroform extract of aerial parts of *Salvia connivens* against *Spodoptera frugiperda*.

Treatment (ppm)	Mortality %		Duration (days)		Pupal Weight (mg)
	Larvae	Pupae	Larvae	Pupae	
5000	90 ± 6.9^{a}	10 ± 6.9^{a}	56.5 ± 4.5^{a}	ND	98 ± 3^{a}
4000	80 ± 9.2^{a}	20 ± 9.2^{a}	50 ± 1.2^{a}	ND	105.8 ± 7.7^{a}
2000	70 ± 10.5^{ab}	15 ± 8.2^{a}	31.3 ± 2.4^{b}	13.7 ± 0.3^{a}	140.2 ± 10.8^{b}
1000	55 ± 11.4^{ab}	15 ± 8.2^{a}	29.4 ± 0.7^{b}	11 ± 0.4^{b}	164.5 ± 11.9^{bc}
500	35 ± 10.9^{bc}	0 ± 0^{a}	23.7 ± 1.1^{c}	9.8 ± 0.2^{c}	207.5 ± 11.2^{bc}
0	10 ± 6.9^{c}	0 ± 0^{a}	21.8 ± 0.7^{c}	9.6 ± 0.2^{c}	229.8 ± 4.6^{c}
LC_{50}	1504.03 (893.5–2114.6) ppm				

Average values (±SE) followed by the same letter are not significantly different ($p < 0.05$, Tukey's test). ND, no data because all individuals presented mortality of 100%.

3.2. Identification of the Major Components of the Chloroform Extract of Aerial Parts of S. connivens

Seventeen fractions were separated from the chloroformic extract of *S. connivens* in the thin layer chromatography column. A light-green solid was formed in the 11th and 12th fractions. The liquid fractions were mixed and analyzed. The peak intensities in the chromatogram, mass spectrometry plot, and isotopic profile MFG, showed eight possible compounds (Figure 2, Table 2) at 10.053, 10.168, 11.933, 12.759, 12.760, 12.762, 13.566, and 14.836 min, corresponding to 6E-Octen-2,4-dinoic acid (C_8H_6O), S-2-Hidroxyglutarate ($C_5H_8O_5$), glutaral ($C_5H_8O_2$), 2-octenedioic acid ($C_8H_{12}O_4$), 3-hydroxyphenylglycol ($C_8H_{10}O_3$), pyrocatechol ($C_6H_6O_2$) (Figure 3), nonanal ($C_9H_{18}O$) (Figure 4), and indanone (C_9H_8O).

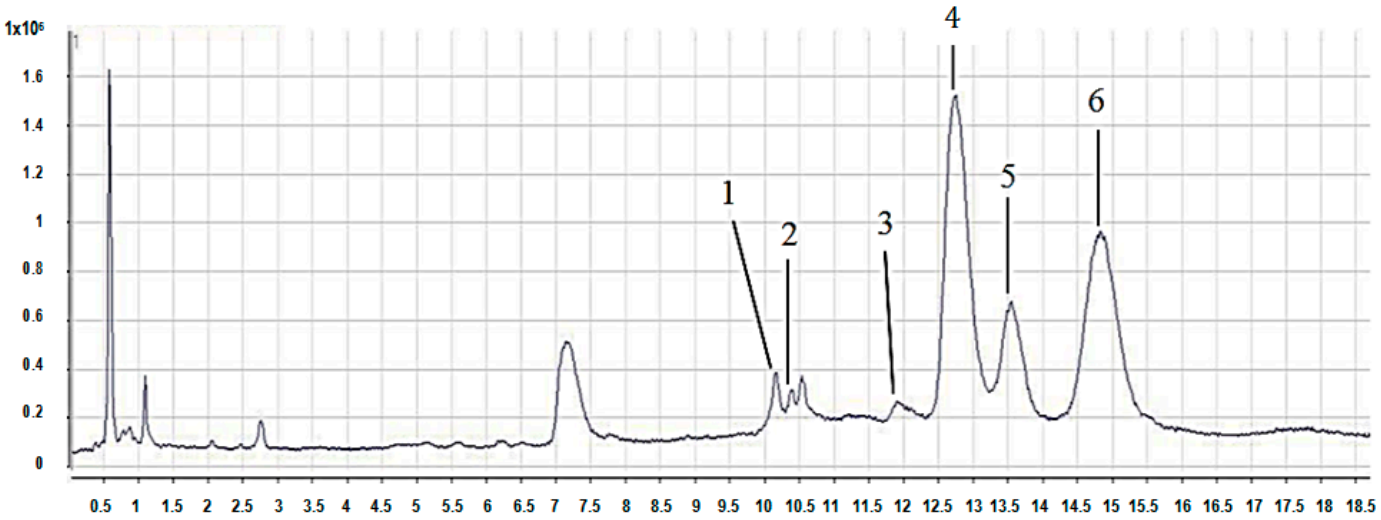

Figure 2. Chromatogram from the 11th and 12th fractions from the chloroformic extract of aerial parts of *Salvia connivens.* The "X axis is the area under the curve; the "Y" axis is retention time.

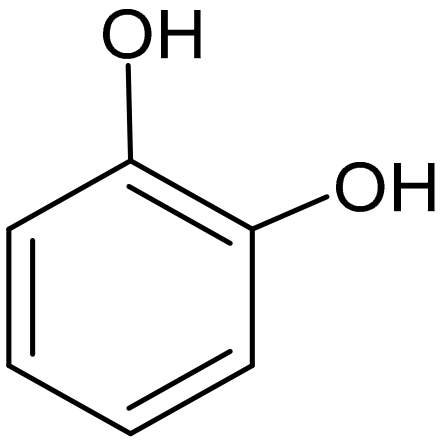

Figure 3. Pyrocatechol.

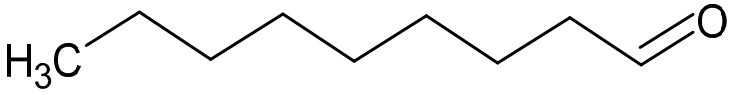

Figure 4. Nonanal.

Table 2. Main compounds in the 11th and 12th fractions of the chloroformic extract of aerial parts of *Salvia connivens*.

No.	Name	Formula	Mass	Mass (MFG)	RT (min)	DB	MFG	No. of Isomers
1	6E-Octen-2,4-dinoic acid	$C_8H_6O_2$	134.03	134.03	10.05	97.83	97.84	6
2	S-2-Hidroxyglutarate	$C_5H_8O_5$	148.03	148.03	10.16	57.70	57.72	2
3	Glutaral	$C_5H_8O_2$	100.05	100.05	11.93	99.76	99.76	2
4	Pyrocatechol	$C_6H_6O_2$	110.03	110.03	12.76	95.06	95.06	4
5	Nonanal	$C_9H_{18}O$	142.13	142.13	13.56	96.88	96.88	1
6	Indanone	C_9H_8O	132.05		14.83	90.42		

RT: retention time; DB: correlation percentage compared with the Metlin database; MFG: correlation percentage compared with the MFG database.

Nonanal was selected because it was the only compound with only one isomer. Pyrocatechol had four isomers; however, it was selected because of its abundance and commercial availability. In contrast, 3-hydroxyphenyl glycol was not selected because it had four isomers and was not commercially available. Indanone, even with a peak in the chromatogram, was not selected because the MFG and number of isomer data were not found, making it difficult to ensure its identification.

3.3. Biological Response from Pyrocatechol against S. frugiperda

There was no significant difference ($p < 0.05$) between larval and pupal mortality compared with the control (Table 3). However, there was a significant difference between the treatments and the control in larval duration. The larval duration was 1.7, 2.6, 2.7, 2.6, and 4.9 days longer at 80, 120, 400, 600, and 1000 ppm, respectively, compared with the control ($p < 0.0001$). The pupal duration was increased by 1.5 and 1.6 days at 600 and 1000 ppm, respectively, compared with the control ($p < 0.0001$). Regarding pupal weight, we observed a positive correlation ($p < 0.05$) between the pupae weight and concentration of pyrocatechol; the data show increases of 6.3, 6.4, 6.6, 11.8, and 14% at 80, 120, 400, 600, and 1000 ppm compared with the control ($p < 0.0001$), suggesting that this compound has a fagostimulant activity.

Table 3. Insecticide and insectistatic activity from pyrocatechol against *Spodoptera frugiperda*.

Treatment (ppm)	Mortality %		Duration (days)		Pupal Weight (mg)
	Larvae	Pupae	Larvae	Pupae	
1000	15 ± 8.2^{a}	25 ± 9.9^{a}	30.2 ± 0.2^{a}	15.2 ± 0.3^{a}	246.8 ± 0.7^{a}
600	10 ± 6.9^{a}	20 ± 9.2^{a}	27.9 ± 0.5^{b}	15.1 ± 0.3^{ab}	242.1 ± 0.4^{b}
400	10 ± 6.9^{a}	20 ± 9.2^{a}	28.0 ± 0.5^{b}	14.60 ± 0.4^{abc}	230.8 ± 0.4^{c}
120	5 ± 5^{a}	20 ± 9.2^{a}	27.9 ± 0.2^{b}	14.1 ± 0.2^{bc}	230.5 ± 0.4^{c}
80	5 ± 5.00^{a}	15 ± 8.2^{a}	27 ± 0.2^{b}	14.1 ± 0.2^{bc}	230.1 ± 0.3^{c}
0	10 ± 6.9^{a}	10 ± 6.9^{a}	25.3 ± 0.3^{c}	13.6 ± 0.3^{c}	216.5 ± 0.5^{d}
LC_{50}	3339.2 (ND) ppm				

Average values (± SE) followed by the same letter are not significantly different ($p < 0.05$, Tukey's test).

3.4. Biological Response from Nonanal against S. frugiperda

The insecticidal effect of nonanal against *S. frugiperda* began at 80 ppm and was increased significantly ($p < 0.0001$) by 65, 70, 70, and y 90% at 120, 400, 600, and 1000 ppm (Table 4); however, pupal mortality was not different between treatments. Regarding insectistatic activity, nonanal increased larval duration in 3.2, 3.9, 5.1, 6.6, and 10.8 days at 80, 120, 400, 600, and 1000 ppm, respectively, compared with the control. However, there were no differences in pupae development. Finally, feeding inhibition was observed with the reduction in pupal weight. A significant negative correlation ($p < 0.0001$) between nonanal concentrations and pupal weight was found with decreases of 3.4, 2.9, 5.8, 8.8, and 24% at 80, 120, 400, 600, and 1000 ppm, respectively, compared with the control.

Table 4. Insecticide and insectistatic activities from nonanal against *Spodoptera frugiperda*.

Treatment (ppm)	Mortality %		Duration (days)		Pupal Weight (mg)
	Larvae	Pupae	Larvae	Pupae	
1000	90 ± 6.9 [a]	10 ± 6.9 [a]	47 ± 1.0 [a]	-	187.5 ± 1.5 [a]
600	70 ± 10.5 [a]	10 ± 6.9 [a]	42.8 ± 0.5 [b]	17 ± 0.6 [a]	225 ± 0.7 [b]
400	70 ± 10.5 [a]	5 ± 5.00 [a]	41.3 ± 0.5 [bc]	16.8 ± 0.7 [a]	232.3 ± 1.2 [b]
120	65 ± 10.9 [a]	5 ± 5.00 [a]	40.1 ± 0.6 [bc]	16.6 ± 0.6 [a]	239.4 ± 0.7 [c]
80	55 ± 11.4 [a]	5 ± 5.00 [a]	39.4 ± 0.5 [c]	16.1 ± 0.5 [a]	238.4 ± 1.1 [d]
0	5 ± 5 [b]	0 ± ND [a]	36.2 ± 0.4 [d]	16 ± 0.4 [a]	246.7 ± 0.6 [e]
LC_{50}	200 (7.9–338.6) ppm				

Average values (±SE) followed by the same letter are not significantly different ($p < 0.05$, Tukey's test).

4. Discussion

Zavala-Sánchez et al. [24] evaluated the insecticidal and insectistatic activities from the chloroformic extracts of the aerial parts of *Salvia ballotiflora*, *S. connivens*, *S. keerlii*, and *S. microphilla*. They found that *S. frugiperda* larvae mortality was higher with *S. microphilla* than with *S. connivens*, *S. keerlii*, and *S.ballotiflora*. With LC_{50} of 916, 936, 1527, and 1685 ppm, respectively, the pupal mortalities were 83.3, 75, 62.5, and 54.2% with *S. microphylla*, *S. connivens*, *S. keerlii*, and *S.ballotiflora*. Insectistatic activity began at 500 ppm with all species; larval phase duration increased by 7.6, 6.5, 5.2, and 2.6 days with the *S. connivens*, *S. microphylla*, *S. ballotiflora*, and *S. keerlii* extracts, respectively. Pupal phase duration increased by 2.3, 2, 1.4, and 1.2 days with *S. keerlii*, *S. microphylla*, *S. connivens*, and *S. ballotiflora*, respectively. Finally, pupal weight was reduced by 16.4, 13.2, 12.3, and 9.8% with *S. keerlii*, *S. ballotiflora*, *S. microphylla*, and *S. connivens*, respectively. The LC_{50} in this work was higher than that reported by previous authors who used the chloroformic extract of the same plant genus, and the insectistatic activity began at 1000 ppm instead of 500 ppm. The higher concentration for insectistatic activity found in our study might be related to the fact that the plant material was collected from the same place but during different years, affecting the quality and abundance of secondary metabolites with insectistatic and insecticidal activity [19]. Despite the higher concentration for insectistatic activity, the results suggest that the chloroformic extracts of *Salvia connivens* have insecticidal and insectistatic activities.

Romo-Asunción et al. [19] evaluated the insecticidal and insectistatic activities of the n-hexane extract of the aerial parts of *S. microphylla* against *S. frugiperda*; the LC_{50} was 456.2 ppm. The larval mortality at 500 ppm was 65%, and pupal mortality was 82.5%. Furthermore, [19] evaluated the insecticidal and insectistatic activities from the chloroformic extracts and found that insectistatic activity began at 500 ppm, increasing the larval phase duration by 2 days and the pupal phase duration by 12.1 days, while pupal weight was reduced by 14.1% compared with the control. Apparently, these species present a higher activity than the present investigation's results, although the different species prevent direct comparisons.

Šućur et al. [15] showed that the aqueous extract from aerial parts of *S. sclarea* at 1000 ppm in adults from *T. vaprariorum* had 56.7% mortality after 120 h. Irfan et al. [25] evaluated the methanolic and aqueous extract of leaves and flowers of *S. officinalis* against *Sitophilus oryzae* (Coleoptera: Curculionidae), obtaining 100, 80, and 60% mortality at concentrations of 15,000, 10,000, and 5000 ppm, respectively, with the methanolic extract of leaves; 60, 20, and 40% at the same concentrations with the methanolic extract from flowers; 60, 40, and 20% at the same concentrations with the aqueous extract of leaves; and 40, 20, and 0% at the same concentrations with the flowers aqueous extract. Therefore, there is evidence that aqueous and organic extracts from some *Salvia* species have insecticidal activity.

In the case of the composition, Baricevic et al. [26] analyzed the chloroformic extract from leaves of *S. officinalis*, obtaining a chromatographic profile with the most pronounced band corresponding to 48% of ursolic acid and 1.5% of carnosol. Alimpić et al. [27] determined the composition from the dichloromethane extract of aerial parts of *Salvia amplexicaulis,* obtaining hyperoside (5.4%), coumarin (2.9%), and Genkwanin 5-o-(6′-o-malonyl-glucoside) as major components. Duletić-Laušević et al. [28] found 18.06% and 7.71% in the dichloromethane extract from aerial parts of *Salvia fruticosa* and *Salvia lanigera* hyperoside, respectively. Furthermore, genkwanin glycosides (1.21%) appeared in the *S. lanigera* extract. Some chloroformic and dichloroformic *Salvia* species extracts contain flavonols, flavones, and polyphenols.

Nonanal has been found to be a constituent of other plants from the *Salvia* genus, such as the essential oils of *Salvia farinacea*, *S. madrensis*, *S. splendens*, *S. leucantha*, and *S. longispicata*, with 6.0, 0.8, 0.8, 0.2, and 0.1%, respectively [29].

There is evidence that nonanal is present as a metabolite in other species from the *Salvia* genus. For instance, 0.1% of the relative concentration percentage of nonanal was detected in *Salvia scabra* essential oil obtained by hydrodistillation in a Clevenger apparatus [30]. Another example of *Salvia* taxa with nonanal in the essential oil is *Salvia argentea,* which contained 0.8% [31]. Bader et al. [32] analyzed the composition of *Salvia samuelssonii* essential oil from two different regions of Jordan: As-Subayhi (sample 1) and Al-Adasiyyah (sample 2). These authors reported nonanal as a compound in both essential oils, with 0.6% in sample 1 and 1% in sample 2.

Pyrocatechol has not been reported in other *Salvia* species. However, pyrocatechol in this species has been reported as pyrocatechol equivalents such as in methanolic extracts obtained by ultrasonic bath of aerial parts from *Salvia ringens*, *S. solaria*, and *S. nemorosa,* which contained 33.19, 26.75, and 21.47 g of dry weight, respectively [33]. Halfon et al. [34] analyzed the total phenolics in pyrocatechol equivalents from the aqueous, ethanolic, and aqueous extracts from *Salvia cassia*, obtaining 44.65, 39.87, and 21.34 mg equivalents per mg of extract.

Pyrocatechol has been reported to have biological activity. Kocacaliskan et al. [35] evaluated the effect of pyrocatechol against plant pathogens that are commonly found in soil. The activity was measured in diameter (mm) of the inhibition zones present on agar medium in Petri plates. In the case of the bacteria *Pseudomonas putida*, *Corynebacterium xerosis*, and *Pseudomonas pyocyanea*, the inhibitions were 31.3, 21.6, and 20.6 mm, respectively, with 0.1 mL from a 10 mM pyrocatechol solution. In contrast, for the fungi *Fusarium oxysporum* and *Penicillium italicum*, the inhibition zones were 29.8 and 26.5 mm, respectively, with the same volume of solution. Ibrahim et al. [36] evaluated the antimicrobial activity of pyrocatechol against *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus cereus*, obtaining minimum inhibitory concentrations of 0.67, 0.67, 1.3, 1.6, and 1.8 mg mL^{-1}, respectively. These results suggest that pyrocatechol has antibacterial and antifungal activities, showing that this compound is efficacious against different types of bacteria.

The research results cited above are consistent with the findings of the current investigation, where pyrocatechol had biological but not insecticidal activity against bacteria and fungi. However, it had insectistatic activity, increasing the durations of the larval and pupal periods and probably stimulating the feeding habits.

Other studies of biological effects include Zhang et al. [37], who reported the total inhibition of mycelial growth of *Penicillium cyclopium*, a phytopathogenic fungi, at 350 ppm. Fernando et al. [38] tested nonanal against *Sclerotinia sclerotiorum* and observed inhibition of the germination of the mycelial plug, suggesting that this molecule has antifungal effects.

Xiu et al. [39] found an attractant effect of nonanal at 10 mg mL^{-1} in adults of *Harmonia axyridis* (Coleoptera: Coccinellidae), a predatory insect used as a biological control against *Myzus persicae* and *Aphis gossypii* (Hemiptera: Aphididae). Galassi et al. [40] studied the response of *Pediculus humanus capitis* (Phthiraptera: Pediculidae) to nonanal, finding that it has an attractant effect at 0.001 mg ml^{-1} but repellency at 10 mg mL^{-1}. Gosset et al. [41]

found that *Solanum tuberosum* emitted green leaf volatile compounds, including nonanal, under the attack of *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) as a defense method; however, it acts as an attractant for predators of this insect.

The studies cited above show that nonanal is part of green leaf volatiles in some plant species, acting mainly as an attractant to some insects (mainly predators) and as a repellent against others. However, to the best of our knowledge, this is the first report of nonanal activity against the fall armyworm.

Finally, the results show that LC_{50} was lower in nonanal (200 ppm), followed by $CHCl_3$Sc extract (1504.03 ppm) and pyrocatechol (3339.3 ppm). This could indicate that nonanal has an integral part in the insecticidal and insectistatic effects of $CHCl_3$Sc extract against *S. frugiperda*.

5. Conclusions

In the present study, the chloroformic extract from aerial parts of *Salvia connivens* had insecticidal and insectistatic activity against *S. frugiperda* larvae. Pyrocatechol had only insectistatic activity. Moreover, nonanal showed both activities against the *S. frugiperda* insect pest.

The study of plant allelochemicals has been the subject of many studies because of the importance of these substances as an alternative pest management method. In this sense, the potential use of chloroformic extract from aerial parts of *S. connivens* could be possible if this plant was cultivated because the extract could be standardized with nonanal as a biological marker.

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