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Biological Activity of Phytochemicals from Agricultural Wastes and Weeds on *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)

Analleli Jiménez-Durán [1], Josefina Barrera-Cortés [1,*], Laura Patricia Lina-García [2], Rosa Santillan [3], Ramón Marcos Soto-Hernández [4], Ana C. Ramos-Valdivia [1], Teresa Ponce-Noyola [1] and Elvira Ríos-Leal [1]

1 Centro de Investigación y de Estudios Avanzados del, Departamento de Biotecnología y Bioingeniería, Instituto Politécnico Nacional (Cinvestav-IPN), Unidad Zacatenco, Ciudad de México 07360, Mexico; anallel.jimenez@cinvestav.mx (A.J.-D.); aramos@cinvestav.mx (A.C.R.-V.); tponce@cinvestav.mx (T.P.-N.); erios@cinvestav.mx (E.R.-L.)
2 Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos, Chamilpa C.P., Cuernavaca Morelos 62209, Mexico; lina_g63@yahoo.com
3 Departamento de Química, Cinvestav-IPN, Unidad Zacatenco, Ciudad de México 07360, Mexico; rsantill@cinvestav.mx
4 Laboratorio de Fitoquímica, Campus Montecillo, Colegio de Postgraduados, Texcoco 56230, Mexico; msoto@colpos.mx
* Correspondence: jbarrera@cinvestav.mx; Tel.: +52-55-5747-3800

Abstract: *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) is a polyphagous insect pest native to America. Due to its capacity for adaptation and migration, it is currently located in Africa, Asia, and Oceania, where it threatens agricultural crops. The ability of *S. frugiperda* to develop resistance to insecticides is one of the reasons for the continuous search for more effective, low-cost, and environmentally friendly control products. In the present work, the insecticidal activity of ethanolic and hexane extracts obtained from fresh and dehydrated leaves of *Piper auritum* Kunth (Piperales: Piperaceae), *Piper umbellatum* L. (Piperales: Piperaceae), and *Cedrela odorata* L. (Sapindales: Meliaceae) was studied against first instar larvae of *S. frugiperda*. The ethanolic extracts of the dehydrated leaves of *C. odorata* and *P. auritum* presented insecticidal activity as high (100% mortality at a concentration of 92 mg/cm^2) as that obtained with the positive control, *Melia azedarach* L. (Sapindales: Meliaceae). The GC-MS analysis of the extracts revealed the presence of phytochemicals classified mainly into the groups of monoterpenes, sesquiterpenes, diterpenes, phenylpropanoids, alcohols, and fatty acids. *P. auritum* grows and propagates rapidly. In addition, due to its low toxicity in mammals and non-target insects, it is a plant with the potential to be used as a botanical insecticide. The exposure of *S. frugiperda* larvae to low concentrations of ethanolic extract of *P. auritum* allowed us to observe their biological activity in the development of this insect. The LC_{50} was 22.1 mg/cm^2. At sublethal concentrations (LC_{21} and LC_{35}) the low fertility of the emerging adults was noticeable.

Keywords: biocontrol; fertility; Meliaceae; phytochemicals; phytolarvicide; Piperaceae

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1. Introduction

Spodoptera frugiperda (J.E. Smith) (Noctuidae: Lepidoptera) is a polyphagous and prolific insect with the ability to adapt to different habitats and migrate [1–3]. Until 2016, *S. frugiperda* was considered an endemic pest of the tropical and subtropical areas of America. However, it is currently found in Africa (48 countries), Asia (18 countries), and Australia, where the hot climate throughout the year has favored its proliferation, threatening agricultural production [4–7] (http://www.fao.org/fall-armyworm/Monitoring-tools/faw-map/en/, accessed on 12 December 2021). Recently, 353 species of host plants of *S. frugiperda* belonging to 76 families were identified, predominantly Poaceae, Asteraceae, and Fabaceae [8,9]. Nonetheless, corn, sorghum, rice, and cotton continue to be the most

attacked crops by this larva [8,10]. The uncontrolled proliferation of *S. frugiperda* has reduced the yield of maize crops by up to 60%, while this number climbs to 100% in tropical areas [2,11,12].

Botanical pest control was one of the first strategies to protect crops [13,14]; however, since 1940, these were rapidly displaced by pesticides due to the high effectiveness of the latter [15,16]. The indiscriminate use of pesticides and their slow degradation rate has favored the development of resistance in pest insects, among which *S. frugiperda* stands out. This fact and the carcinogenic toxic properties detected in some insecticides have been the reason for the continuous search and development of insecticides of low toxicity for humans and which are environmentally friendly, such as bioinsecticides and botanicals [6,17,18]. In general, botanicals are easily degradable products whose complex phytochemical composition favors various modes of action, and, with some exceptions, have low toxicity to non-target organisms. [14,15,18,19]. Examples of toxic phytochemicals are the following: rotenone (lethal dose in humans, 300–500 mg/g), aconitine, and nicotine [20–22].

Numerous plant species have been evaluated to combat *S. frugiperda* [3,23–25]. Rioba and Stevenson (2020) [6] reviewed the potential of 69 plant species, most of them studied in the United States and South America. The biological activity of these plants is diverse, highlighting the prolongation of the larval stage, impaired larval development, larval death, and acute toxicity [14]. The non-specific toxicity of some of these plants, and the lack of information regarding their active components, demands careful evaluation before their application in the control of insect pests.

In the search for Mexican plants with insecticidal properties, Hernández-Carlos and Gamboa-Angulo (2019) [26] identified 85 plant species belonging to 26 families, of which, Asteraceae, Lamiaceae, Meliaceae, Annonaceae, Chenopodiaceae, Fabaceae, and Rutaceae predominated. The plants were evaluated in 20 insects, of which *S. frugiperda* was one of the target insects. These authors identified 43 metabolites with biological activity against the genus *Spodoptera* including terpenes, flavonoids, stilbenes, fatty acids, and some alkaloids. The metabolites with the highest toxicity (LD_{50} < 65 ppm) were isolated from plants of the Meliaceae, Asteraceae, Fabaceae, and Asparagaceae families [27–29].

Piperaceae is a family of plants that includes many species (approx. 3000), most of them within the genus *Piper* (approx. 1500 taxa [30]). Due to their importance in ethnomedicine and traditions, the plants of the *Piper* genus are among the most studied and, currently, more than 667 metabolites with biocidal activity are known [31]. Fungicidal, bactericidal, nematicidal, and acaricidal activities of *Piper* plants have been reported. However, there are few studies on their biological activity on insect pests such as *S. frugiperda* [6,32]. As mentioned above, the use of phytochemical extracts in the control of insect pests has the advantage of different modes of action; since the interaction between pesticides and pests is of a biochemical type, a greater number of target sites reduces the probability of rapid development of resistance in insects [14,18,33].

The present research aimed to evaluate the larvicidal activity of phytochemicals extracted from three plants that grow in the State of Oaxaca, Mexico, on *S. frugiperda*. The plants studied were *Cedrela odorata* L. (Sapindales: Meliaceae), *Piper auritum* Kunth (Piperales: Piperaceae), and *Piper umbellatum* L. (Piperales: Piperaceae). *Melia azedarach* L. (Sapindales: Meliaceae), one of the plants best known for its insecticidal properties and that also grows in the Oaxaca State, was used as a positive control. The *C. odorata* plant was selected because it is a tree grown commercially in Mexico for timber production and a significant fraction of it (branches, bark, and leaves) is discarded. *P. auritum* and *P. umbellatum* plants have a wide variety of properties, including their use as home remedies and as condiments in Central American countries [34–36]. However, due to their extensive proliferation, they have become invasive plants that affect croplands. To the best of our knowledge, the insecticidal activity of *C. odorata*, *P. auritum*, and *P. umbellatum* on *S. frugiperda* has not been reported. As such, the possibility of having large volumes of these plants available, and the possibility of processing them to produce bioinsecticides, provides us the opportunity to contribute to the development of alternative and effective

bioinsecticides to combat *S. frugiperda*, a pest insect with a great capacity to damage extensive hectares of agricultural crops.

2. Materials and Methods

2.1. Plants

Cedrela odorata, *P. umbellatum*, *M. azedarach*, and *P. auritum* were the plants selected for this research, with *M. azedarach* being the positive control. The leaves of the first two species were collected from Santos Reyes Nopala, Oaxaca, a municipality located at an altitude of 474 m (16°06′23″ N, 97°08′39″ W; https://www.los-municipios.mx/municipio-santos-reyes-nopala.html, accessed on 12 December 2021). *Piper auritum* and *M. azedarach* leaves were collected from the municipality of San Pedro Atoyac, Oaxaca, which is located at an altitude of 242 m (16°29′17″ N, 97°59′14″ W; https://www.los-municipios.mx/municipio-san-pedro-atoyac.html, accessed on 12 December 2021). The leaves of the *C. odorata* and *M. azedarach* plants were taken from branches that were removed from the lower part of trees at approximately 10–15 m and 20–25 m, respectively. In the case of the *Piper* plants, the whole leaves of the shrubbery were used. The height of the shrubbery was 1.2 m, approximately, in the case of *P. auritum* and, 0.8–0.9 m in the case of the *P. umbellatum* plants. The family and species of the collected plants were identified by M.Sc. Ernestina Cedillo Portugal, at the Autonomous University of Chapingo, Mexico. Samples of each plant were deposited at Jorge Espinosa Salas herbarium of the same University with the following registration numbers: 25,944 (*P. umbellatum* L.), 25,972 (*P. auritum* Kunth), 25,945 (*M. azedarach* L.), and 25,946 (*C. odorata* L.). Each plant species collected (on 23 March 2018) was divided into two batches for fresh ground leaves (FL) and dried ground leaves (DL) of each one. The leaves were dried at room temperature and then reduced in size to 34 mesh (0.45 mm) with a blender (Oster Model BPST02-B00-013). The fresh leaves were frozen with liquid nitrogen before grinding. The powders obtained from the fresh and dried leaves of each plant were poured into pre-weighed amber vials for storage at −80 °C until use.

2.2. Extraction of Phytochemical Compounds

Phytochemical extraction was conducted in batches of 5 g leaf powder soaked in 30 mL of solvent, either ethanol (Macron Fine Chemicals™, Palo Alto, CA, USA, grade ACS reagent) or hexane (J.T. Baker, 98.5%) contained in a 200 mL beaker [37]. Extraction was carried out for 10 min at 25–30 °C using an ultrasonic homogenizer Hielscher UP200Ht (200 W, 26 kHz) implemented with a S26D7 sonotrode (Hielscher Ultrasonics GmbH, Teltow, Germany). The phytochemicals in the solution were separated by filtration (Whatman filter No. 3) and the extraction was repeated two more times using 30 mL of fresh solvent each time. The filtrates were mixed and concentrated in a rotary evaporator (Büchi model R-124, Flawil, Switzerland). Next, they were poured into pre-weighed amber vials to be stored at −4 °C until use. The dry mass of extracted phytochemicals was determined by the weight difference, and these data were used to calculate the yield of crude extract per dried leaf-mass.

2.3. Analysis by GC-MS of Phytochemicals

The ethanolic and hexanic extracts of the tested plants were filtered (0.2 µm GHP Acrodisc 13 PALL) and analyzed by gas chromatography coupled to mass spectrometry (GC-MS). This was performed (liquid samples of 3 µL) on a PerkinElmer Clarus 580/Clarus SQ8S implemented with a 30 m × 0.32 mm × 0.25 µm Elite-5MS capillary column (PerkinElmer, Waltham, MA, USA) (5% phenyl 95% dimethylpolysiloxane). The temperature program was divided into two ramps after an initial temperature of 100 °C maintained for 3 min; ramp 1: 50 °C/min to 150 °C; ramp 2: 10 °C/min up to 260 °C. Helium was used as the carrier gas at 0.8 mL/min and the ionization energy was 70 eV. The mass range for MS was 40–350 m/z. Major peaks were identified using the NIST database [38].

2.4. Screening of Phytochemical Extracts to Select the One Most Suitable in the Control of S. frugiperda

The larvicidal activity of the crude ethanolic and hexanic extracts obtained from the fresh and dried leaves of each plant species was conducted in vitro by bioassays with first instar larvae of *S. frugiperda*. The larvae were obtained from the Biotechnology Research Center of the Autonomous University of the State of Morelos, Mexico. Bioassays were set up in 24-well polystyrene plates (Mexico Thermo Scientific Nunc; 1 mL of volume per well and the well surface of 1.9/cm^2) half-filled with a sterile synthetic diet (composition per kilogram of diet: agar, 10 g; soy flour, 50 g; corn flour, 96 g; yeast extract, 40 g; wheat germ, 4 g; sorbic acid, 2 g; choline chloride, 2 g; ascorbic acid, 4 g; p-hydroxybenzoic acid methyl ether, 2.5 g; Vanderzant vitamin mix for insects, 15 mL; formaldehyde, 2.5 mL; and ground corn, 20 g).

From each of the four extracts obtained per plant (two ethanolic and two hexane), four solutions of 2 mL each, in concentrations of 5, 10, 50, and 100 µg/µL were prepared, and 35 µL aliquots taken from each solution were poured into each well of the polystyrene plates previously prepared with the gelled synthetic diet. Aliquots with the same distilled water or solvent volumes were added to the negative control experiments; the positive control was the bioassay implemented with the *M. azedarach* extracts. After evaporation of the solvent and water, one *S. frugiperda* first instar larva was placed into each well of the 24-well polystyrene plates (see the experimental design in Figure S1). The 24-well polystyrene plates were covered with plastic film to prevent larvae escape, to which some perforations were made to allow fresh air to enter the wells. Larvae were reared at 25 ± 2 °C, relative humidity of 80 ± 5%, and 18/6 photoperiod. Dead and surviving larvae were recorded every 24 h for seven days [39]. On the seventh day, the weight (OHAUS Explorer brand analytical balance) and size (Vernier; Scale Regla Dial Caliper mm metric) of the surviving larvae were recorded. The bioassays with each of the four extracts obtained from each plant and their solutions were implemented in quadruple with 12 larvae per replicate, 48 larvae per treatment; 3104 larvae were used in this bioassay.

2.5. Exposure of S. frugiperda Larvae to Low Concentrations of the Ethanolic Extract of P. auritum Dehydrated Leaves

This study was conducted with the crude ethanolic extract obtained from dehydrated *P. auritum* leaves, the extract that presented a lethal activity as high as that obtained with the positive control, *M. azedarach*. First instar larvae of *S. frugiperda* were exposed to the selected extract at concentrations of 2, 9, 18, and 37 µg/cm^2. The experimental design was performed in quadruplicate (12 larvae per replicate, 48 larvae per treatment) following the experimental procedure described in the previous section; sterilized water and ethanol were used as controls. The surviving larvae exposed for 7 days to the *P. auritum* ethanolic extract were transferred individually to 200 mL plastic containers to continue their growth with the same gelled artificial diet but free of phytochemicals [40]. The larvae were checked every 24 h for pupation or until mortality occurred. Pupae were placed in 10 cm Petri dishes, on a cotton pad moistened with sterilized tap water. Emerging adults were placed in pairs (male and female) in a 1 L container, lined with filter paper and covered with a cloth to allow air to enter. Adults were fed with 10% sugar water. Eggs deposited on the filter paper were removed from the container and placed in a Petri dish previously prepared with a cotton pad moistened with sterilized tap water. The surviving specimens' development was followed daily and the effects of the *P. auritum* extract on pupae formation (length, weight, and duration) and adult emergence were recorded. This latter activity was conducted by observation under a microscope (Olympus CH30, Japan) at 10× and 20×.

The oviposition activity index (*OAI*) was calculated as follows [41]:

$$OAI = \frac{NT - NC}{NT + NC} \quad (1)$$

where *NT* is the number of eggs in treatments and *NC* is the total number of eggs in the control treatment. The oviposition activity index ranged from −1 to +1, with 0 indicating a neutral response. Positive index values indicate an attractant effect of phytochemical extracts and negative index values indicate a deterrent effect.

2.6. Statistical Analysis

A mixed experimental design (Figure S1) was implemented to study the larvicidal effect of the ethanolic and hexanic extracts obtained from the fresh and dehydrated leaves of the tested plants (*C. odorata*, *P. auritum*, and *P. umbellatum*; *M. azedarach*, positive control) to which *S. frugiperda* was exposed at four different concentrations (92, 184, 921, and 1842 $\mu g/cm^2$). The reported values are the means of the results obtained from four replicates. The effect of the variables studied, and their interactions were corroborated by a mixed-design analysis (mixed-design ANOVA model), and the difference between means was determined by the Bonferroni test for a $p < 0.05$. The normality distribution, homogeneity, and sphericity of experimental data were previously verified to transform data or select any of the corrections suggested for the ANOVA, if necessary. The normal distribution of the dependent data was verified from the Shapiro–Wilk test and the sphericity by Mauchly's test. The sublethal effects of *P. auritum* ethanolic extract in the biological cycle of *S. frugiperda* were determined from an experimental design of one factor at four levels. This study was carried out in a quadruplicate with 12 larvae per replicate; 288 larvae were used in this bioassay. The reported results are the averages of the four replicates. The difference between averages in this test was determined by a one-way ANOVA and the Tukey's HSD test for $p < 0.05$; the normal distribution of the dependent data was verified from the Shapiro–Wilk test. The lethal concentration of *P. auritum* ethanolic extract was determined by the probit method. The Kaplan–Meier model was used to analyze the effect of the *P. auritum* ethanolic extract concentration on the life cycle of *S. frugiperda*. PASW Statistics 18 version 18.0.0 USA (2009) was used for statistical analysis.

3. Results

3.1. Phytochemical Extracts

The ethanol and hexane extract yields obtained from fresh and dehydrated leaves of the four plants tested are presented in Table 1. The mixed ANOVA indicates that there are significant differences in the phytochemical extraction yield due to solvent ($F = 265.063$; $df = 1, 4$; $p < 0.001$), leaf type ($F = 169.736$; $df = 1, 4$; $p < 0.001$) and plant species ($F = 31.762$; $df = 3, 12$; $p < 0.001$). Significant differences were also found in the double and triple interactions of the plant species with the solvent and the leaf type. In general, the yields from dehydrated leaf extracts were higher by an average ratio of 1.7 ± 0.9 ($n = 48$) than those obtained with fresh leaves. As for solvents, ethanol favored the extraction of phytochemicals from the Meliaceae plants by an average ratio of 3.8 ± 1.2 ($n = 24$). In the case of the two Piperaceae plants species, the change in solvent showed no differences, except for the fresh leaves of *P. auritum*, for which twice the number of metabolites (average ratio of 2.2 ± 0.2, $n = 12$) were extracted with ethanol.

Table 1. Yield (% ± SE) of phytochemicals extracted with ethanol and hexane from fresh and dehydrated leaves of Meliaceae and Piperaceae plants.

Solvent	Leaves	Meliaceae		Piperaceae	
		C. odorata	*M. azedarach*	*P. auritum*	*P. umbellatum*
Ethanol	Fresh	6.7 ± 0.4^{b}	6.2 ± 1.3	$3.4 \pm 0.02^{d,e}$	$5.2 \pm 0.5^{b,c,d}$
	Dehydrated	11 ± 0.5^{a}	$4.2 \pm 0.6^{c,d}$	$5.9 \pm 0.9^{b,c}$	6.3 ± 0.7^{b}
Hexane	Fresh	1.3 ± 0.19^{f}	1.4 ± 0.1^{f}	$1.6 \pm 0.1^{d,e}$	$5.2 \pm 0.1^{b,c}$
	Dehydrated	$3.5 \pm 0.2^{d,e}$	$1.7 \pm 0.1^{d,e}$	$5.4 \pm 0.3^{b,c}$	6.5 ± 0.1^{b}

All values are the average % (wte/wtm) of dry matter. SE = standard error; wte = extract weight; wtm = plant dry leaf weight. Three-way ANOVA. Average values (±SE) that do not share an equal letter ($^{a–f}$) are significantly different according to Tukey's test ($\alpha = 0.05$).

The chromatographic program implemented allowed for identifying 30 compounds in the ethanol extracts and 35 in the hexane extracts, where 14 of the 65 compounds were common to both (Tables 2 and 3). The compounds extracted were classified into monoterpenes, sesquiterpenes, diterpenes, triterpenes, phenylpropanoid, alcohols, and fatty acids. Compounds classified into the aldehyde and alkene groups were only extracted with ethanol, while esters and alkanes were extracted with hexane. The 65 compounds listed in Tables 2 and 3 were found in concentrations above 1%.

Table 2. Compounds (≥1%) obtained with ethanol from the dehydrated leaves of the selected plant species.

	% Peak Area *			
Compounds	*Cedrela odorata*	*Melia azedarach*	*Piper auritum*	*Piper umbellatum*
Monoterpenes				
Cis-2,6-Dimethyl-2,6-octadiene	-	-	-	1.29
Menthol	-	1.70	-	-
Sesquiterpenes				
β-cis-caryophyllene	4.70	-	-	-
β-Cubebene	-	-	-	2.49
δ-Cadinene	-	-	-	1.19
β-Sesquiphellandrene	14.19	-	-	-
γ-Muurolene	13.83	-	-	-
β-Bisabolene	6.81	-	-	-
Farnesene	3.40	-	-	-
δ-Elemene	3.17	-	-	-
Farnesol	-	-	1.10	-
Spathulenol	-	-	1.06	1.03
Farnesyl acetate	1.56	-	-	-
Ledol	1.16	-	-	-
Diterpene				
Phytol	-	5.86	2.34	5.93
Triterpene				
Squalene	-	-	1.86	-
Phenylpropanoid				
Safrole	1.80	1.58	32.10	-
Alcohol				
1-Heptadecanol	-	-	4.21	-
1-Hexadecanol	-	-	2.10	-
1-Tetradecanol	-	-	-	1.74
1-Naphthalenol	-	-	-	2.02
Z-2-Dodecenol	3.85	7.92	-	-

Table 2. *Cont.*

	% Peak Area *			
Compounds	***Cedrela odorata***	***Melia azedarach***	***Piper auritum***	***Piper umbellatum***
Aldehyde				
Tetradecanal	-	-	-	5.39
Alkyne				
1-Octadecyne	-	-	3.48	-
Fatty acid				
Dihomo-γ-linolenic acid	-	1.39	-	4.92
n-Hexadecanoic acid	5.04	4.97	-	4.75
Octadecanoic acid	-	-	-	1.71
1-Cyclopentyl-4-n-octyldodecane	-	-	-	1.07
Methyl linoleate	2.67	4.24	-	-
Pentadecanoic acid	1.33	1.39	-	-
Number of peaks (%area)	30 metabolites (77.0%)	13 metabolites (32.7%)	14 metabolites (53.6%)	27 metabolites (44.2%)

* The values are the percentage of each peak concerning the total peaks identified by GC-MS. The table only includes peaks with an area higher than 1%. (-) Averages that the phytochemical compound has not been observed.

Table 3. Compounds (≥1%) obtained with hexane from the dehydrated leaves of the selected plant species.

	% Peak Area *			
Compounds	***Cedrela odorata***	***Melia azedarach***	***Piper auritum***	***Piper umbellatum***
Monoterpenes				
Terpinolene	-	-	1.243	-
Sesquiterpenes				
β-cis-caryophyllene	5.74	-	2.55	2.96
Caryophyllene oxide	1.07	-		3.07
β-Cubebene	13.25	-	1.54	22.43
δ-Elemene	-	-	3.400	-
(-)-β-Elemene	3.71	-	-	-
γ- Elemene	1.11	-	-	-
Farnesol	-	-	1.28	-
Spathulenol	-	-	2.38	-
Farnesyl acetate	-	-	1.15	-
Ledol	1.14	-	-	-
Nerolidol		-	-	5.02
Diterpenoids				
Phytol	10.11	15.94	1.45	3.31
Triterpene				
Squalene	-	-	1.13	-
Phenylpropanoid				
Safrole	1.25	12.52	53.00	2.16
Alcohol				
1-Heptacosanol	-	1.12		-
1-Tetradecanol	-		3.38	-
3,7,11-Trimethyl-1-dodecanol	-	1.31		-
Ester				
(Z,E)-Tetradeca-9,12-dienylacetate	-	1.21		-
(2-dodecen-1-yl) Succinic anhydride	-	-	1.27	-
Ethyl stearate	-	-	-	2.96
Geranyl acetate	-	1.33	-	-

Table 3. *Cont.*

Compounds	% Peak Area *			
	Cedrela odorata	*Melia azedarach*	*Piper auritum*	*Piper umbellatum*
Alkane				
Tetratetracontane	9.25		2.36	3.81
3-Ethyl-5-(2-ethylbutyl) octadecane	3.05	1.40	-	-
4-Methyltridecane	2.49		-	-
Fatty acid				
Linolenic acid	18.11	29.74	-	-
Oleic acid	-	1.83	-	-
Stearic acid	-	1.39	-	4.91
Dihomo-γ-linolenic acid	-	-	-	8.22
Dipalmitin	-	-	-	2.37
Methyl arachidonate	-	-	-	1.69
Methyl linoleate	-	-	1.03	-
Pentadecanoic acid	-	1.09	-	-
Palmitic acid	7.16	17.75	-	-
Ethyl palmitate	-	-	1.64	2.33
Number of peaks (% area)	30 metabolites (88.8%)	18 metabolites (89.9%)	25 metabolites (87.1%)	32 metabolites (87.2%)

* The values are the percentage of each peak concerning the total peaks identified by GC-MS. The table only includes peaks with an area higher than 1%. (-) Averages that the phytochemical compound has not been observed.

Most of the phytochemicals extracted with ethanol were identified in the extracts of *C. odorata* (43%) and *P. umbellatum* (40%). However, major components were only identified in one or two groups. Sesquiterpenes predominated in *C. odorata* at 48.8% and fatty acids at 9%. In *P. umbellatum*, fatty acids, diterpenes (phytol), and aldehydes (tetradecanal) were found at 12.4%, 5.9%, and 5.4%, respectively. Safrole represented 32% of the extract from dehydrated *P. auritum* leaves and it was the major component in the ethanol extract. In *Melia azedarach* (the control plant), phytol, (Z)-2-dodecenol, and fatty acids were found at 6%, 8%, and 12%, respectively.

The comparison of Tables 2 and 3 shows that hexane extracted a more significant number of phytochemicals from the Piperaceae plants and in a higher concentration when compared to the extract concentration obtained with ethanol. In *P. umbellatum*, the β-cubebene concentration increased from 2.5% to 22.4%, and that of dihomo-γ-linolenic acid increased from 4.9% to 8.2%. It is important to highlight the significant increase in safrole, from 32% to 53%, in *P. auritum*, and the increase in the number of sesquiterpenes extracted, from 2 to 6. In *C. odorata*, fewer sesquiterpenes were isolated. However, phytol, linoleic acid, and the group of alkanes were identified and quantified in concentrations of 10.1%, 18.1%, and 14.8%, respectively. These three percentages correspond to 55.5% of the total phytochemicals identified in the hexane extract of *C. odorata*. In the case of *M. azedarach*, hexane extraction yielded phytol, safrole, and fatty acids at concentrations of 17.3%, 12.5%, and 51.8%, respectively. The predominant fatty acids were linolenic acid (29.7%) and palmitic acid (17.7%). The concentrations reported in percentages in Tables 2 and 3 were determined regarding the total area under the curve. Supplementary Figures S2–S5 show the ethanol and hexane extract chromatograms obtained from dehydrated leaves of the plants studied. These figures clearly show the number of compounds and the relative concentrations (area under the peaks) at which they were found in the extracts.

3.2. Screening of Phytochemical Extracts to Select the One Most Suitable for the Control of S. frugiperda

The mixed ANOVA of *S. frugiperda* mortality, by effect of plant species ($F = 349.028$; $df = 3, 13$; $p < 0.001$), leaf type ($F = 66.128$; $df = 1, 15$; $p < 0.001$), and solvent ($F = 1415.061$; $df = 1, 15$; $p < 0.001$), showed significant differences according to the Pillai's trace correction

for $\alpha < 0.05$. The double, triple, and quadruple interactions were significantly different, with a $p < 0.003$ and F values from 5.322 to 95.139, with the following plant-species–leaves and leaves–solvent double interactions the only exceptions. Ethanolic extracts obtained from fresh and dehydrated leaves of the studied plants showed higher activity (average mortality of 76.2 ± 32.6%, n = 160) on *S. frugiperda* larvae than the hexane extracts (average mortality of 48.6 ± 35.9%, n = 160) obtained from the same plants (Figure 1). The ethanolic extracts of *M. azedarach* (the positive control) were the most lethal, with mortality percentages of 100%, even at 92 μg/cm^2, the lowest extract concentration evaluated; the mixed ANOVA of the triple interaction between the plant species, solvent, and the extract concentration was significant with the following statistic parameters: F = 5.322; df = 12, 45; $p < 0.001$. At 920 and 1842 μg/cm^2, the ethanolic extracts of the three tested plants control *S. frugiperda* at 100%. When the ethanolic extract concentration was additionally reduced by one order of magnitude, the lethal activity on *S. frugiperda* of these extracts remained above or close to 50%. Hexane extracts from fresh and dehydrated leaves from the studied plants allowed good control (mortality above 50%) on *S. frugiperda* when supplied at 920 and 1842 μg/cm^2 concentrations. However, at the two lowest concentrations of 92 and 184.2 μg/cm^2, larvicidal activity decreased to 20% for both Piperaceae plants, as well as with the hexane extract obtained from the fresh leaves of *C. odorata* (the following are the statistical parameters of the triple interaction between the plant species, leaf type, and extract concentration: F = 5.718; df = 12, 45; $p < 0.001$).

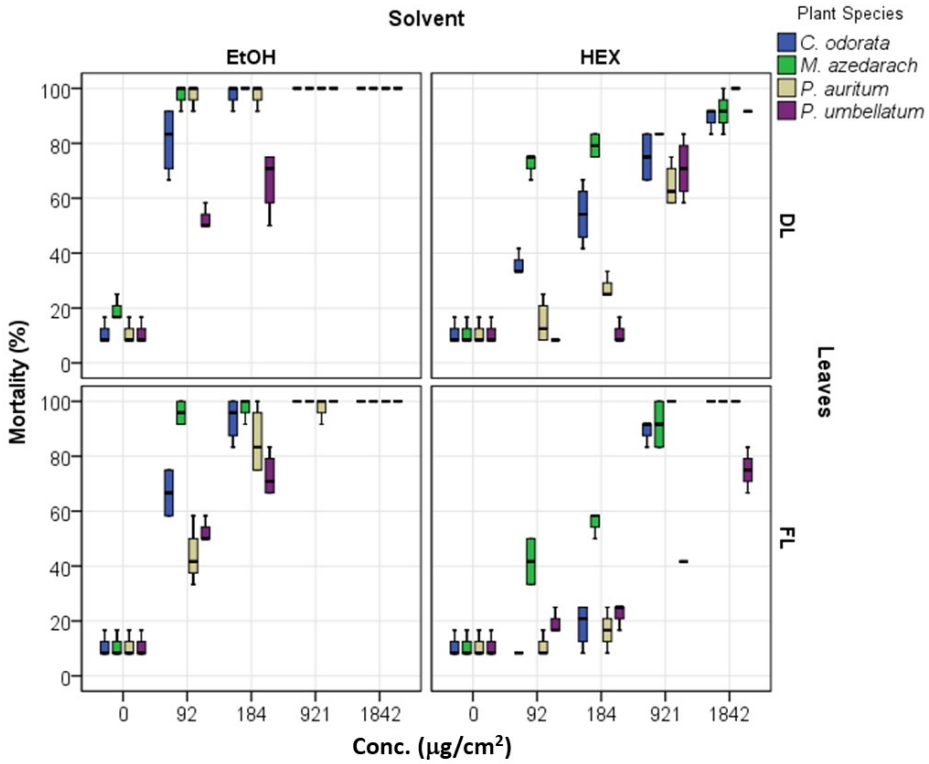


Figure 1. Mortality (average (%) ± SE) of first instar larvae of *Spodoptera frugiperda* by the effect of the concentration (μg/cm^2) of phytochemical extracts obtained with ethanol (graphs on the left) and hexane (graphs on the right) from dehydrated (DL, graphs at the top) and fresh leaves (FL, graphics at the bottom) from four different plants. Mixed-design ANOVA. The Pillai correction indicates differences in larval mortality due to the main factors (extract concentration, solvent, leaf type, and plant species). The double, triple, and quadruple interactions were significantly different, with plant species–leaves and leaves–solvent double interactions the only exceptions. The difference in average was determined by the Bonferroni test for a $p < 0.05$. Boxes represent mean and 95% confidence intervals, and tails are maximum and minimum values.

The surviving larvae for the tested extracts showed a shortening in size that decreased as a function of extract concentration. The lower number of bars in the graphs corresponding to ethanol (graphs on the left of Figure 2), most of them with average values lower than 5, indicates the greater damage caused by ethanol extracts in the *S. frugiperda larvae*. The mixed ANOVA indicated differences in the larva size by effect of the following main factors: plant species ($F = 57.348$; df = 3, 13; $p < 0.001$), leaf type ($F = 66.933$; df = 1, 15; $p < 0.001$), solvent ($F = 399.599$; df = 1, 15; $p < 0.001$), and concentration ($F = 548.915$; df =4, 15; $p < 0.001$); the double, triple, and quadruple interactions among factors also showed significant differences, except for the double interaction between the plant species and solvent ($F = 0.987$; df = 3, 13; $p = 0.429$). The greatest size shortening was observed in larvae exposed to the ethanolic extracts, and the larvae most affected were those exposed to the *M. azedarach* extracts, as indicated by the Greenhouse–Geisser correction (statistical factors of the triple interaction between plant species, leaf type, and extract concentration: $F = 9.531$; df = 1.855, 27.826; $p = 0.001$). The fresh-leaf hexane extracts of the studied plants produced visible larval shortening, but only at 920 and 1842 μg/cm^2 concentrations (statistical factors of the triple interaction between leaf type, solvent, and extract concentration: $F = 11.223$; df = 4, 15; $p < 0.001$), as shown in Figure 2.

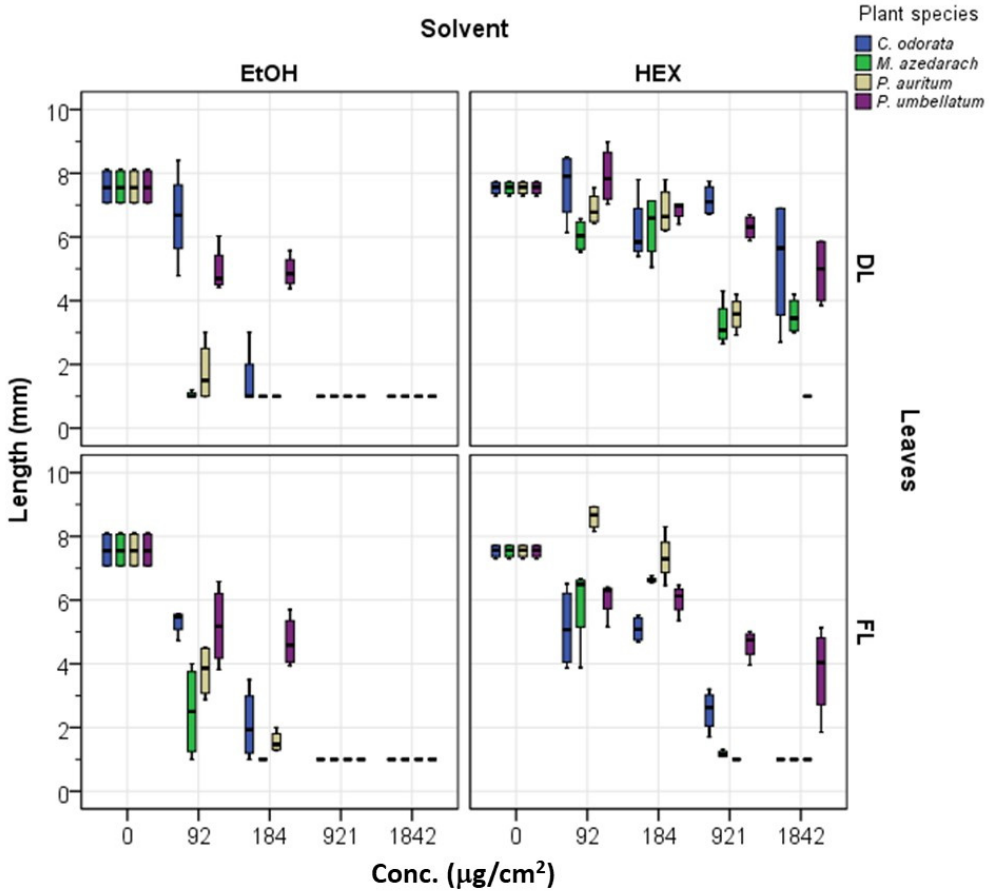


Figure 2. Length (average (mm) ± SE) of *Spodoptera frugiperda* larvae exposed for seven days to phytochemical extracts obtained with ethanol (graphs on the left) and hexane (graphs on the right) solvents from dehydrated (DL, graphs at the top) and fresh leaves (FL, graphics at the bottom) from four different plants. The mixed ANOVA indicated differences in the larva size by the effect of the main factors (extract concentration (μg/cm^2), solvent, leaf type, and plant species) and their double, triple, and quadruple interactions, with the double interaction between the plant species and solvent factors the exception. The difference between averages was determined by the Bonferroni test for a $p < 0.05$. Boxes represent mean and 95% confidence intervals, and tails are maximum and minimum values.

The weight variation in the larvae exposed to the different extracts was congruent with their respective variations in size. With the ethanol extracts at the highest concentration, *S. frugiperda* larval weight was close to zero; however, at 92 and 184.2 μg/cm^2, the larvae weight was around 50% compared to the weight of the negative control. The extract concentration (F = 275.148; df = 4, 15; $p < 0.0001$) and solvent (F = 69.302; df = 1, 15; $p < 0.001$) factors produced the greatest impact on larval development (Figure 3). However, although the mixed ANOVA of the larvae weight showed significant differences, the latter was low for the intra-subject effects tested, with F values in the range of 2.514–23.869, for a $p < 0.039$.

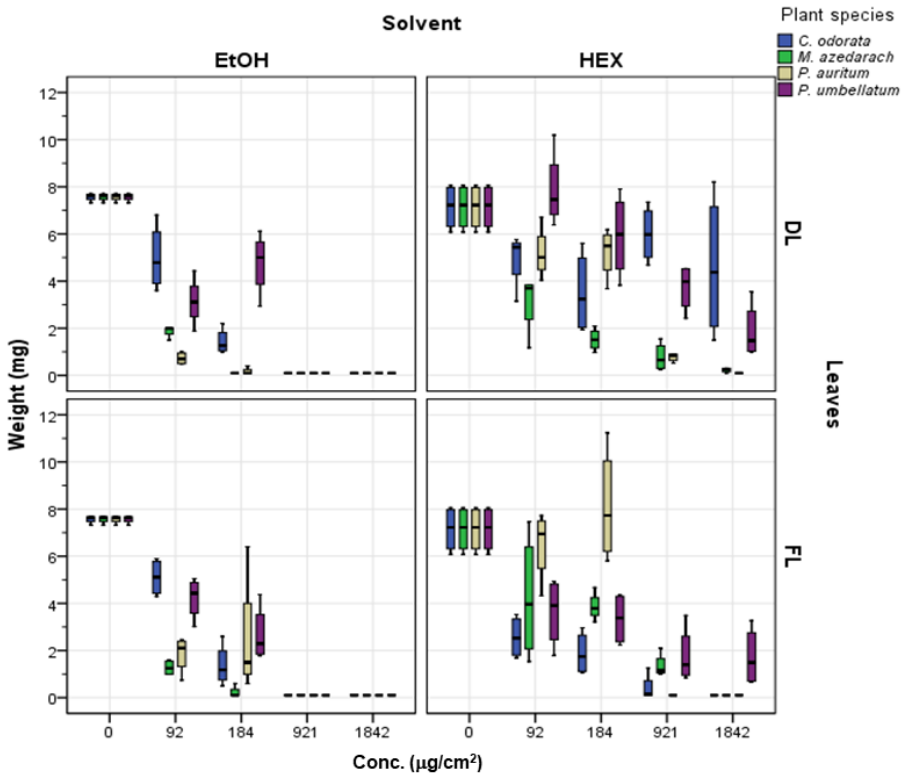


Figure 3. Weight (average (mg) ± SE) of *Spodoptera frugiperda* larvae exposed for seven days to phytochemical extracts obtained with ethanol (graphs on the left) and hexane (graphs on the right) from dehydrated (DL, graphs at the top) and fresh leaves (FL, graphics at the bottom) from four different plants. The mixed ANOVA indicated differences in the larvae weight by the effect of the main factors (extract concentration (μg/cm^2), solvent, leaf type, and plant species) and their double, triple, and quadruple interactions. The difference between averages was determined by the Bonferroni test for a $p < 0.05$. Boxes represent mean and 95% confidence intervals, and tails are maximum and minimum values.

3.3. Development of S. frugiperda Larvae Exposed to Ethanol Extract of P. auritum

The ethanolic extract from dehydrated *P. auritum* leaves was one of the most effective at controlling *S. frugiperda* first instar larvae in the 92–1840 μg/cm^2 (5–100 μg/mL) concentration range (Figure 1). At lower concentrations (2–37 μg/cm^2) the extract controlled the *S. frugiperda* larvae between 22% and 58% over seven days (effect of the extract concentration: F = 156.511; df = 5, 36; $p < 0.0001$), as shown in Figure 4. During the following two weeks, the surviving larvae, maintained on a free phytochemical diet, developed deformations on their cuticles. The damage magnitude increased with the *P. auritum* extract concentration to which the *S. frugiperda* larvae were exposed. On day 28, the larval mortality percentages were increased by 60.5 ± 10%, 43 ± 4%, and 16.2 ± 4% in the treatments corresponding to the extract concentrations of 9 μg/cm^2, 18 μg/cm^2, and 37 μg/cm^2, respectively (effect of

the day: F = 73.346; df = 1, 36; $p < 0.0001$). The lethal concentration calculated by the probit analysis is shown in Table 4.

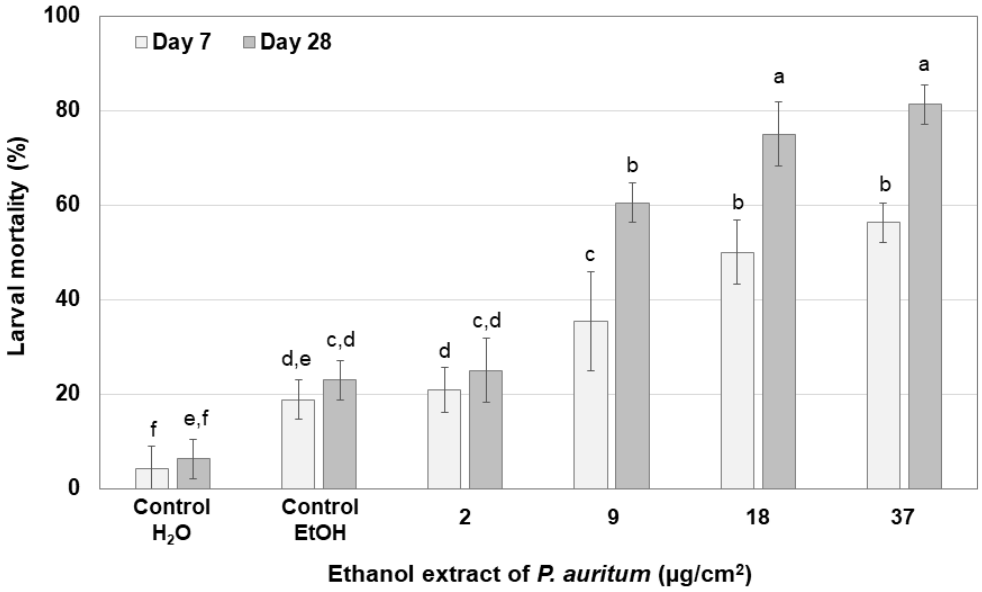


Figure 4. Mortality (average (%) ± SE) of first instar larvae of *Spodoptera frugiperda* exposed to ethanolic extracts of dehydrated *Piper auritum* leaves in the concentration range of 2–37 µg/cm^2. Mortality was recorded at 7 and 28 days. Bars topped with the same letter (a–d) do not significantly differ (Tukey's test for an $\alpha = 0.05$; Shapiro–Wilk normality test).

Table 4. Lethal concentration of ethanolic extracts from dehydrated *Piper auritum* leaves in *Spodoptera frugiperda* first instar larvae exposed over 7 and 28 days.

	Conc.	95% Confidence Limits for µg/µL		Conc.	95% Confidence Limits for mg/cm^2	
	µg/µL	Lower Limit	Upper Limit	mg/cm^2	Lower Limit	Upper Limit
Exposure of *S. frugiperda* for 7 days (probit analysis. $\chi^2 = 3.409$; df = 14; Sig.: 0.998)						
LC_{21}	0.11	0.01	0.22	1.9	0.3	4.1
LC_{35}	0.39	0.17	0.67	7.3	3.1	12.4
LC_{50}	1.20	0.70	3.25	22.1	13.0	60.0
LC_{56}	1.72	0.97	6.33	31.8	17.9	116.6
Exposure of *S. frugiperda* for 28 days (probit analysis. $\chi^2 = 3.251$; df = 14; Sig.: 1.000)						
LC_{26}	0.04	0.03	0.11	0.77	0.63	2.04
LC_{50}	0.27	0.18	0.38	4.90	3.29	7.03
LC_{60}	0.49	0.32	0.81	9.02	5.86	14.91
LC_{76}	1.28	0.80	2.67	23.51	14.78	49.21
LC_{83}	1.98	1.20	4.49	36.46	22.12	82.78

The data in Figure 4 (larval mortality versus *P. auritum* extract concentration) were correlated using a logarithmic model. There was a concentration above which the larvicidal activity gradually decreased (effect of the interaction between the concentration and day factors: F = 8.44; df = 5, 36; $p < 0.0001$). In this figure, the letters at the upper end of the bars, corresponding to the 7th and 28th days, corroborate this type of correlation. At a 2 µg/cm^2 concentration, the lethality of the extract was similar to that produced by control 2 (solvent).

The ethanolic extract of the dehydrated *P. auritum* leaves affected the survival of *S. frugiperda*, prolonging the larval and pupal stages and reducing the duration of the adult stage, and the extract concentration determined the degree of effect. The cumulative survival of the larval, pupae, adult stages, and the total development time of *S. frugiperda* is

shown in Figure 5. Although all surviving larvae pupated, the percentage of pupal death was higher than 50% in all treatments of the larvae exposed to the LC_{35} extract and greater than 80% concerning control 1 (Table 5). The adults in the LC_{50} and LC_{56} treatments died on day three and those of the LC_{35} treatment on eight days (Figure 5c). Only the adults from the larvae exposed to LC_{21} oviposited. However, the number of eggs was 37% lower than those of the water control. The hatching time was three days and did not present significant differences compared to control 1. The infertility degree of adults that emerged from the LC_{21}–LC_{56} treatments is presented in Table 6. This is represented by the −1 value of the oviposition activity indexes (*OAI*) in Table 6. At concentrations of 9 μg/cm^2 (LD_{21}) and above, results show that *P. auritum* ethanol extracts can effectively control *S. frugiperda* larvae and decrease the probability of new generations of the pest to develop.

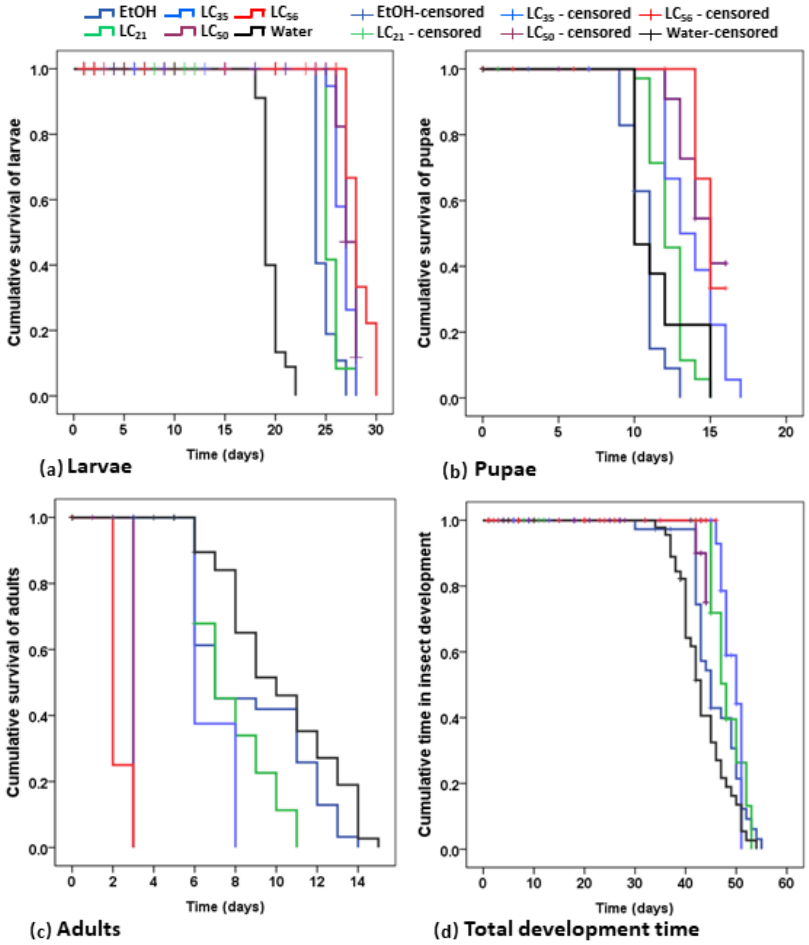


Figure 5. Kaplan–Meier curves comparing the duration of the life cycle stages of *Spodoptera frugiperda* first instar larvae exposed to ethanolic extracts of dehydrated *Piper auritum* leaves at concentrations of LC_{21} (2 μg/cm^2), LC_{35} (9 μg/cm^2), LC_{50} (18 μg/cm^2), and LC_{56} (37 μg/cm^2). (**a**) Larvae, (log rank (Mantel–Cox), χ^2 = 296.68, df = 5, Sig. 0.000); (**b**) pupae, (log rank (Mantel–Cox), χ^2 = 64.99, df = 5, Sig. 0.000); (**c**) adults, (log rank (Mantel–Cox), χ^2 = 214.59, df = 5, Sig. 0.000); (**d**) total development time of *S. frugiperda* (log rank (Mantel–Cox), χ^2 = 26.22, df = 5, Sig. 0.000).

Table 5. Pupal mortality (% ± SE) induced by the ethanolic extract of *Piper auritum* in the concentration range of 2–37 μg/cm^2 (LC_{21}–LC_{56}).

Piper auritum Lethal Conc.	Pupal Mortality (% ± SE)		
	Within the Same Treatment *	Compared to Control 1 **	Compared to Control 2 ***
LC_{21}	24.3 ± 1.0 ^{b}	45.4 ± 10.3 ^{c}	29.3 ± 2.1 ^{b}
LC_{35}	59.0 ± 4.9 ^{a}	82.6 ± 2.3 ^{b}	77.4 ± 0.6 ^{a}
LC_{50}	55.0 ± 7.1 ^{a}	87.6 ± 2.7 ^{a,b}	84.0 ± 0.9 ^{a}
LC_{56}	58.3 ± 11.8 ^{a}	90.0 ± 0.7 ^{a}	86.8 ± 3.0 ^{a}
Control 1 (H_2O)	0 ^{c}	0 ^{e}	
Control 2 (EtOH)	16.1 ± 0.9 ^{b,c}	35.3 ± 9.6 ^{d}	16.0 ± 0.9 ^{b}

All values are the average pupal mortality percentage. SE = standard error. One-way ANOVA with the extract concentration as a factor. Per column: average pupal mortalities that do not share equal letters ($^{a–e}$) are significantly different according to Tukey´s test (α = 0.05). * (F = 25.35; df = 5, 18; $p < 0.0001$); ** (F = 364.11; df = 5, 18; $p < 0.0001$); *** (F = 179.64; df = 4, 15; $p < 0.0001$).

Table 6. Fertility and oviposition activity index (*OAI*) of *Spodoptera frugiperda* larvae exposed to ethanolic extracts of dehydrated *Piper auritum* leaves.

Piper auritum Lethal Conc.	Fertility (No. Eggs ± SE)	OAI ± SE
LC_{21}	647 ± 33 ^{a}	−0.24 ± 0.01 ^{a}
LC_{35}	14 ± 0 ^{b}	−0.99 ± 0.02 ^{b}
LC_{50}	0 ± 0 ^{b}	−1 ± 0 ^{b}
LC_{56}	0 ± 0 ^{b}	−1 ± 0 ^{b}
Control 1 (H_2O)	1017 ± 7 ^{a}	0 ± 0
Control 2 (EtOH)	757 ± 118 ^{a}	−0.16 ± 0.07 ^{a}

SE = standard error. One-way ANOVA with the extract concentration as a factor. Per column: average activity values (±SE) that do not share an equal letter ($^{a, b}$) are significantly different according to Tukey´s test (α = 0.05).

3.4. Morphological Abnormalities of S. frugiperda Larvae Exposed to P. auritum Ethanolic Extracts

The *P. auritum* ethanolic extract produced significant abnormalities in all stages of the *S. frugiperda* life cycle, and the magnitude of these abnormalities was associated with the extract concentration (Figure 6). Larval death was the first indicator of the biocidal activity of the *P. auritum* extracts, but in surviving larvae, small dark spots appeared on the head, abdomen, or anal protuberance of the larval cuticle. Cuticle darkening spread rapidly in larvae exposed to extracts at 18 and 37 μg/cm^2 (LD_{50} and LD_{56}) concentrations, accompanied by visible dehydration of the cuticle. In larvae exposed to the *P. auritum* extract at concentrations of 2 and 9 μg/cm^2 (LD_{21} and LD_{35}), cuticle darkening was less intense, but the larva exhibited swollen dorsal segments.

The surviving larvae exposed to *P. auritum* extract of 2 μg/cm^2 (LD_{21}) developed into pupae. However, of the 75% of adults that emerged, 37% had wing abnormalities: thin hind wings, wrinkled wings, or no wings at all. In larvae treated with *P. auritum* extracts from 9 to 37 μg/cm^2 (LD_{35} to LD_{56}), all the surviving larvae pupated. However, they presented notable anomalies, which could explain the deformations of the very few adults that emerged (1.5 ± 0.5). The main anomaly in the pupae was incomplete or fragile tissue formation observed in the cuticle of the pupa. The cuticle was observed to be thin, dehydrated, and wrinkled; the absence of a cuticle in some specimens allowed us to see a white mass protruding from the pupa surface. Abnormal adults showed wrinkled or very short wings, even with a missing leg or antenna; in one case, only half of an adult emerged.

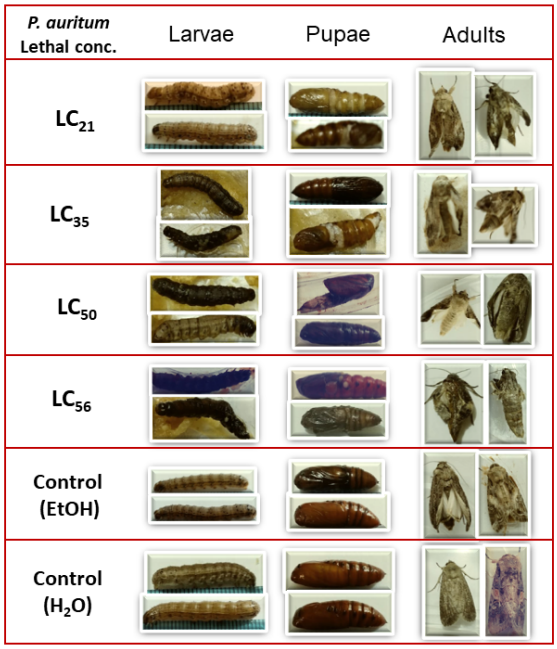


Figure 6. Pupae and adults of surviving larvae exposed to ethanol extracts of dehydrated leaves of *Piper auritum* within the concentration range of 2–37 μg/cm^2 (LC_{21}–LC_{56}).

4. Discussion

Ethanol and hexane are two solvents commonly used for extracting phytochemical metabolites of interest in biological control [37,42,43]. In the present work, the highest yields of phytochemicals were obtained from the dehydrated leaves of Meliaceae plants and using ethanol as a solvent. Concerning the Piperaceae plants, the yield of phytochemicals did not change when using ethanol or hexane as solvent. However, the chromatographic analysis identified different molecular structures in both extracts, particularly in the groups of sesquiterpenes, alcohols, and fatty acids [44]. Piperaceae plants are known for their high content of essential oils, of which safrole is one of the predominant phytochemicals in *P. auritum*. Safrole was extracted with hexane more than with ethanol, which is consistent with that reported in the literature [36,45]. Phytol (diterpene) and safrole (phenylpropanoid) were identified in the hexane extract of all plants and, to a lesser extent, in the ethanolic extracts of three of the four plants studied. The extraction of these two components with solvents of different polarities was previously reported by Lucena et al. [46].

Cedrela odorata and *P. auritum* belong to families of plants whose insecticidal activity has already been reported against different insect pests [36,47,48]. In the present study, the bioassay implemented, allowed us to observe that the ethanolic extracts of the dehydrated leaves of both plants, even in the lowest concentration evaluated (92 mg/cm^2 = 5000 mg/L), have a similar lethality against *S. frugiperda* as that obtained with *M. azedarach*, the positive control. The lethal concentration of 5000 mg/L is high, but the first bioassay was intended only to select the plant with potential use as a bioinsecticide. On the other hand, the lethal concentration of crude extracts is usually high. Bullangpoti et al. (2012) [49] reported that 12,000 mg/L of an ethanolic extract of the dehydrated leaves of *M. azedarach* were necessary to reach 100% mortality in the third instar larvae of *S. frugiperda*. Regarding the insecticidal activity of plants of the genus *Piper*, Lucena et al. (2017) [46] reported mortality percentages of 31% in *S. frugiperda* larvae exposed to 15,000 mg/L of an ethanolic extract of dehydrated leaves of *Piper aduncum* L. (Piperaceae). Celis et al. (2014) [35] reported mortality percentages of 80.3% in second instar larvae of *S. frugiperda* exposed to ethanolic extracts of *Piper arboreum* Aubl. (Piperaceae) and *Piper el-bancoanum* Trel. and Yunck (Piperaceae) in concentrations of 50 and 200 mg/L. Compared to these *Piper* plants, the lethality of *P. auritum* in *S. frugiperda* is higher than that of *P. aduncum* but lower

when compared with the following plants: *P. arboreum* Aubl plants. (Piperaceae) and *P. el-bancoanum* Trel. and Yunck (Piperaceae).

The decrease in size and weight of larvae, as observed here, are antifeedant effects already reported in *S. frugiperda* larvae exposed to botanical insecticides. Such morphological changes have been attributed, among other factors, to a deficient absorption of necessary nutrients for the development of the insect [50]. At a dose of 1000 mg/kg of a *M. azedarach* fruit extract, Scapinello et al. [51] reported a decrease in weight and length of 65.3% and 29.2%, respectively, in 7-day-old *S. frugiperda* larvae. A greater effect, a decrease of 73 ± 0.05% (weight) and 35 ± 0.04% (height), was observed by Cárdenas et al. (2012) [52], in neonate *S. frugiperda* larvae fed with 1000 mg/L of an ethyl acetate extract obtained from *Bursera copallifera* (D.C.) Bullock (Burseraceae) leaves. The morphological effect observed in those larvae was associated with the acetylcholinesterase inhibition (IC_{50} = 367 mg/L) analyzed in vitro by these authors. Dowd et al. (2011) [53] reported an effect of 20–50% in the development of first instar larvae of *S. frugiperda* exposed to doses of 1000 mg/L of saponins extracted from *Glycine max* (Fabaceae) and *Quillaja saponaria* (Mol.) (Quillajaceae). The saponin biological activity has been attributed to their surfactant properties and their interaction with the cholesterol in insects [18,54]. Saponins have been found in *P. auritum* [55]. However, in the ethanolic extracts of the leaves of this plant, they have not been identified [56].

P. auritum is a plant known for its medicinal and antimicrobial properties attributed to its secondary metabolites consisting mainly of flavonoids, triterpenoids, alkaloids, amides, saponins, amines, and propenylphenols [36,56]. Some of these metabolites can induce malformations in insects by attacking vulnerable targets such as cytochrome P450 monooxygenases and carboxylesterases enzymes [49,51,57]. In the present work, the effect on the development of *S. frugiperda* larvae exposed to the ethanolic extract of *P. auritum* (100–2000 mg/L (9–37 mg/cm^2)) could be explained by its composition, consisting of different types of terpenes (phytol), alcohols, and safrole as the majority constituent [58]. Phytol has been reported to inhibit insect development, either through growth regulation or as an antifeedant compound [32]. Safrole can cause neurotoxic problems by ingestion or contact with larvae, as reported by Lima et al. (2009) [33]. Bhardwaj et al. [59] reported 100% mortality in second instar larvae of *Spodoptera litura* (Fabricius, 1775) (Noctuidae: Lepidoptera) exposed to safrole at 5 mg/mL. In larvae of the sixth instar of *Spodoptera littoralis* (Boisduval, 1833) (Noctuidae: Lepidoptera) exposed to 5 mg/cm^2 of safrole, Andrés et al. [60] reported 80.5 ± 7.2% antifeedant inhibition. In the present work, the low oviposition observed in the adults of *S. frugiperda* of the LC_{21} and LC_{35} treatments is an effect already reported in insects exposed to extracts of plants of the genus *Piper* [23,58].

The drawback of chemical pesticides is their high non-specific toxicity and non-biodegradability. Regarding the extracts of the *P. auritum* plant studied, its degradative properties and low toxicity for mammals and non-target insects could be of interest for its application in the field without regulatory restrictions because it is a plant commonly used as a condiment in Mexican cuisine. The LD_{50} in mice administered with *P. auritum* hexane and methanolic extracts has been reported at 1264 and 2900 mg/kg, respectively [61]. According to Lorke's method, these LD_{50} values are of low toxicity. The LC_{50} (22.1 µg/cm^2; 1200 mg/L) of the ethanolic extract from dehydrated *P. auritum* leaves studied here is below the LD_{50} determined to be toxic in mice. Concerning the other plants, at doses of 2000 mg/kg p.o., the *P. umbellatum* ethanolic hydrate did not alter the behavior of mice [62]. No behavioral change was observed in mice subjected to an oral administration of 250 mg/kg of *C. odorata* bark ethanolic hydrate. At a concentration of up to 5000 mg/kg, the mice presented ailments that were reversed within the following 4–8 h [63]. The ethanolic extracts from *M. azedarach* fruits and flowers, in an oral dose of 1500 mg/kg, did not cause toxic effects in mice.

5. Conclusions

The ethanolic extracts of the dried leaves of *C. odorata* and *P. auritum* showed effective insecticidal properties against *S. frugiperda*, one of the most voracious larvae of economically important crops such as corn. Of these two plants, the insecticidal activity of *P. auritum* was as high as that observed with *M. azedarach*, one of the plants already known for its insecticidal properties against a wide variety of insect pests, including *S. frugiperda*. The study of the biological activity of the ethanolic extract of *P. auritum* in the life cycle of *S. frugiperda* allowed us to observe its antifeedant effect, recorded through the fragile tissue formed in the larvae and pupa cuticle, and the deformation of adults that emerged. *Piper auritum* is a plant used in different parts of the world as a condiment and home remedy. These properties and the low toxicity of the *P. auritum* extracts in mammals and non-target insects could facilitate the regulation of this plant for its use as a botanical insecticide. Furthermore, *P. auritum* is a plant that is easily cultivated and propagated; thus, its potential use as a botanical insecticide is promising.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/su132413896/s1, Figure S1: Experimental design, Figure S2: Chromatograms of the ethanolic extracts of dehydrated leaves of (a) *C. odorata* and (b) *M. azedarach*, Figure S3: Chromatograms of the ethanolic extracts of dehydrated leaves of (a) *P. auritum* and (b) *P. umbellatum*, Figure S4: Chromatograms of the hexane extracts of dehydrated leaves of (a) *C. odorata* and (b) *M. azedarach*, Figure S5: Chromatograms of the hexane extracts of dehydrated leaves of (a) *P. auritum* and (b) *P. umbellatum.*

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