Insect Growth Regulatory Activity of Some Extracts and Compounds from *Parthenium argentatum* on Fall Armyworm *Spodoptera frugiperda*

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The methanolic extract from aerial parts of *Parthenium argentatum*, afforded argentatin A and B. These compounds were evaluated for their effect on the fall armyworm (*Spodoptera frugiperda*). Toosendanin, a commercial insecticide derived from *Melia azedarach* was used as positive control. When tested for activity, using neonate larvae into the no-choice artificial diet bioassays, argentatin A, argentatin B and methanol extract caused significant growth inhibitory activity with GC_{50} of 17.8, 36.1 and 6.4 ppm at 7 days, respectively, and increased the development time of surviving larvae in a concentration-dependent manner with RGI values of 0.40, 0.60 and 0.26, at 25.0, 25.0 and 5.0 ppm, respectively. In addition, it was possible to observe in most of the treated groups a significant delay in the time of pupation, adult emergence and deformities. Acute toxicity against adults of *S. frugiperda* was also found, MeOH extract had the most potent activity with LD_{50} value of 3.10 ppm. In addition, MeOH extract and argentatin A caused acetylcholinesterase inhibition of 93.7% and 90.0%, at 5.0 and 50.0 ppm, respectively; whereas argentatin B had only slight inhibitory activity. Therefore, the MeOH extract was identified as insecticidal extract from *P. argentatum* with activity at concentrations above 15.0 ppm.

Introduction

Recent studies have demonstrated that many plant species produce and accumulate a large variety of secondary metabolites which provide defense against insect predators (Guella *et al.*, 1996; Marvier, 1996). Examples are *Azadirachta indica* and *Derris elliptica*, which produce the very well known insecticides azadirachtin and rotenone, respectively (Kraus, 1995; and Gomes *et al.*, 1981).

The main characteristics that account for the successful use of these secondary metabolites as natural insecticides are that, most of them have low mammalian toxicity, lack of neurotoxic activity, low persistence in the environment, and biodegradability, which made them lesser aggressive to the environment than the synthetic ones (Camps, 1988; Berembaum, 1989; and Castillo *et al.*, 1998). As result of these facts the trend on the research in this field have been directed in finding new insecticides from natural sources (Jacobson, 1989; and Singh *et al.*, 1997); these characteristics may enhance their value as botanical pesticides (Isman *et al.*, 1996; Gonzalez and Estévez-Braun, 1998; and Valladares *et al.*, 1997).

Our studies on the resin from "Guayule" *Parthenium argentatum* Gray, Asteraceae, a shrub endemic to the Chihuahuan desert and used as an important source of natural rubber, have revealed the presence of large quantities of cycloarte-type triterpenes. We had reported the isolation of the triterpenes argentatins B, C and D (Romo de Vivar *et al.*, 1990; and Matsubara and Romo de Vivar, 1985). We also reported the antimicrobial activity of argentatin A (Martínez-Vázquez *et al.*, 1986 and 1994), the non-competitive inhibition of estrogens by argentatins A, B and D (Calzada *et al.*, 1995) and the synthesis of carbenoxolone analogs from argentatin B (Martínez *et al.*, 1993). There are other reports of constituents of *P. argen-*

Abbreviations: AChE, acetylcholinesterase; ATC, acetylthiocholine; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); RGI, relative growth index; GI, growth index.

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tatum and their biological activities, including antifungal eudesmanoids (Maatooq *et al.*, 1996), fungistatic sesquiterpenoids (Maatooq and Hoffman, 1996), allergic contact dermatitis of guayulin A and effective fungicides sesquiterpene esters (Martínez-Vazquez *et al.*, 1986).

The present paper deals with the effects of MeOH extract of guayule as well as its isolates: the cycloarten-type triterpenes argentatin A (1) and B (2) against Fall armyworm (*Spodoptera frugiperda* (J. E. Smith), Lepidoptera: Noctuidae).

Aspects such as insecticide and growth regulatory activities, rate of development, time of pupation, adult emergence and deformity were evaluated and compared with those of toosendanin **3**, a known growth inhibitor against *Spodoptera litura* (Isman *et al.*, 1996), *Peridroma saucia* (Chen *et al.*, 1995) and *Ostrinia nubilalis* (Jiménez *et al.*, 1997).

Materials and Methods

Plant material

P. argentatum (Asteraceae) was collected in Matehuala, State of San Luis Potosí, Mexico, in February 1997. A voucher sample was deposited at National Herbarium, Instituto de Biología, UNAM. (Voucher: J. L. Villaseñor).

Apparatus

^{1}H NMR spectra were recorded at 300 and 500 MHz, ^{13}C NMR at 75 and 125 MHz respectively, on Varian VXR-300S and VXR-500S spectrometers, chemical shifts (ppm) are related to $(CH_3)_4Si$ as internal reference. $CDCl_3$ and acetone-d_6 from Aldrich Chemical Co. were used as solvents, and coupling constants are quoted in Hz. IR spectra were obtained as KBr pellets on Perkin Elmer 283-B and FT-IR Nicolet Magna 750 spectrophotometers. Electron impact mass spectra were taken on a JEOL JMS-SX102A instrument (70 eV). UV spectra were determined on a Shimadzu UV-160 spectrophotometer; $CHCl_3$ was used as solvent. Optical rotations were measured on a JASCO DIP-360 spectropolarimeter; $CHCl_3$ was used as solvent. Melting points were obtained on a Fisher-Johns hot-plate apparatus and remain uncorrected. Nunc 24-well polystyrene multidishes were purchased from Cole-Parmer. LAB-LINE Chamber model CX14601A, with adjustable Hi-Lo protection thermostat safeguard samples.

A Spectronic Instruments model GENESYS 5 spectrophotometer was used to carry out the spectrophotometric measurements in the acetylcholinesterase activity. The centrifuge used in this study was B. Braun model SIGMA 2–15.

Chemicals and solvents

All used reagents were commercially available. Thiamin, sorbate, methyl-paraben, ascorbate, acetic acid, acetaldehyde, acetylcholinesterase (AChE), acetylthiocholine (ATC), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), choline-chloride, calcium pantothenate, niacinamide, riboflavin, folic acid, biotin and vitamin B-12 were purchased from Sigma Chemical Co. Methanol, ethyl acetate, $CuSO_4$, KCl, $NaHCO_3$, $MgCl_2$, NH_4Cl, pyridine, acetic anhydride, Silica-gel GF_{254} analytical chromatoplates, Silica gel grade 60, 70–230, 60 Å, for column chromatography were purchased from Merck. Pre-coated TLC plates SIL G-100 UV_{254}, 1.0 mm, preparative were purchased from Macherey-Nagel, Düren, Germany.

Extraction and isolation of triterpenes, bioassay guided fractionation

Milled aerial parts (stem and leaves) of *P. argentatum* (Gray) (Asteraceae) (10.1 kg) were extracted exhaustively with methanol. The solvent was removed *in vacuo* with a rotatory evaporator leaving 945.9 g of extract. Then the oily residue (682 g) was partitioned between n-hexane (4 X 50 ml) (F_0-1) and ethyl acetate (4 × 50 ml) (F_0-2). Elimination of the solvent yielded the residues F_0-1 (325 g) and F_0-2 (284 g).

The MeOH extract and the primary fractions (F_0-1 and F_0-2) showed significant insect growth inhibitory activities. Fraction F_0-1 was chromatographed on a silica-gel column (300 × 65 mm, Si Gel grade 60, 70–230 mesh, 1000 g, and gravity flow) and eluted with n-hexane/ethyl acetate mixtures with increasing polarity starting with hexane 100%. Inhibitory active samples were collected from several fractions eluted with hexane-EtOAc (9:1 v/v). These fractions were purified by crystallography procedures to give argentatin B (12.4 g). On the other hand fraction F_0-2 when chromato-

graphed on silica-gel and eluted with n-hexane-EtOAc (2:1 v/v) mixture afforded several fractions with inhibitory activity which were combined to yield a mixture of argentatin A, incalin and other compounds in minor amounts identified as guayulins and argentatin D (4.28 g). Further purification by TLC (preparative plates Macherey-Nagel, precoated TLC plates Sil-G-100, UV_{254}, 1.0 mm, eluted with n-hexane/ethyl acetate (80:20 v/v) solvent system, using UV_{254} detection) yielded argentatin A (485 mg). The identification of argentatins was made by spectroscopic methods and direct comparison with authentic samples (Rodríguez-Hahn *et al.*, 1970; Romo de Vivar and Matsubara, 1986; and Romo de Vivar *et al.*, 1990), as the argentatins A (**1**) and B (**2**) were purified in sufficient amount these were used in the bioassays. Toosendanin (**3**) was a gift from Prof. M. B. Isman (Department of Plant Science, University of British Columbia, Vancouver, British Columbia, Canada).

Bioassays with fall armyworm

Larvae used for the experiments were obtained from the culture at the Centro de Investigación en Biotecnología at the Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, México, maintained under previously described conditions (Aranda *et al.*, 1996). An artificial diet containing 800 ml of sterile water, 10.0 g of agar, 50.0 g of soy meal, 96.0 g of corn meal, 40.0 g of yeast extract, 4.0 g of wheat germ, 2.0 g of sorbic acid, 2.0 g of choline chloride, 4.0 g of ascorbic acid, 2.5 g of *p*-hydroxybenzoic acid methyl ester, 7.0 ml of Wesson salt mixture, 15.0 ml of Vanderzant vitamin mixture for insects, 2.5 ml of formaldehyde, 0.1 unit of streptomycin, 5.0 g of aureomycin, and 20.0 g of milled ear of corn grain (for 1 kg of diet) were used for the bioassay, which was prepared by the procedure described earlier (Mihm, 1987). 24-well polystyrene multidishes were filled with the liquid diet, then left for twenty minutes at room temperature under sterile conditions. The 3.4 ml wells measure 17 mm in depth × 15 mm in diameter with a 1.9 cm^2 culture area. All test compounds were dissolved in 95% ethanol and layered on top of each well with the artificial diet using up to six concentrations (see Table I) and a control (1 ml 95% ethanol) allowing evaporation of solvent (in addition was used 1.0 and 3.5 ppm for MeOH extract, since this extract showed the highest inhibitory activity in the preliminary bioassay). For each concentration used and control, a single *S. frugiperda* neonate first instar larva was placed on the diet mixture in each dish of well for 7 days (3 Nunc of 24-well polystyrene multidishes, in total 72 larvae for each one concentrations). After 7 days, surviving larvae were measured and weighed and then transferred to separate vials containing fresh stock diet. Larval weight gains and mortality were recorded after 21 days of incubation, since pupation average is 23 ± 1 days. Other life cycle measurements were recorded, such as time to pupation, weight of pupae, mortality of larvae and adult emergence and deformities. All treatments were carried out in a controlled environment chamber with an 18L: 6D photoperiod, at 25 °C day and 19 °C night temperature regime, and a relative humidity of 80% ± 5%. There were three replications for each assay. Control assays (24-wells) contained the same numbers of larvae, volume of diet, and ethanol as the test solutions (Céspedes *et al.*, 2000).

Acute toxicity on Spodoptera frugiperda

Acute toxicity was determined by topical application to larvae of last stage of *S. frugiperda.* The larvae of *S. frugiperda* were iced to stop their movement and treated on their abdomens with each of the test compounds, at concentrations of 5.0; 10.0; 15.0; 25.0; 35.0 and 50.0 ppm; in addition was used 1.0 and 3.5 ppm for the MeOH extract. In 4.5 ml of acetone with 10 ml microsyringe. Control was treated with 4.5 ml of acetone. After 24 hrs the survival were recorded. Five larvae were used for each concentration, respectively. LD_{50} is the lethal dose for 50% mortality.

Inhibition of acetylcholinesterase

An enzyme extract containing acetylcholinesterase (AChE) was obtained according to the method of Grundy and Still, (1985). About 100 adults were frozen at −20 °C for 7 days. The heads of frozen adults were detached, then milled and homogenized in 20 ml of 0.1 M phosphate buffer at pH 8.0. The crude homogenate was centrifuged at 15000×*g* for 30 min, and the supernatant was used for the enzyme activity. ATC (cholinesterase substrate) was dissolved in 0.1 M phosphate buffer

(pH 8.0). DTNB (3-carboxy-4-nitrophenyldisulfide), Ellman's reagent a sensitive sulfhydryl, 39.6 mg of this compound was dissolved in 10 ml of 0.1 M phosphate buffer at pH 7.0, and 15.0 mg of $NaHCO_3$ was added.

Inhibition of AChE was determined according to the Ellman's procedure (colorimetric method) (Ellman *et al.*, 1961) using both the control (MeOH) and test solutions. The reaction mixture contained 0.2 ml of the enzyme solution and 0.1 ml of DTNB added to 2.4 ml of 0.1 M phosphate buffer (pH 8.0). The reaction mixtures were added to each of the test compounds dissolved in 50 ml of EtOH. The control solution was similarly prepared by the addition of 50 ml of EtOH. Both control and each of the test solutions were preincubated at 25 °C for 10 min. After preincubation, the enzyme reaction was started by the addition of 40 ml of ATC followed by incubation at 25 °C for 20 min. After 20 min, the absorbance at 420 nm was measured spectrophotometrically and compared with that of the control immediately after adding an enzyme to the above reaction mixtures. Reading was repeated for 5 min at 30 sec intervals to verify that the reaction occurs linearly. Blank reaction was measured by substituting saline for the enzyme. AChE activity was calculated with the absorption coefficient 1.36 mmol/min. All experiments were repeated three times and the results were analyzed by SAS ANOVA and GLM procedures.

Relative growth index and growth index

The relative growth index (RGI) and growth index (GI) were calculated according to Zhang *et al.* (1993).

Data analysis

Data for all the live insect bioassays were analyzed by SAS ANOVA and GLM procedures ($p < 0.05$), and GC_{50}, LD_{50}, and MC_{50} values for each activity were calculated by Probit analysis on the basis of the percentage of mortality obtained from the concentration of the compounds. Differences between treatment means were established with a Student-Newman-Keuls (SNK) test. LD_{50} and MC_{50} are the concentration producing 50% mortality.

Results and Discussion

Insecticidal activity against larvae

The insecticidal effects of the argentatins A (**1**), B (**2**), toosendanin **3** and methanolic extract against larvae of first instar of *Spodoptera frugiperda* are outlined in Table I. Argentatin B and toosendanin at concentration of 35.0 ppm show

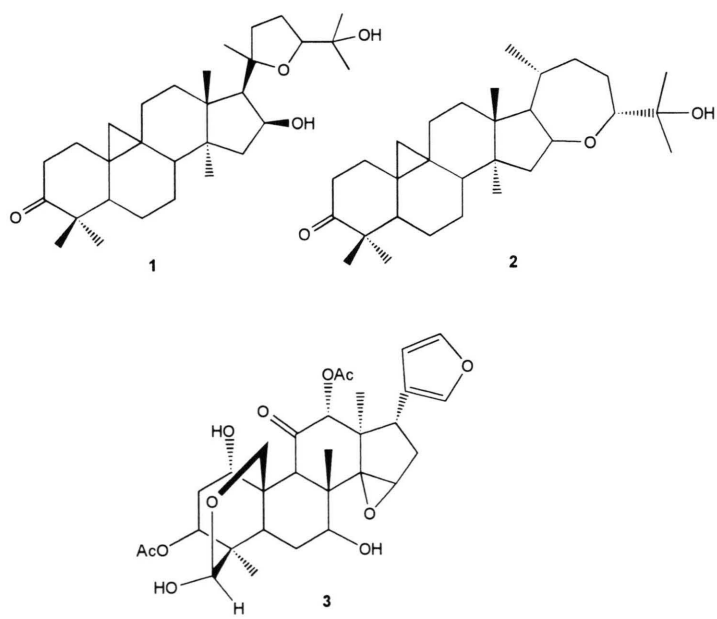

Fig. 1. Chemical structures of argentatin A (**1**), argentatin B (**2**) and toosendanin (**3**).

Table I. Growth inhibitory effects of MeOH extract, compounds 1, 2 and toosendanin on fall armyworm growth bioassay[a].

Treatment	µg/ml (ppm)	Mean weight gained [mg][b]	% of weight[c]	Mean length gained [cm]	% of length[c,e]	LC_{50} (ppm)[d]	Mortality(%)	MC_{50} (ppm)[f]
Control		81.3 ± 8.4[a]	100	0.95 ± 0.2	100		8.33	
Argentatin A	5.0	11.3 ± 2.8[b]	13.8	0.75 ± 0.18	78.9	17.8	12.2	21.3
	10.0	10.5 ± 3.1[b]	12.2	0.65 ± 0.20	68.2		23.3	
	15.0	9.2 ± 0.6[b]	11.3	0.55 ± 0.07	58.3		35.7	
	50.0	3.8 ± 0.5[c]	4.7	0.20 ± 0.05	21.0		97.5	
Argentatin B	5.0	42.5 ± 2.0[a]	52.3	0.96 ± 0.21	101.1	36.1	14.6	37.0
	25.0	11.6 ± 2.1[b]	14.2	0.74 ± 0.15	77.9		28.9	
	35.0	5.3 ± 0.5[b]	6.5	0.51 ± 0.10	53.7		49.5	
	50.0	4.5 ± 0.51[b]	5.5	0.24 ± 0.15	25.1		77.1	
MeOH	1.0	79.5 ± 2.3[a]	97.8	0.94 ± 0.15	98.9	6.4	12.5	6.9
Extract	3.5	78.1 ± 5.5[a]	96.1	0.78 ± 0.30	82.6		25.3	
	5.0	69.7 ± 6.3[a]	85.7	0.59 ± 0.21	62.2		36.5	
	25.0				0		100	
	35.0				0		100	
	50.0				0		100	
Toosendanin	5.0	5.1 ± 2.5[a, b]	6.27	0.90 ± 0.23	94.7	29.0	20.1	33.7
	10.0							
	15.0	2.3 ± 1.7[c]	2.82	0.77 ± 0.18	81.0		29.5	
	50.0	0.89 ± 0.75[c]	1.09	0.19 ± 0.15	20.0		70.1	

[a] Values taken after 7 days of incubation, mean of three replicates. [b] Means followed by the same letter within a column after ± standard error values are not significantly different in a Student-Newman-Keuls (SNK) test at $P < 0.05$ (treatments are compared to control). [c] percentage with respect to control. [e] Mean length total increase from eclosion. [d] The LC_{50} is the inhibitory concentration for reduction of first instar larval length rates by 50% in "no choice" test calculated by ANOVA program ($P < 0.05$). [f] The MC_{50} is the concentration producing 50% mortality.

significant mortalities in this larval stage (> 49%). However, Argentatin A produced higher mortality (80.2%) at the same concentration. On the other hand the methanolic extract show highest insecticidal activity producing 96.5% of larval mortality at 10.0 ppm. Notoriously, when larvae were fed with a diet containing 15.0 ppm or higher doses of methanolic extract all of the larvae died (Table I). It is important to note that the 50% lethal concentration (MC_{50}) of larvae at 7 days for this extract was found at 6.9 ppm of diet concentration.

Insect growth inhibitory activity

The two compounds from *P. argentatum* tested (argentatin A and B) inhibited specifically each larval stage (up 75%), i.e. growth when incorporated into diets at 35.0 and 50.0 ppm (% of length) and at 5.0 and 10.0 ppm (% of weight), respectively (Table I). At 23 days, this growth reduction was clearly significant between 25.0 and 50.0. However, only toosendanin **3** showed the highest larval growth inhibition at the same concentrations (Table II).

The percentage of larvae that reached pupation decreased in all tested compounds in comparison to control. Thus, argentatin A (**1**) and B (**2**) showed significant delayed of survival pupation by 12% at 25.0 and 35.0 ppm, respectively. The most important effect was observed with MeOH extract and toosendanin, at 10.0 and 15.0 ppm, which reduced survival pupation by 4.0% in both cases, respectively. Significant delays in time to pupation (26 days) were observed at 25.0 and 35.0 ppm for **1** and **2**, respectively. Furthermore, **1** and MeOH extract, significantly reduced pupae weights at 35.0, 5.0 and 10.0 ppm, respectively. While, toosendanin showed the greatest effect at 10.0 ppm (Table III).

Percentage of emergence, as compared to the pupal stage, showed further reductions with compounds **1** and **2** at 25.0 ppm with 15.7 and 16.7% of emergence, respectively. However, the MeOH extract and toosendanin drastically reduced the percentage of adult emergence to 0% at 10.0 and 35.0 ppm, respectively.

Table II. Activity of argentatin A, argentatin B, toosendanin and MeOH extract of *P. argentatum* on larval growth parameters of *S. frugiperda*[a].

Treatment	Concentration (ppm)	Mean weight Gained (mg)[b]	% c	Mean Length Gained (mm)	% [c]
Control		481.6 ± 6.59[a]	100.0	30.1 ± 4.1	100.0
Argentatin A	5.0	199.6 ± 3.6[b]	41.4	16.5 ± 1.1	54.8
	10.0	130.1 ± 1.5[b]	27.0	11.8 ± 0.8	39.2
	15.0	44.9 ± 5.5[b]	9.3	9.1 ± 3.5	30.2
	50.0	11.3 ± 1.2[c]	2.3	6.5 ± 0.8	21.6
Argentatin B	5.0	216.1 ± 0.7[b]	44.9	24.6 ± 0.4	81.7
	10.0	161.1 ± 0.4[b]	33.4	22.6 ± 0.7	75.1
	15.0	134.5 ± 0.8[b]	27.9	22.1 ± 0.2	73.4
	50.0	20.2 ± 0.3[a]	4.2	10.1 ± 0.1	33.6
MeOH extract	1.0	391.1 ± 4.5[b]	81.2	18.9 ± 0.7	62.8
	3.5	311.2 ± 6.1[b]	64.6	13.2 ± 0.5	43.8
	5.0	289.1 ± 4.3[b]	60.0	10.4 ± 0.6	34.5
	10.0	180.3 ± 2.3[b]	37.4	3.4 ± 0.4	11.3
	35.0				
Toosendanin	5.0	21.5 ± 2.5[a, b]	4.58	7.0 ± 0.5	25.0
	10.0				
	15.0	17.0 ± 3.1[b]	3.62	6.1 ± 0.6	21.7
	50.0	6.5 ± 1.5[c]	1.35	1.9 ± 0.3	6.3

[a] Values taken at 23 days before pupation, mean of three replicates. [b] Means followed by the same letter within a column after ± standard error values are not significantly different in a Student-Newman-Keuls (SNK) test at $P < 0.05$ (treatments are compared by concentration to control). [c] Percentage with respect to control.

Table III. Activity of compounds 1–3 and MeOH extract on pupation and emergency parameters of S. *frugiperda*.[a]

Treatment	Concentration (ppm)	Mean time of pupation [days]	Mean weight of pupae [mg][c]	SP[e] (%)	Mean emergence [days]	Mean adult weight [mg]	Emergences[f] (%)
Control		22.0	281.5 ± 10.1[b]	87.5	31.5	51.0 ± 2.1	83.3
Argentatin A	5.0	22.0	174.0 ± 1.0[b]	65.0	31.5	21.0 ± 1.0	70.8
	10.0	23.0	117.0 ± 2.0[b]	60.0	31.5	20.0 ± 0.5	47.8
	15.0	23.0	116.0 ± 3.1[b]	22.0	31.5	28.0 ± 0.3	35.5
	50.0	27.5[b]	81.4 ± 0.3[c]	12.0	33.0[b]	15.0 ± 0.1	10.5
Argentatin B	5.0	22.0	233.1 ± 1.3[b]	36.8	32.0	47.0 ± 0.3	88.3
	10.0	23.0	218.4 ± 2.1[b]	28.3	35.0[b]	43.0 ± 0.6	74.0
	15.0	24.0	217.4 ± 3.1[b]	24.0	33.0[b]	35.0 ± 0.4	55.9
	50.0	26.5[b]	110.0 ± 2.5[b]	12.0	36.0[b]	10.0 ± 0.1	10.5
MeOH extrc.	1.0	22.0	231.5 ± 4.5[b]	25.0	31.0	22.4 ± 0.3	55.3
	3.5	24.5[b]	200.5 ± 3.1[b]	12.5	35.0[b]	19.1 ± 0.4	44.1
	5.0	26.5[b]	101.5 ± 2.7[c]	12.0	36.0[b]	13.5 ± 0.2	35.0
	10.0	28.0[b]	36.0[d]	4.17	–	–	0
Toosendanin	5.0	23.5	95.0 ± 2.1[c]	17.9	36[b]	13.1 ± 0.3	16.7
	10.0	24.0 [b]	71.3 ± 1.8[c]	17.9	36[b]	12.1 ± 0.3	16.7
	15.0	24.0[b]	45.1[d]	4.17	36[b]	10.1 ± 0.1	4.17
	50.0	26.0[b]	15.0[d]	4.17	–	–	0

[a] Mean of three experiments. [b] Means within a column are significantly different from control in a Kruskal-Wallis chi-squared approximation test at $P < 0.005$. [c] Means followed by the same letter within a column after ± standard error values are not significantly different in a Student-Newman-Keuls (SNK) test at $P < 0.05$ (treatments are compared by concentration to control). [d] These values correspond to one survival larva. [e] SP: Survival Pupation= Number of survival pupae × 100 / Total larvae for pupation. [f]% = Number of adults emerged × 100 / Total Number of pupae.

Growth inhibition and regulatory growth index

In many of the treatments, mean adult weight was significantly delayed in the average time to reach the adult stage relative to control larvae. GI and RGI clearly showed (Table IV) that the stronger effect was shown by MeOH extract, with RGI value of 0.26 at 5.0 ppm. Toosendanin also showed a pronounced effect with RGI of 0.51 and 0.10 at 15.0 and 25.0 ppm, respectively (Table IV). These parameters together with the LD_{50} values (Table V), to corroborate the highest effect that showed the methanolic extract, since it caused the greatest inhibitory effect with a 36.2 and 37.4% of growth inhibition in weight at 7 and 23 days, respectively (Table I and II) and 22.2 and 11.3% of growth inhibition in length at the 7 days and 23 days, respectively. In addition, this extract, at 15.0 ppm, was noteworthy insecticidal with a 100% of death (Tables I, III and IV).

It is important to note that similar insect growth regulatory activity on *Ostrinia nubilalis* (European corn borer) was studied by Jiménez *et al.*, (1997). These authors reported that the nortriterpenoids humilinolide A, B, C, and D, cedrelanolide and toosendanin induced larval mortality between 5.0 to 50.0 ppm ranges with percentages between 36 to 60%, respectively. However, our compounds showed lower range between 5.0 to 25.0 ppm.

Table IV. GI and RGI of S. *frugiperda* as a function of increased concentrations of argentatin A (1), argentatin B (2) and MeOH extract from *P. argentatum* and toosendanin (3).[a]

Compounds	Concentration (ppm)	GI[b]	RGI[c]
Control		0.99 ± 0.045[a]	
Argentatin A	5.0	0.89 ± 0.044[b]	0.90
	10.0	0.84 ± 0.085[b]	0.85
	15.0	0.69 ± 0.055[b]	0.70
	50.0	0.15 ± 0.028[c]	0.15
Argentatin B	5.0	0.99 ± 0.050[b]	1.00
	10.0	0.92 ± 0.046[b]	0.93
	15.0	0.80 ± 0.047[b]	0.95
	50.0	0.15 ± 0.038[c]	0.20
MeOH extrc.	1.0	0.99 ± 0.050[b]	1.00
	3.5	0.74 ± 0.037[b]	0.75
	5.0	0.26 ± 0.015[c]	0.26
	10.0	0.03 ± 0.015[c]	0.03
	35.0	0.00	0.00
	50.0	0.00	0.00
Toosendanin	5.0	0.99 ± 0.050[b]	1.00
	10.0	0.77 ± 0.060[b]	0.77
	15.0	0.51 ± 0.040[b]	0.51
	50.0	0.00	0.00

[a] Mean of three replicates. [b] Means followed by the same letter within a column after ± standard error values are not significantly different in a Student-Newman-Keuls (SNK) test at $P < 0.05$ (treatments are compared by concentration to control).
[c] $RGI_{treatment} = GI_{treated} / GI_{control}$.
(GI = Growth index = Number survival larvae/total larvae used).

Table V. Acute toxicity of compounds 1–3 and MeOH extract against larvae of last stage of S. *frugiperda.*[a]

Compounds	Concentration (ppm)	% Survival[b]	LD_{50}[c]
Control		100	
Argentatin A	5.0	76.0 ± 5.5[b]	12.4
	10.0	65.0 ± 6.5[b]	
	15.0	40.0 ± 3.75[b]	
	50.0	11.0 ± 2.1[c]	
Argentatin B	5.0	85.0 ± 4.25[b]	19.8
	25.0	42.0 ± 2.1[b]	
	35.0	28.0 ± 1.4[b]	
	50.0	17.0 ± 0.85[c]	
MeOH extrc.	1.0	75.0 ± 4.75[b]	3.1
	3.5	55.0 ± 3.75[b]	
	5.0	19.0 ± 3.95[b]	
	25.0	0.0	
	35.0	0.0	
	50.0	0.0	
Toosendanin	5.0	82.0 ± 4.8[b]	11.5
	10.0	52.0 ± 3.9[b]	
	15.0	35.0 ± 3.75[b]	
	50.0	0	

[a] After 24 hrs, survival of adults was recorded (percent relative to controls). [b] Mean of three replicates. Means followed by the same letter within a column after ± standard error values are not significantly different in a Student-Newman-Keuls (SNK) test at $P < 0.05$ (treatments are compared by concentration to control). [c] The LD_{50} is the lethal concentration producing 50% mortality.

Acute toxicity on larvae of last stage of S. frugiperda

Argentatin A and argentatin B showed moderated acute toxicity with 11.0 and 17.0% of survival at 50.0 ppm, respectively. However, toosendanin and MeOH extract from *P. argentatum* showed a potent acute toxicity (0% of survival) on larvae of last stage of *S. frugiperda* at 35.0 and 15.0 ppm, respectively. The LD_{50} values of **3** and MeOH extract were 11.5 and 3.1 ppm, respectively (Table V). In order to determine the site of inhibition on the insect growth regulatory activity (IGR) and the acute toxicity, the effect of argentatins A and

B, MeOH extract, and toosendanin on acetylcholinesterase activity was studied.

Inhibition of acetylcholinesterase

According to Ellman's method (1961), was investigated the mode of action of insect growth regulatory activities by studying the inhibitory activity of argentatin A, argentatin B, MeOH extract and toosendanin on AChE and their effect are outlined in Table VI. In similar form to the acute toxicity argentatin A, MeOH extract and toosendanin showed the greatest effect with 90.2, 83.5 and 96.3% of inhibition at 50.0, 5.0 and 25.0 ppm, respectively; unlike, argentatin B that showed an inhibitory effect by 62.0%, at 50.0 ppm. As shown in Table VI argentatin A, toosendanin, and MeOH extract were strong inhibitor of acetylcholinesterase activity. Toosendanin at 15.0 ppm showed stronger inhibition than the argentatins (Fig. 2). While MeOH extract showed similar inhibitory activity with toosendanin, above 25.0 ppm (Fig. 2).

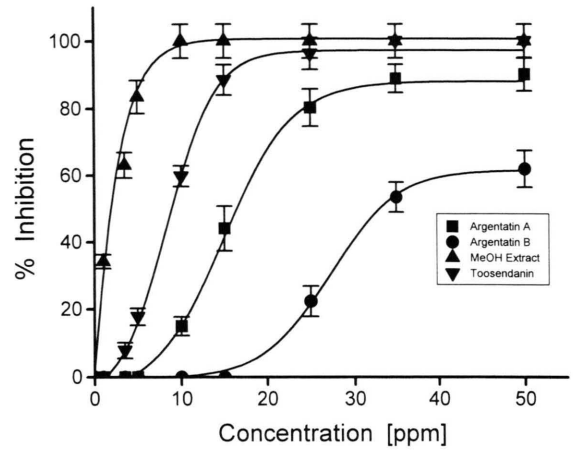

Fig. 2. Dose-dependent inhibition of acetylcholinesterase activity by major guayule compounds, toosendanin and MeOH extract. Each value represents mean ± S. E. (n=5). The inhibition efficacy was expressed as a percentage of enzyme activity inhibited compared with the control value (100%) of argentatin A (■), argentatin B (●), toosendanin (▼) and MeOH extract (▲). Acetylcholinesterase activity of the 100% control correspond to 24.3 mmol ATC split/mg protein × min.

Table VI. Inhibitory activity of compounds 1–3 and MeOH extract against acetylcholinesterase from adult heads of S. *frugiperda*.[a]

Compounds	Concentration (ppm)	% Inhibition[c]
Control		[b]
Argentatin A	5.0	[b]
	10.0	15.0 ± 2.5
	15.0	44.1 ± 6.7
	50.0	90.2 ± 4.9
Argentatin B	5.0	[b]
	25.0	22.4 ± 4.5
	35.0	53.5 ± 4.5
	50.0	62.0 ± 5.5
MeOH extrc.	1.0	34.2 ± 2.1
	3.5	63.1 ± 3.8
	5.0	83.5 ± 4.9
	25.0	100
	35.0	100
	50.0	100
Toosendanin	3.5	7.9 ± 2.3
	5.0	17.8 ± 2.5
	10.0	59.8 ± 3.1
	15.0	88.5 ± 4.5
	50.0	100

[a] After incubation for 20 min, changes in absorbance at 420 nm were recorded and compared with control. [b] No effect was observed. [c] Means of three replicates each value represent ± S. E. (n = 5).

In addition, MeOH extract, toosendanin and argentatin A inhibited AChE activity in a dose-dependent manner (Fig. 2) confirming that these compounds are the active inhibitors of acetylcholinesterase in *Parthenium argentatum*.

The compounds with furanic groups showed to have more potent inhibitory activity. In other words, the presence of a ring with 7 members group decreases the strength of these compounds on inhibition of AChE. We suggested that inhibitory activities of MeOH extract were caused not by one strong inhibitor but by synergistic activity. Which confirm the results obtained by Ortego *et al*, (1999), with furanic limonoids as azadirone and a mixture of 1,7-di-O-acetylhavanensin and 3,7-di-O-acetylhavanensin from *Trichilia havanensis* (Meliaceae), those compounds, significantly reduce digestive protease and esterase activities in the midgut of Colorado potato beetle (*Leptinostarsa decemlineata*) (Ortego *et al.*, 1999). Inhibition of AChE activities by terpenoids have been reported on related insecticidal effects (Grundy *et al.*, 1985; and Ryan *et al.*, 1988). Therefore, the plant terpenes may be available as an AChE antagonist (Miyazawa *et al.*, 1997).

These facts show that acute toxicity and growth inhibition observed may be due to the inhibition

of acetylcholinesterase. Since, this target was demonstrated also for *Spodoptera litura* larvae and adult migratory grasshopper's *Melanoplus sanguinipes*. Since, the bark extract of *Melia toosendan*; whose major component is toosendanin; inhibited midgut esterase activity (Feng *et al.*, 1995) and also on *Leptinostarsa decemlineata* (Ortego *et al.*, 1999).

In summary, the insecticidal activity of MeOH extract from aerial parts of *P. argentatum* may be due to a synergistic effects shown by the principal components of the mixture in the test systems used in this investigation. Comparison of insecticidal activities of EtOH extracts from *Azadirachta indica* and *Melia azedarach* (Mikolajczak *et al.*, 1989) showed an inhibitory effect of 100 and 58% at 16 and 80 ppm, respectively, which is indicative of the potency of the MeOH extract from *P. argentatum*.

Thus, the effect of the argentatin A and B on reducing insect growth, increasing development time and mortality of *S. frugiperda* is similar to that of limonoids such as gedunin and cedrelone (Arnason *et al.*, 1993; and Govindachari *et al.*, 1995). The mode of action of these compounds is being investigated and may be due to a combination of antifeedant action as midgut esterase inhibition and postdigestive toxicity, as found for other limonoids (Champagne *et al.*, 1992; Nakatani *et al.*, 1994; and Isman *et al.*, 1996) and extracts (Feng *et al.*, 1995). In addition, the presence of a furanyl group seems to be important for these activities as showed for the most potent compounds in this study argentatin A and toosendanin. Furthermore, a great inanition observed may be due to the inhibition by acetylcholinesterase as well.

The activity of this neotropical plant and their metabolites and MeOH extract is comparable to the commercial insecticide toosendanin, which suggests potential for further development of these materials. However, no neotropical triterpene has been found with the outstanding activity of azadirachtin (Champagne *et al.*, 1992).

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