

Full Length Research Paper

## Activity of *Ricinus communis* (Euphorbiaceae) against *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

M. A. Ramos-López<sup>1\*</sup>, S. Pérez G.<sup>2</sup>, C. Rodríguez-Hernández<sup>3</sup>, P. Guevara-Fefer<sup>4</sup> and M. A. Zavala-Sánchez<sup>2</sup>

<sup>1</sup>Doctorado en Ciencias Biológicas, Universidad Autónoma Metropolitana, Edificio A, II piso, Area de posgrados de CBS, Calzada del Hueso 1100, Col. Villa Quietud, México, D.F., CP. 04960.

<sup>2</sup>Universidad Autónoma Metropolitana-Xochimilco. Calzada del Hueso 1100, Col. Villa Quietud, México, D.F., CP. 04960.

<sup>3</sup>Colegio de Postgraduados en Ciencias Agrícolas, Campus Montecillos Km 36.5 Carretera México-Texcoco, Texcoco, Edo. Mex, CP 56230.

<sup>4</sup>Universidad Nacional Autónoma de México, Facultad de Ciencias, Universidad 3000, Circuito Exterior s/n, Ciudad Universitaria, México, D. F., CP 04510.

Accepted 19 January, 2010

One of the most studied plant species with insecticidal properties is the castor bean *Ricinus communis*. However, its activity against *Spodoptera frugiperda* is unclear. Therefore, to determinate the insecticidal and insectistatic activities of methanol, hexane and ethyl acetate extracts of the seeds and leaves of *R. communis*, castor oil and ricinine were tested at different concentrations against *S. frugiperda*. This study demonstrated for the first time, that the castor oil and ricinine are active ingredients of *R. communis* that acts against *S. frugiperda* and that each of the seed extracts exhibited better insecticidal and insectistatic activity than the leaf extracts. The half maximum larvae viability concentration (LVC<sub>50</sub>) were  $0.38 \times 10^3$  ppm for the ricinine,  $0.75 \times 10^3$  ppm for a methanol extract of seeds,  $1.97 \times 10^3$  ppm for an ethyl acetate seed extract,  $2.69 \times 10^3$  ppm for the castor oil,  $4.83 \times 10^3$  ppm for a methanol extract of leaves,  $5.07 \times 10^3$  ppm for an ethyl acetate extract of leaves,  $9.95 \times 10^3$  ppm for a hexane extract of seeds and  $10.01 \times 10^3$  ppm for a hexane extract of leaves.

**Key words:** *Ricinus communis*, *Spodoptera frugiperda*, insecticidal, insectistatic, castor oil, ricinine.

### INTRODUCTION

Insects represent one of the major causes of crop and grain loss worldwide (Ferry et al., 2004). Synthetic chemical pesticides have been the most widely adopted method for field and post-harvest protection of crops against insect pests; however, there are many problems associated with the extensive use of these compounds, such as build-up of pesticide resistance, negative impact on natural enemies,

in addition to negative environmental and health impacts. These facts, combined with consumer demand for residue-free food and the increasingly stringent environmental regulations governing pesticide use (Isman, 2000), have resulted in renewed interest by agrochemical companies in the development and use of plants and their natural products for pest management.

The fall armyworm *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) has been identified as a polyphagous insect and several pest of many crops (Sparks, 1979) especially maize in Latin America (Andrews, 1988); yield reductions in maize due to feeding of the *S. frugiperda* have been reported as high as 34% (Cruz et al., 1996).

*Ricinus communis* L. (Euphorbiaceae), the castor bean, has been used to control insect pests in several crops. Aqueous castor bean leaf extract has been shown

\*Corresponding author. E-mail: [agromyke@yahoo.com](mailto:agromyke@yahoo.com). Tel/ Fax: +52 (55) 54 83 74 10.

**Abbreviations:** MeOH, Methanol; Hx, hexane; AcoEt, ethylacetate; Sd, seed extract; Lf, leaf extract; CO, castor oil; Ric, ricinine; VC, variability coefficient; LVC<sub>50</sub>, half maximum larvae viability concentration; ANOVA, analysis of variance.

to possess insecticidal activity against *Callosobruchus chinensis* (Coleoptera: Bruchidae) (Upasani et al., 2003), *Cosmopolites sordidus* (Coleoptera: Curculionidae) (Tinzaara et al., 2006), *Culex pipiens*, *Aedes caspius*, *Culiseta longiareolata* and *Anopheles maculipennis* (Diptera: Culicidae) (Aouinty et al., 2006); whereas amethanolic leaf extract had insecticidal activity against *C. chinensis* (Upasani et al., 2003). In addition, both aqueous and acetone leaf extracts had different activity against *Acromyrmex lundii* (Hymenoptera: Formicidae) (Caffarini et al., 2008). Castor oil had insecticidal activity against *Zabrotes subfasciatus* (Coleoptera: Bruchidae) (Mushobozzy et al., 2009). Furthermore, Rodríguez-Hernández (2005) found that the aerial parts also had insectistatic activity against the same insect in a corn crop and because *S. frugiperda* represents a pest of many crops (Kumar and Mihm 2002; Molina et al., 2003), it has been used as a model species for testing for the insecticidal and insectistatic activities of many plants (Céspedes et al., 2005).

Earlier studies of aerial parts of this plant have reported the presence of ricinine (Kang et al., 1985; Leite et al., 2005), N-demetilricinine (Skursky et al., 1969; Kang et al., 1985), six flavonoids: glycosides kaempferol-3-O- $\beta$ -D-xylopyranoside, kaempferol-3-O- $\beta$ -D-glucopyranoside, quercetin-3-O- $\beta$ -D-xylopyranoside, quercetin-3-O- $\beta$ -D-glucopyranoside, kaempferol-3-O- $\beta$ -rutinoside, quercetin-3-O- $\beta$ -rutinoside (Kang et al., 1985), as well as gallic acid, gentistic acid and rutin (Chen et al., 2008). Also, it was found that the seeds contain castor oil (Achaya et al., 1964; Kim, 2001), ricin (Achaya et al., 1964; Darby et al., 2001), the protein allergens Ric c 1 and Ric c3 (Bashir et al., 1998; Pantoja-Uceda et al., 2003) and ricinine (Bashir et al., 1998; Yudalshev, 2001). In the present study, the activity of methanol (MeOH), hexane (Hx), ethyl acetate (AcoEt) extracts of seeds (MeOH-Sd; Hx-Sd; AcoEt-Sd respectively) and leaves (MeOH-Lf; Hx-Lf; AcoEt-Lf, respectively) of *R. communis*, as well as castor oil (CO) and ricinine (Ric) were evaluated against *S. frugiperda*.

## MATERIALS AND METHODS

### Plant material

Seeds and leaves of *R. communis* were collected in Ecatepec of Morelos, State of México, México, in December, 2007. Species identity was authenticated by Stephen D. Koch (Ph.D) and a voucher (CHAPA-001) was deposited in the Herbario-Hortorio of Colegio de Postgraduados en Ciencias Agrícolas. Leaves and seeds of the plant were dried in the shade.

### Extracts preparation

Dried and powdered leaves or seeds (1 kg) were extracted with hexane (3.0 L) under reflux for 4 h. The extracts were then filtered. The leaf or seed were extracted with methanol or ethyl acetate (3.0 L). The extracts were filtered and the solvents removed under reduced pressure using a rotatory evaporator. The yields of the

extracts were: Hx-Sd, 373.1 g; Hx-Lf, 21.1 g; MeOH-Sd, 233.8 g; MeOH-Lf, 98.2 g; AcoEt-Sd, 286.2 g; AcoEt-Lf, 83.3 g.

### Isolation of ricinine

Dried and powdered leaves or seeds of *R. communis* (4 kg) were extracted with Hx (12 L), then was filtered and extracted with MeOH (12 L). The solvent was evaporated and the residue was chromatographed on silica gel (70-230 mesh) and eluted with chloroform, increasing the polarity with methanol. Fourteen (14) fractions were obtained according to the technique of Kang et al. (1985). Fractions 7, 8 and 9 produced a white solid (0.0013% to leaves and 0.022% to seeds), which was chromatographed again.

$^1\text{H NMR } \delta$  (ppm) 7.76 (d, 1H,  $J = 7.7$  Hz, H-6), 6.22 (d, 1H,  $J = 7.7$  Hz, H-5), 4.02 (s, 3H, 230 CH<sub>3</sub>O) and d 3.53 (s, 3H, CH<sub>3</sub>N).  
 $^{13}\text{C NMR } \delta$  (ppm): 36.75 (NMe), 56.72 (OMe), 86.74 (C-3), 113.49 (CN), 144.14 (C-6), 160.48 (C-2), 171.68 (C-4).

### Insects

First instar larvae of *S. frugiperda* were obtained from the Entomology Laboratory of International Maize and Wheat Improvement Center, "El Batán", Texcoco, State of México, México.

### Artificial diet

1 Kg of artificial diet were prepared with: yeast 40 g, lepidopteran diet (Product # F0635S.W. Corn Borer, Bio-Serv, Canada) 60 g, powdered maize 100 g, sterile maize ear 20 g, neomycin sulphate 6 g, vitamin mix fortification lepidoptera, Bio-Serv 10 g, sorbic acid 1.7 g, methyl p-hydroxybenzoate 1.7 g, agar 10 g, formaldehyde 2.5 ml, ethanol 17 ml, distilled water 800 ml. All the components were mixed (Bergvinson and Kumar, 1997).

### Bioassay

Groups of 100 larvae were randomly selected for bioassays to each concentration. Preliminary screening of MeOH, Hx, AcoEt extracts from seeds and leaves, CO and Ric were carried out at seven concentrations ranging from 0.016 to 24,000 ppm; each extract was mixed with the larval diet ingredients during preparation. Based on preliminary screening results, all of the extracts were subjected to concentration-response bioassays for insecticidal or insectistatic activities against *S. frugiperda*. Samples of seven concentrations ranging from 16 to 16,000 ppm were used for CO, Ric, MeOH-Sd, AcoEt-Sd and AcoEt-Lf; whereas concentrations ranging from 160 to 24,000 ppm were used for MeOH-Lf, Hx-Sd and Hx-Lf. Each treatment utilized a negative control diet; media were poured into 100 acrylic glasses (Bio-Serv N° 9051) that were left solidify at room temperature for 24 h. First instar *S. frugiperda* larvae were then transferred individually and the glasses were closed with a top (Bio-Serv N° 9049). All samples were maintained at  $27 \pm 2^\circ\text{C}$ ,  $70 \pm 5\%$  relative humidity and 14/10 h light/dark. The pupae were weighed 24 h after pupation and then moved to another glass for development to the adult stage. The following parameters were evaluated: the length of the larval and pupal period; the larval and pupal viability, as well as the pupal weights at 24 h. According to Rodríguez-Hernández and Vendramim (1996), extension of the larval phase by a treatment is termed "growing inhibition", extension of the pupae phase duration is termed "developing inhibition" and reduction of pupal weight (relative to the control) is termed "feeding inhibition". The half maximum viability larvae concentration (VLC<sub>50</sub>) represents the concentration at which 50% of the larvae lived during all larvae phase.

**Table 1.** Mean ( $\pm$  SE) larvae and pupae duration, larvae and pupae viability and pupae weight of *S. frugiperda* with methanol extract of the seeds and leaves (MeOH-Sd; MeOH-Lf) of *R. communis*.

Concentration (ppm)	Duration (d)		Viability (%)		Pupae weight (mg)
	Larvae	Pupae	larvae	Pupae	
<b>MeOH-Sd</b>					
Control	22.6 $\pm$ 0.20 d	12.1 $\pm$ 0.09 b	94 $\pm$ 1.87 a	95.7 $\pm$ 1.08 a	235.0 $\pm$ 2.63 a
16	22.7 $\pm$ 0.21 d	12.3 $\pm$ 0.08 b	94 $\pm$ 1.87 a	95.7 $\pm$ 1.97 a	234.4 $\pm$ 2.63 a
112	23.1 $\pm$ 0.22 cd	12.3 $\pm$ 0.09 b	93 $\pm$ 1.23 a	95.7 $\pm$ 2.06 a	233.0 $\pm$ 3.10 a
160	23.9 $\pm$ 0.23 bc	12.3 $\pm$ 0.10 b	90 $\pm$ 1.58 a	95.6 $\pm$ 2.08 a	228.7 $\pm$ 2.61 a
560	24.7 $\pm$ 0.27 b	12.5 $\pm$ 0.12 ab	75 $\pm$ 2.74 b	94.1 $\pm$ 2.11 a	226.8 $\pm$ 2.67 a
1 600	27.4 $\pm$ 0.55 a	12.9 $\pm$ 0.23 a	36 $\pm$ 1.87 c	76.9 $\pm$ 2.21 b	209.5 $\pm$ 3.75 b
9 600	&	‡	0*	0*	‡
16 000	&	‡	0*	0*	‡
**LVC <sub>50</sub>			0.75 $\times$ 10 <sup>3</sup>	ppm	
VC	10.8	7.5	26.9	6.5	11.5
<b>MeOH-Lf</b>					
Control	20.5 $\pm$ 0.38 d	11.5 $\pm$ 0.12 b	96 $\pm$ 1.87 d	96.9 $\pm$ 1.30 a	233.0 $\pm$ 3.13 a
160	22.0 $\pm$ 0.41 cd	11.5 $\pm$ 0.11 b	96 $\pm$ 1.87 d	96.9 $\pm$ 2.09 a	227.4 $\pm$ 2.80 ab
560	22.2 $\pm$ 0.48 c	11.6 $\pm$ 0.12 b	94 $\pm$ 1.87 d	95.7 $\pm$ 1.07 a	225.9 $\pm$ 2.77 ab
1 600	27.0 $\pm$ 0.47 b	11.7 $\pm$ 0.16 ab	79 $\pm$ 1.87 c	83.5 $\pm$ 2.58 b	219.2 $\pm$ 2.75 bc
4 000	29.2 $\pm$ 0.44 a	11.8 $\pm$ 0.16 ab	70 $\pm$ 1.58 b	81.4 $\pm$ 1.53 b	217.7 $\pm$ 2.99 bc
8 000	30.0 $\pm$ 0.44 a	12.3 $\pm$ 0.19 a	54 $\pm$ 1.87 a	59.3 $\pm$ 2.50 c	211.3 $\pm$ 3.16 c
16 000	32.7 $\pm$ 0.80	13.3 $\pm$ 0.34	37 $\pm$ 2.00 *	40.0 $\pm$ 1.51	198.0 $\pm$ 4.49
24 000	&	‡	0*	0*	‡
**LVC <sub>50</sub>			4.83 $\times$ 10 <sup>3</sup>	ppm	
VC	21.9	9.9	20.1	16.9	12.3

\*Values were not used in statistical analysis; & = Not one of the individuals completed its larval stage; ‡ = There were no pupae; VC = Variability coefficient; \*\*LVC<sub>50</sub> was calculated with the larvae mortality; different letters represent statistically significant differences; SE = standard error of mean.

### Statistical analysis

For statistical analyses, one way ANOVA analysis and (0.05%) TUKEY test with SAS software (Delwiche and Slaughter, 2002). The half maximum viability larvae concentration (LVC<sub>50</sub>) was calculated using Probit program (Raymond, 1985).

### RESULTS

In the experiments of insecticidal activity, the activity of the methanol seeds and leaves extracts are summarized in Table 1. The MeOH-Sd produced 0% larvae viability at concentrations ranging from 16,000 and 9,600 ppm; at concentrations of 1,600 and 560 ppm, larval viability rates were 36 and 75%. With this extract, pupal viability was 76.9% at 1,600 ppm. A larval viability rate of 0% was achieved with MeOH-Lf at a concentration of 24,000 ppm. At concentrations of 16,000, 8,000 and 4,000 ppm, the larval viability rates were 37, 54 and 70%, respectively; whereas the pupal viability rates at 16,000, 8,000, 4,000 and 1,600 ppm were 40, 59.3, 81.4 and 83.5%, respectively. Hx-Sd caused 11, 43, 65, 73 and 79% larval viability at concentrations of 24,000, 16,000, 9,600, 4,000 and 1,600 ppm, respectively; whereas at concentrations

of 24,000 and 16,000 ppm, were observed 54.5 and 88.4% pupal viability. With Hx-Lf, the larval viability rates were 19, 46 and 76% at concentration of 24,000, 16,000 and 9,600 ppm, respectively; whereas the pupal viability was 68.4% at a concentration of 24,000 ppm (Table 2). The rates of larval viability with AcoEt-Sd were 11, 19 and 60% at 16,000, 9,600 and 1,600 ppm, respectively; the pupal viability rates were 54.5 and 78.9% at concentrations of 16,000 and 9,600 ppm, respectively. AcoEt-Lf caused 34, 50 and 64% larval viability at concentrations of 16,000, 9,600 and 1,600 ppm, respectively, and 16,000 ppm resulted in 85.3% pupal viability (Table 3). A larval viability rate of 0% was achieved with CO at a concentration of 16,000 ppm and at concentrations of 9,600, 1,600 and 560 ppm, the larval viability rates were 45, 69 and 80%, respectively; whereas the pupal viability rates at 9,600 ppm was 88.9%. The Ric caused 0% larvae viability at concentrations ranging from 1,600 to 16,000 ppm; at concentrations of 560 and 160 ppm, larval viability rates were 44 and 60%, and pupal viability was 54.5% at 560 ppm (Table 4).

In the experiment of insectistatic activity, the MeOH-Sd treatment at 1,600 ppm showed growing inhibition and developing inhibition of *S. frugiperda*: it prolonged the

**Table 2.** Mean ( $\pm$  SE) larvae and pupae duration, and viability and pupae weight of *S. frugiperda* with hexane extract of the seeds and leaves (Hx-Sd; Hx-Lf) of *R. communis*.

Concentration (ppm)	Duration (d)		Viability (%)		Pupae weight (mg)
	Larvae	Pupae	Larvae	Pupae	
<b>Hx-Sd</b>					
Control	22.8 $\pm$ 0.23 f	12.1 $\pm$ 0.11 b	92 $\pm$ 2.55 a	97.8 $\pm$ 1.26 a	230.1 $\pm$ 2.30 a
160	23.6 $\pm$ 0.22 ef	12.1 $\pm$ 0.10 b	90 $\pm$ 2.74 ab	96.7 $\pm$ 2.03 a	230.1 $\pm$ 2.30 a
560	24.6 $\pm$ 0.30 de	12.2 $\pm$ 0.11 ab	85 $\pm$ 2.24 ab	96.5 $\pm$ 1.48 a	229.0 $\pm$ 2.40 a
1 600	25.7 $\pm$ 0.34 cd	12.2 $\pm$ 0.12 ab	79 $\pm$ 4.00 bc	94.9 $\pm$ 1.45 ab	225.3 $\pm$ 2.50 ab
4 000	26.5 $\pm$ 0.43 c	12.3 $\pm$ 0.14 ab	73 $\pm$ 2.55 cd	94.5 $\pm$ 0.25 abc	222.7 $\pm$ 2.80 ab
9 600	28.1 $\pm$ 0.43 b	12.4 $\pm$ 0.14 ab	65 $\pm$ 3.54 d	92.3 $\pm$ 1.33 bc	217.8 $\pm$ 2.79 b
16 000	30.1 $\pm$ 0.51 a	12.7 $\pm$ 0.17 a	43 $\pm$ 2.00 e	88.4 $\pm$ 3.21 c	214.2 $\pm$ 4.00 b
24 000	31.6 $\pm$ 0.86*	13.2 $\pm$ 0.48*	11 $\pm$ 1.00 *	54.5 $\pm$ 3.33*	210.5 $\pm$ 3.00*
**LVC <sub>50</sub>			9.95 $\times$ 10 <sup>3</sup>	ppm	
VC	14.2	8.6	22.7	16.9	10.3
<b>Hx-Lf</b>					
Control	21.6 $\pm$ 0.18 e	12.1 $\pm$ 0.12 a	93 $\pm$ 3.00 a	97.8 $\pm$ 1.23 a	238.4 $\pm$ 3.06 a
160	21.9 $\pm$ 0.25 de	12.1 $\pm$ 0.15 a	92 $\pm$ 2.55 a	97.8 $\pm$ 1.30 a	237.8 $\pm$ 2.87 a
560	22.1 $\pm$ 0.21 de	12.2 $\pm$ 0.14 a	92 $\pm$ 3.00 a	97.8 $\pm$ 1.34 a	234.4 $\pm$ 3.16 ab
1 600	22.6 $\pm$ 0.24 cd	12.3 $\pm$ 0.13 a	88 $\pm$ 2.55 ab	97.7 $\pm$ 2.11 a	229.3 $\pm$ 3.53 ab
4 000	23.1 $\pm$ 0.25 c	12.3 $\pm$ 0.13 a	88 $\pm$ 2.55 ab	96.6 $\pm$ 2.12 a	226.6 $\pm$ 3.02 ab
9 600	24.5 $\pm$ 0.27 ab	12.4 $\pm$ 0.12 a	76 $\pm$ 2.45 b	96.1 $\pm$ 2.50 a	223.1 $\pm$ 3.02 bc
16 000	25.6 $\pm$ 0.41 a	12.5 $\pm$ 0.15 a	46 $\pm$ 2.92 c	89.1 $\pm$ 3.22 a	219.1 $\pm$ 4.14 bc
24 000	27.1 $\pm$ 0.51*	13.1 $\pm$ 0.24*	19 $\pm$ 1.87 *	68.4 $\pm$ 2.86*	211.3 $\pm$ 6.43*
**LVC <sub>50</sub>			10.01 $\times$ 10 <sup>3</sup>	ppm	
VC	11.1	10.0	20.5	5.3	12.9

\*Values were not used in statistical analysis; VC = Variability coefficient; \*\*LVC<sub>50</sub> was calculated with the larvae mortality; different letters represent statistically significant differences; SE = standard error of mean.

larval and pupal phases to 4.8 d and 0.8 d, at the same concentration; the treatment also resulted in feeding inhibition because the pupae weighed was only 89.1% of the control group. MeOH-Lf caused growing inhibition (in the larval phase) to increase to 12.2, 9.5, 8.7, 6.5 and 1.7 d at 16,000, 8,000, 4,000, 1,600 and 560 ppm, respectively, and developing inhibition increased the pupal phase to 1.8 and 0.8 d at 16,000 and 8,000 ppm. The weights were inhibited by 15, 9.3, 6.6 and 5.9% compared to the control at 16,000, 8,000, 4,000 and 1,600 ppm, respectively (Table 1). Hx-Sd resulted in growing inhibition at 24,000, 16,000, 9,600, 4,000, 1,600 and 560 ppm, prolonging the larval phase to 8.8, 7.3, 5.3, 3.7, 2.9 and 1.8 d, respectively. Development inhibition increased the pupal phase to 1.1 and 0.6 d and feeding inhibition because the weight reached 91.5, 93.1 and 94.7% compared with the control weight at 24,000, 16,000 and 9,600 ppm, respectively. Hx-Lf increased the larval phase to 5.5, 4.0, 2.9, 1.5 and 1 d at 24,000, 16,000, 9,600, 4,000 and 1,600 ppm, respectively, and the pupal phase was extended 1 d at 24,000 ppm. Hx-Lf caused feeding inhibition to reach 88.6, 91.9 and 93.6% of the weight compared with the control at 24,000, 16,000 and 9,600 ppm, respectively (Table 2). AcoEt-Sd increased the larval phase to 11.1, 8.4, 4.1, 1.9 and 1.5 d

at 16,000, 9,600, 1,600, 560 and 160 ppm, respectively; the extract increased the pupal phase to 1.5 and 0.8 d at 16,000 and 9,600 ppm. The pupal weights were 76.9, 89.7 and 95% at 16,000, 9,600 and 1,600 ppm, respectively. AcoEt-Lf increased the larval phase to 7.5, 5.4, 3.9, 2.5 and 1.9 d at 16 000, 9 600, 1 600, 560 and 160 ppm, respectively; whereas the pupal phase was increased to 0.9 d at 16,000 ppm and the pupal weights were 88.6, 91.1 and 95.9% of the weight of the control (Table 3). CO resulted in growing inhibition at 9,600, 1,600 and 560 ppm, prolonging the larval phase to 3.8, 2.8 and 1.7 d, respectively. Development inhibition increased the pupal phase to 0.8 d at 9,600 ppm and weight was 89.9 and 91% of the control weight at 9,600 and 1,600 ppm. Ric increased the larval phase to 14.1, 6.6 and 3.1 d at 560, 160 and 112 ppm, respectively and the pupal phase was extended 2.1 d at 560 ppm. It caused feeding inhibition because the weight was only 78.4 and 89.1% of the control weight at 560 and 160 ppm (Table 4).

## DISCUSSION

Mushobozy et al. (2009) demonstrated that castor oil had

**Table 3.** Mean ( $\pm$  SE) larvae and pupae duration, larvae and pupae viability and pupae weight of *S. frugiperda* with ethyl acetate extract of the seeds and leaves (AcoEt-Sd; AcoEt-Lf) of *R. communis*.

Concentration (ppm)	Duration (d)		Viability (%)		Pupae weight (mg)
	Larvae	Pupae	Larvae	Pupae	
<b>AcoEt-Sd</b>					
Control	22.4 $\pm$ 0.21 c	12.0 $\pm$ 0.10 a	93 $\pm$ 1.26 a	97.8 $\pm$ 1.33 a	249.4 $\pm$ 2.73 a
16	22.6 $\pm$ 0.21 c	12.1 $\pm$ 0.09 a	93 $\pm$ 2.00 a	96.7 $\pm$ 1.32 a	248.0 $\pm$ 2.42 ab
112	22.8 $\pm$ 0.23 c	12.1 $\pm$ 0.09 a	90 $\pm$ 3.54 a	96.7 $\pm$ 1.35 a	246.9 $\pm$ 2.80 ab
160	23.9 $\pm$ 0.31 b	12.2 $\pm$ 0.10 a	86 $\pm$ 1.87 a	95.3 $\pm$ 1.17 a	244.0 $\pm$ 2.72 ab
560	24.3 $\pm$ 0.27 b	12.2 $\pm$ 0.11 a	85 $\pm$ 2.24 a	95.3 $\pm$ 2.21 a	242.1 $\pm$ 2.59 ab
1 600	26.5 $\pm$ 0.27 a	12.5 $\pm$ 0.12 a	60 $\pm$ 3.54 b	91.7 $\pm$ 2.46 a	237.0 $\pm$ 2.44 b
9 600	30.8 $\pm$ 0.61*	12.8 $\pm$ 0.18*	19 $\pm$ 1.87*	78.9 $\pm$ 5.59*	223.7 $\pm$ 4.15*
16 000	33.5 $\pm$ 0.99*	13.5 $\pm$ 0.43*	11 $\pm$ 1.0 *	54.5 $\pm$ 3.33*	191.7 $\pm$ 3.26*
**LVC <sub>50</sub>			1.97 $\times$ 10 <sup>3</sup>	ppm	
VC	11.1	7.5	15.0	4.1	9.8
<b>AcoEt-Lf</b>					
Control	23.0 $\pm$ 0.22 f	12.1 $\pm$ 0.12 b	94 $\pm$ 1.87 a	97.9 $\pm$ 1.33 a	238.7 $\pm$ 2.32 a
16	23.4 $\pm$ 0.23 f	12.1 $\pm$ 0.11 b	93 $\pm$ 2.55 a	96.8 $\pm$ 1.33 a	238.7 $\pm$ 2.44 a
112	23.9 $\pm$ 0.23 ef	12.1 $\pm$ 0.11 b	93 $\pm$ 2.92 a	96.8 $\pm$ 2.01 a	238.1 $\pm$ 2.54 a
160	24.9 $\pm$ 0.36 de	12.2 $\pm$ 0.13 b	89 $\pm$ 1.87 a	96.6 $\pm$ 1.40 a	235.5 $\pm$ 2.80 a
560	25.5 $\pm$ 0.40 cd	12.2 $\pm$ 0.11 b	88 $\pm$ 2.00 a	96.6 $\pm$ 1.42 a	233.6 $\pm$ 2.17 a
1 600	26.9 $\pm$ 0.46 bc	12.3 $\pm$ 0.13 ab	64 $\pm$ 1.87 b	93.8 $\pm$ 2.73 a	228.8 $\pm$ 3.48 ab
9 600	28.4 $\pm$ 0.54 b	12.6 $\pm$ 0.14 ab	50 $\pm$ 3.16 c	92.0 $\pm$ 2.42 a	217.4 $\pm$ 2.82 bc
16 000	30.5 $\pm$ 0.55 a	13.0 $\pm$ 0.20 a	34 $\pm$ 2.92 d	85.3 $\pm$ 2.22 b	211.6 $\pm$ 3.96 c
**LVC <sub>50</sub>			5.07 $\times$ 10 <sup>3</sup>	ppm	
VC	14.5	8.9	30.0	6.6	10.7

\*Not taken values to statistical analysis; VC = Variability coefficient; \*\*LVC<sub>50</sub> was calculated with the larvae mortality; different letters represent statistically significant differences; SE = standard error of mean.

insecticidal activity against *Z. subfasciatus*; moreover, ricinine had insecticidal activity against *Myzus persicae* (Homoptera: Aphididae) (Olaifa et al., 1991) and *Atta sexdens rubropilosa* (Hymenoptera: Formicidae) (Bigi et al., 2004).

Then the MeOH-Sd induced 0% larval viability at 16,000 and 9,600 ppm (LVC<sub>50</sub> 0.75  $\times$  10<sup>3</sup> ppm) and the insectistatic activity was evident beginning at 160 ppm. These facts suggest that the insecticidal and insectistatic activity of MeOH-Sd could be due to the castor oil and ricinine present on the seeds of *R. communis*. The insecticidal activity of Hx-Sd was low (LVC<sub>50</sub> 9.95  $\times$  10<sup>3</sup> ppm); it was shown only at concentrations of 560 ppm or higher and this activity might be due to castor oil, because ricinine is not soluble in hexane. AcoEt-Sd had an activity intermediate between MeOH-Sd and Hx-Sd (LVC<sub>50</sub> 1.97  $\times$  10<sup>3</sup> ppm); its insectistatic activity was detectable beginning from 160 ppm. This result might be due to the solubility of ricinine in AcoEt is lower than in MeOH and also that castor oil is present in this extract, so that this is the reason, the insecticidal effect of AcoEt-Sd is lower than MeOH-Sd.

The MeOH-Lf extract achieved 0% viability rate against larvae at 24,000 ppm (LVC<sub>50</sub> 4.83  $\times$  10<sup>3</sup> ppm) and it had

insectistatic activity beginning at 560 ppm, which suggests that ricinine is the compound responsible for this activity; ricinine is also present in the leaf (Kang et al., 1985; Upanasi et al., 2003), but in minor concentration than in seed. Hx-Lf exhibited the lowest insecticidal activity (LVC<sub>50</sub> 10.01  $\times$  10<sup>3</sup> ppm), but had insectistatic activity beginning at 1,600 ppm. These results suggest that a compound other than ricinine is responsible for the observed insectistatic activity of these extracts because this alkaloid is not soluble in hexane. AcoEt-Lf had lower activity than MeOH-Lf (LVC<sub>50</sub> 5.07  $\times$  10<sup>3</sup> ppm) and its insectistatic activity was detectable beginning from 160 ppm; it is possible that this activity is due to ricinine because the solubility of the compound is lower in AcoEt than in MeOH. Thus, AcoEt-Lf had less ricinine than MeOH-Lf. When included in the diet of *S. frugiperda*, ricinine induced 0% larval viability at 16,000; 9,600 and 1,600ppm (LVC<sub>50</sub> 0.38  $\times$  10<sup>3</sup> ppm) and the insectistatic activity was evident beginning at 112 ppm, then when the castor oil included, it induced 0% larval viability at 16,000 ppm (LVC<sub>50</sub> 2.69  $\times$  10<sup>3</sup> ppm) and the insectistatic activity was detectable at 560 ppm.

From our results we demonstrated for the first time, that the castor oil and ricinine are active ingredients of *R.*

**Table 4.** Mean ( $\pm$  SE) larvae and pupae duration, larvae and pupae viability and pupae weight of *S. frugiperda* with castor oil (CO) and ricinine (Ric).

Concentration (ppm)	Duration (d)		Viability (%)		Pupae weight (mg)
	Larvae	Pupae	Larvae	Pupae	
<b>CO</b>					
Control	22.2 $\pm$ 0.14 d	12.1 $\pm$ 0.11 b	94 $\pm$ 2.45 a	97.8 $\pm$ 1.30 a	247.6 $\pm$ 2.78 a
16	22.4 $\pm$ 0.14 cd	12.1 $\pm$ 0.11 b	94 $\pm$ 1.87 a	97.8 $\pm$ 1.30 a	246.7 $\pm$ 2.37 a
112	22.8 $\pm$ 0.17 cd	12.2 $\pm$ 0.11 b	93 $\pm$ 2.00 a	97.8 $\pm$ 1.36 a	245.5 $\pm$ 2.68 a
160	23.3 $\pm$ 0.21 bc	12.3 $\pm$ 0.11 ab	88 $\pm$ 1.23 ab	97.7 $\pm$ 1.40 a	242.7 $\pm$ 3.04 a
560	23.9 $\pm$ 0.27 b	12.3 $\pm$ 0.10 ab	80 $\pm$ 3.16 b	96.3 $\pm$ 1.48 ab	238.2 $\pm$ 2.70 a
1 600	25.0 $\pm$ 0.31 a	12.6 $\pm$ 0.15 ab	69 $\pm$ 1.00 c	95.7 $\pm$ 1.75 ab	225.3 $\pm$ 2.83 b
9 600	26.0 $\pm$ 0.37 a	12.9 $\pm$ 0.18 a	45 $\pm$ 1.58 d	88.9 $\pm$ 3.20 b	222.7 $\pm$ 3.32 b
16 000	&	‡	0*	0*	‡
**LVC <sub>50</sub>			2.69 $\times$ 10 <sup>3</sup>	ppm	
VC	9.7	8.8	21.8	6.0	11.0
<b>Ric</b>					
Control	23.6 $\pm$ 0.44 d	12.2 $\pm$ 0.21 b	88 $\pm$ 4.90 a	90.1 $\pm$ 4.90 b	249.6 $\pm$ 4.43 a
16	23.9 $\pm$ 0.46 d	12.3 $\pm$ 0.20 b	84 $\pm$ 4.00 a	85.7 $\pm$ 5.79 b	248.2 $\pm$ 4.69 a
112	26.7 $\pm$ 0.74 c	12.8 $\pm$ 0.20 b	76 $\pm$ 4.00 ab	78.9 $\pm$ 8.08 ab	241.4 $\pm$ 4.46 ab
160	30.2 $\pm$ 0.74 b	13.1 $\pm$ 0.26 ab	60 $\pm$ 6.33 bc	60 $\pm$ 11.06 ab	222.3 $\pm$ 4.95 b
560	37.7 $\pm$ 1.42 a	14.3 $\pm$ 0.49 a	44 $\pm$ 4.00 c	54.5 $\pm$ 3.34 a	195.6 $\pm$ 8.29 c
1 600	&	‡	0*	0*	‡
9 600	&	‡	0*	0*	‡
16 000	&	‡	0*	0*	‡
**LVC <sub>50</sub>			0.38 $\times$ 10 <sup>3</sup>	ppm	
VC	20.0	11.7	27.3	28.0	8.3

\*Not taken values to statistical analysis; & = Not one of the individuals completed its larval stage; ‡ = There were no pupae  
VC = Variability coefficient; \*\*LVC<sub>50</sub> was calculated with the larvae mortality; different letters represent statistically significant differences; SE = standard error of mean.

*communis* that acts against *S. frugiperda* and that each of the seed extracts exhibited better insecticidal and insectistatic activity than the leaf extracts and corroborate that the insectistatic activity is the principal mode of action of *R. communis* against *S. frugiperda*, in accordance with Rodríguez-Hernández (2005). Continuity of this research is important and thus recommended, because the hexane extract of the leaves showed insecticidal and insectistatic activity against *S. frugiperda*; and the next step would be to isolate the active compounds from this extract, and thereafter standardize the extract with the highest activity.

## ACKNOWLEDGEMENTS

We thank the Consejo Nacional de Ciencia y Tecnología (CONACyT) for the financial support of this research by the agreement PFP-200-93. We also thank the International Maize and Wheat Improvement Center (CIMMYT) and especially Dr. Kevin Pixley for his support and facilitation of the research and Tec. Emiliano Gutiérrez reared the *S. frugiperda* used for this research.

## REFERENCES

- Achaya KT, Craig BM, Youngs CG (1964). The component fatty acids and glycerides of castor oil. *J. Am. Oil. Chem. Soc.* 41: 783-784.
- Andrews KL (1988). Latin American research on *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Florida Entomol.* 71(4): 630-653.
- Aouinty B, Outara S, Mellouki F, Mahari S (2006). Évaluation préliminaire de l'activité larvicide des extraits aqueux des feuilles du ricin (*Ricinus communis* L.) et du bois de thuya (*Tetraclinis articulata* (Vahl) Mast.) sur les larves de quatre moustiques culicidés: *Culex pipiens* (Linné), *Aedes caspius* (Pallas), *Culiseta longiareolata* (Aitken) et *Anopheles maculipennis* (Meigen). *Biotechnol. Agron. Soc. Environ.* 10(2): 67-71.
- Bashir MEH, Hubatsch I, Leinenbach HP, Zeppezauer M, Panzani RC, Hussein IH (1998). Ricin c 1 and Ricin c 3, the allergenic 2S albumin storage proteins of *Ricinus communis*: complete primary structures and phylogenetic relationship. *Int. Arch. Allergy Immunol.* 115(1): 73-82.
- Bergvinson DJ, Kumar H (1997). Cría masiva de insectos en el laboratorio de entomología del CIMMYT (*Diatrea grandiosella*, SWCB; *D. saccharalis*, SBC; *Spodoptera frugiperda*, FAW y *Helicoverpa zea*, CEW). In: Annual Research Progress Report 1996, Maize Entomology. Appendix 7. CIMMYT, Texcoco, México.
- Bigi MF, Torkomian LV, Groote TCS, Hebling MJ, Bueno OC, Pagnocca FC, Fernandes BJ, Vieira CP, Silva MF (2004). Activity of *Ricinus communis* (Euphorbiaceae) and ricinine against the leaf-cutting ant *Atta sexdens rubropilosa* (Hymenoptera: Formicidae) and the symbiotic fungus *Leucoagaricus gogylophorus*. *Pest. Manage. Sci.* 60: 933-938.

- Caffarini P, Carrizo P, Pelicano A, Roggero P, Pacheco J (2008). Effects of acetic and water extracts of *Ricinus communis*, *Melia azedarach* and *Trichillia glauca* on black common cutting ant (*Acromyrmex lundii*). *IDESIA* 26(1): 59-64.
- Céspedes CL, Salazar JR, Martínez M, Aranda E (2005). Insect growth regulatory effects of some extracts and sterols from *Myrtillocactus geometrizans* (Cactaceae) against *Spodoptera frugiperda* and *Tenebrio molitor*. *Phytochemistry*, 66: 2481-2493.
- Chen Z, Zhang J, Chen G (2008). Simultaneous determination of flavones and phenolic acids in the leaves of *Ricinus communis* Linn. by capillary electrophoresis with amperometric detection. *J. Chromatogr.* 863(1): 101-106.
- Cruz I, Oliveira LJ, Oliveira AC, Vasconcelos CA (1996). Efeito do nível de saturação de alumínio em solo ácido sobre os danos de *Spodoptera frugiperda* (Smith JE) em milho. *An. Soc. Entomol. Brasil.* 25: 293-297.
- Darby SM, Miller MI, Allen RO (2001). Forensic determination of ricin and the alkaloid marker ricinine from castor bean extracts. *J. Forensic. Sci.* 46(5): 1033-1042.
- Delwiche LD, Slaughter SJ (2002). *The little SAS book: A primer*. 2<sup>nd</sup> ed. The SAS Institute, Cary NC, USA.
- Ferry N, Edwards MG, Gatehouse JA, Gatehouse AMR (2004). Plant-insect interactions: molecular approaches to insect resistance. *Curr. Opin. Biotechnol.* 15(2): 155-161.
- Isman MB (2000). Plant essential oils for pest and disease management. *Crop Prot.* 19: 603-608.
- Kang S, Cordell G, Soejarto D, Fong H (1985). Alkaloids and flavonoids from *Ricinus communis*. *J. Nat. Prod.* 48(1): 155-156.
- Kim KB (2001). Effects of oil milling steps on residual toxin and antigen activities of castor bean meal. *Food Sci. Biotechnol.* 10(3): 305-310.
- Kumar H, Mihm JA (2002). Fall armyworm (Lepidoptera: Noctuidae), Southwestern corn borer (Lepidoptera: Pyralidae) and sugarcane borer (Lepidoptera: Pyralidae) damage and grain yield of four maize hybrids in relation to four tillage system. *Crop Prot.* 21(2): 121-128.
- Leite AC, Cabral EC, Santos DA, Fernandes J, Vieira PC, Silva MF (2005). Isolamento do alcalóide ricinine das folhas de *Ricinus communis* (Euphorbiaceae) através de cromatografias em contracorrente. *Quim. Nova.* 28(6): 983-985.
- Molina J, Carpenter J, Heinrichs E, Foster J (2003). Parasitoids and parasites of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the Americas and Caribbean Basin: an inventory. *Florida Entomol.* 86(3): 254-289.
- Mushobozy DMK, Nganilevanu G, Ruheza S, Swella GB (2009). Plant oils as common bean (*Phaseolus vulgaris* L.) seed protectants against infestations by the Mexican bean weevil *Zabrotes subfascistus* (Boh.). *J. Plant Prot. Res.* 49(1): 35-39.
- Olaifa JI, Matsumura F, Zeevaart JAD, Mullin CA, Charalambous P (1991). Lethal amounts of ricinine in green peach aphids (*Myzus persicae*) (Sulzer) fed on castor bean plants. *Plant Sci.* 73: 253-256.
- Pantoja-Uceda D, Bruix M, Giménez-Gallego G, Rico M, Santoro J (2003). Solution structure of RicinC3, a S2 albumin storage protein from *Ricinus communis*. *Biochemistry*, 42: 13839-13847.
- Raymond M (1985). Presentation d'un programme d'analyse log-probit por micro-ordinateur. *Cah. ORSTOM. Ser. Entomol. Med. Parasitol.* 23: 117-121.
- Rodríguez-Hernández C, Vendramim DJ (1996). Toxicidad de extractos de Meliaceae en *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Manejo Integrado de Plagas* 42: 14-22.
- Rodríguez-Hernández C (2005). Plantas contra plagas 2: Epazote, hierba de la cucaracha, paraíso, higuera y sabadilla. Colegio de Postgraduados, Red de Acción sobre Plaguicidas y sus Alternativas en México, Red de Acción en Plaguicidas y sus Alternativas para América Latina, Sociedad Mexicana de Agricultura Sostenible, Instituto Tecnológico del Altiplano de Tlaxcala, México.
- Skursky L, Burlison D, Waller G (1969). Interconversion of ricinine and N-demethylricinine in senescent and green castor plant leaves. *J. Biol. Chem.* 244(12): 3238-3242.
- Sparks A (1979). A review of the biology of the fall armyworm. *Florida Entomol.* 62(2): 82-87.
- Tinzaara W, Tushemereirwe W, Nankinga CK, Gold CS, Kashaija I (2006). The potential of using botanical insecticides for the control of the banana weevil, *Cosmopolites sordidus* (Coleoptera: Curculionidae). *Afr. J. Biotechnol.* 5(20): 1994-1998.
- Upasani SM, Kotkar HM, Mendki PS, Maheshwari VL (2003). Partial characterization and insecticidal properties of *Ricinus communis* L. foliage flavonoids. *Pest Manage. Sci.* 59: 1349-1354.
- Yudalshev P (2001). Ricinine and its transformations. *Chem. Nat. Comp.* 37(3): 274-275.