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Full Length Research Paper

Activity of the main fatty acid components of the hexane leaf extract of *Ricinus communis* against *Spodoptera frugiperda*

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The composition of hexane leaf extract of *Ricinus communis* was determined by gas chromatography–mass spectrometry (GC-MS) to contain four fatty acids: linolenic acid (47.76%), linoleic acid (15.28%), palmitic acid (13.01%), and stearic acid (1.73%). The insectistatic and insecticidal activities of the two major components, linolenic acid and linoleic acid, on the larval development of *Spodoptera frugiperda* were assessed. The larval viability fifty (LV_{50}) values of linolenic acid and linoleic acid were 0.849×10^3 ppm and 0.857×10^3 ppm, respectively. Thus, both fatty acids evaluated were found to have the insectistatic and insecticidal activities against *S. frugiperda*.

Key words: Insectistatic activity, insecticide, linolenic acid, linoleic acid, fall armyworm.

INTRODUCTION

The fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) infests over 80 plant species in 23 families (Pashley, 1988), including cotton, corn, and many grass crops (Luttrell and Mink, 1999). To control this insect, plant-derived compounds have been considered as alternatives to synthetic chemical insecticides due to their comparatively lower environmental and health concerns (Koul and Walia, 2009).

In this context, several studies have reported that *Ricinus commnunis* (Euphorbiaceae) is a natural resource of candidate insecticidal compounds; the aqueous extract of *R. communis* has documented insecticide activity against *Aedes caspius, Anopheles maculipennis, Culex pipiens* and *Culiseta longiareolata* (Diptera: Culicidae) (Aouinty et al., 2006), *Callosobruchus chinensis* (Coleoptera: Bruchidae) (Upasani et al., 2003) and *Cosmopolites sordidus* (Coleoptera: Curculionidae) (Tinzaara et al., 2006). It was also found that ricinine, which is present in the seeds and leaf of *R. communis,* had insecticidal activity against *Myzus persicae* (Homoptera: Aphididae) (Olaifa et al., 1991), *Atta sexdens rubropilosa* (Hymenoptera: Formicidae) (Bigi et al., 2004).

Certain fatty acids have also shown activity against insect pests. Ramsewak et al. (2001) reported the insecticidal and insectistatic activities of linoleic acid against *Aedes aegyptii* (Diptera: Culicidae), *Helicoverpa zea* (Lepidoptera: Noctuidae), *Lymantria dispar* (Lepidoptera: Lymantriidae), *Malacosoma disstria* (Lepidoptera: Lasiocampidae), and *Orgyia leucostigma* (Lepidoptera: Lymantriidae). Additionally, studies have shown that oleic acid has insecticidal activity against *A. aegyptii, Anopheles stephensi* (Diptera: Culicidae), and *Culex quinquefasciatus* (Diptera: Culicidae) (Rahuman et al., 2008; Kannathansan et al., 2008). A third fatty acid, linolenic acid, has activity against *Liposcelis bostrychophila* (Psocoptera: Liposcelididae) (Green, 2011) and *Callosobruchus maculates* (Coleoptera: Bruchidae) (Adebowale and Adedire, 2006). Lastly, Figueroa-Brito et al. (2002) reported that linoleic acid, palmitic acid, and stearic acid all exhibit insecticidal

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activity against *S. frugiperda*.

In a previous study, the insectistatic and insecticidal activities of hexane, extract of leaves of *R. communis* evaluated, and it was found that this extract exhibit activity against *S. frugiperda*. In the present research, the composition of *R. communis* hexane extract was determined by gas chromatography–mass spectrometry (GC-MS), and the insectistatic and insecticidal activities of the two main constituents against *S. frugiperda* were assessed.

MATERIALS AND METHODS

Plant material

Leaves of *R. communis* were collected in Ecatepec of Morelos State of Mexico in December 2007. Taxonomic authentication was performed by Dr. Koch from Colegio de Postgraduados en Ciencias Agrícolas (CP), Texcoco, Estado de México, and a voucher (CHAPA-001) was deposited in the Herbario Hortorio of CP.

Hexane extract preparation

Dried and powdered leaves (1 kg) were extracted with 3 L hexane under reflux for 4 h, the extract was filtered, and the solvent was removed under reduced pressure using a rotatory evaporator. The yield of the extract was 2.1%.

Esterification of fatty acids

Fatty acid methyl esters were prepared according to AOAC-IUPAC Method 969.33 (AOAC, 1990). Hexane extract (90 mg) and 1 N solution of NaOH in methanol (4 mL) were combined in a round-bottomed flask, and the solution was heating at boiling point with stirring for 15 min. Next, 5 mL BF_3 in MeOH (15% w/w) was added, and heating continued for 5 min. Isooctane (2 mL) was then added. The mixture was stirred for an additional 5 min and extracted with hexane (2 mL). Finally, the reaction mixture was cooled and 0.2 g NaCl added. The organic phase was dried over anhydrous Na_2SO_4.

Analysis of hexane leaf extract

The resultant fatty acid methyl esters were analyzed on a 6890N gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with an HP-5MS column (30 m length; 25 mm internal diameter; 0.25 µm film thickness) and an Agilent EM 5973 detector maintained at 150°C. The instrument was operated with helium as the carrier gas at a flow rate of 1 mL min^{-1}; the split ratio was 2:1. The column temperature was initially 60°C (for 3 min) and was gradually increased to 170°C, at 3°C min^{-1}; this temperature was held for 1 min. Next, the temperature was raised to 330°C, at a rate of 10°C min^{-1}; this temperature was held for 10 min. The injector temperature was 330°C and 1 µL organic phase was injected in duplicate. Compounds were identified by comparison of observed retention times with those of authentic compounds and with data in the NIST 02 library.

Chemicals

Linoleic and linolenic acids were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Insects

The insect larvae were reared at 25 ± 2°C and 70% relative humidity with 14/10 h light/dark cycles, for 1 kg diet of *S. frugiperda* (Bergvinson, 1997) were used in the next ingredients: distilled water 800 mL, diet (Product# F0635 S. W. Corn Borer, Bio-Serv, Frenchtown, NJ, USA) 60 g, sterile corn spike 20 g, ground corn 100 g, brewer´s yeast 40 g, vitamins (vitamin mix fortification lepidoptera, Bio-Serv) 10 g, agar 10 g, sorbic acid 1.7 g (was dissolved in the ethanol), ethanol 17 mL, formaldehyde 2.5 mL, methyl *p*-hydroxybenzoate 1.7 g and neomycin sulfate 0.6 g.

Bioassay

Bioassays were conducted using groups of 24 randomly selected first instar larvae for each concentration and compound. Preliminary screening of linolenic acid and linoleic acid standards was carried out at seven concentrations ranging from 0.016 to 16,000 ppm, following previous methodology (Santiago et al., 2009). In these assays, the fatty acids were mixed with the larvae diet ingredients during their preparation. Based on the preliminary screening results, a more narrow concentration range of fatty acids was selected for a concentration-response (160 to 1,600 ppm) bioassay according to the method of Rodríguez-Hernández y Vendramim (1996). The larval stage duration and pupal stage duration were recorded, as well as the number of pupae formed (larval viability), the number of adults formed (pupal viability), and the weight of pupae 24 h after formed. The viability larvae fifty (LV_{50}) of a particular compound corresponded to the treatment concentration resulting in fifty percent mortality of the larvae population of *S. frugiperda* during the larval stage.

Statistical analysis

The completely random experimental design was used. Analysis of variance (ANOVA) and Tukey's test were performed using the SAS statistical analysis program (Delwiche and Slaughter, 2002), and the LV_{50} was calculated using probit (Raymond, 1985).

RESULTS

Identification of the main components

GC/MS analysis of hexane extract of *R. communis* leaves identified 4 fatty acids (Table 1) that correspond to 77.78% of the extract material. The main components were linolenic acid (47.76%), linoleic acid (15.28%), palmitic acid (13.01%) and stearic acid (1.73%).

Activity of linolenic acid

The insectistatic activity of linolenic acid started to manifest at 160 ppm, at which the concentration of the duration of the larval stage increased to 2.6 day (Table 2). At 400 ppm, an increase in the duration of the pupal stage to 0.9 d was observed, and pupal weight was

Table 1. Relative fatty acids of hexane extract of *R. communis.*

Number	Fatty acid	Time retention (min)	Peak area (%)
1	Palmitic acid	41.63	13.01
2	Linoleic acid	44.85	15.28
3	Linolenic acid	44.97	47.76
4	Stearic acid	45.38	1.73

The results are the average of three repetitions.

Table 2. Larvae and pupae viability of *S. frugiperda* with linolenic acid.

Concentration (ppm)	Viability (%)		Duration (d)		Pupal weight
	Larval	Pupal	Larval	Pupal	(mg)
Control	91.7 ± 4.8	95.8 ± 2.2	22.6 ± 0.2	12.2 ± 0.2	243.0 ± 4.3
16	91.7 ± 4.8	95.8 ± 2.2	22.8 ± 0.2	12.2 ± 0.2	241.2 ± 4.2
56	91.7 ± 8.3	90.9 ± 3.1	22.8 ± 0.2	12.4 ± 0.2	238.9 ± 3.0
120	75 ± 10.8	88.3 ± 3.4	23.3 ± 0.3	12.4 ± 0.3	234.1 ± 4.6
160	70.8 ± 11.2	82.4 ± 5.8	25.2 ± 0.3*	12.6 ± 0.2	232.6 ± 5.0
400	62.5 ± 14.2*	73.3 ± 5.3*	26.9 ± 0.4*	13.0 ± 0.3*	206.8 ± 8.3*
960	45.8 ± 8.0*	63.6 ± 5.0*	29.5 ± 0.8*	13.3 ± 0.3*	171.9 ± 10.8*
1,600	33.3 ± 12.5*	50.0 ± 11.8*	34.3 ± 1.7*	14.1 ± 0.5*	114.4 ± 16.7*
LV_{50}	0.849×10^3 ppm	-	-	-	-
VC	27.2	12.6	12.5	9.2	7.7

Results are the mean of at least 24 determinations ± standard error; VC= Variability Coefficient; * = Significant different with the control at $P < 0.05$.

reduced by 14.9%, and with 960 ppm the pupal stage was increased 1.1 d and 1.9 d at 1600 ppm.

Insecticidal effects were observed at 400 ppm, at which concentration 62.5% larval viability (37.5% mortality) and 73.3% pupal viability were recorded. The LV_{50} of this acid was 849 ppm. Thus, both insectistatic and insecticide activities of linolenic acid against *S. frugiperda* were detected.

Activity of linoleic acid

The insectistatic activity of linoleic acid started to manifest at 160 ppm, at this concentration, the duration of the larval stage increased to 3.1 d (Table 3). At 400 ppm, pupal weight decreased by 9.7% compared to untreated controls, and with 960 ppm, an increase in the duration of the pupal stage to 0.8 d was observed.

At 400 ppm the insecticidal effects were observed, at which concentration 62.5% larval viability and 80% of pupal viability was recorded. The LV_{50} of linoleic acid was 857 ppm. The results indicated the insectistatic and insecticidal activities of this acid against *S. frugiperda*.

DISCUSSION

In this work, linolenic acid, linoleic acid, palmitic acid, and stearic acid were found to be the main components of the hexane extract of *R. communis* leaves. These results are contrary to a study published by Bigi et al. (2004), which found this extract to contain 10 components, principally palmitic acid (81.0%), stearic acid (6.6%), and pentadecanoic acid (6.4%). This fact may be due to weather, soil, geographic condition of growth, and the variety of each species studied.

The insecticidal activity of linoleic acid has been previously reported by Ramsewak et al. (2001); an LD_{50} value of 100 µg mL^{-1} was observed when fourth instar larvae of *A. aegyptii* were exposed to the compound. Growth inhibition at the same acid concentration was evident when tests were performed using larvae of *H. zea*, *L. dispar*, *M. disstria*, and *O. leucostigma*; the levels of inhibition after 1 week were 88, 93, 81, and 77%, respectively. While this acid were shown insecticide and insectistatic activities with others insects pest, this is the first report that shows these activities against *S. frugiperda*.

On the other hand, there are no report about insectistatic or insecticidal activities of the linolenic acid, so this is the first report in this sense, because our results showed that at 160 ppm, this acid affect the larval duration and at 400 ppm the activity was in the larval viability, pupal viability, pupal duration and pupal weight; this values were different to that reported previously to

Table 3. Larvae and pupae viability of *S. frugiperda* with linoleic acid.

Concentration (ppm)	Viability (%)		Duration (d)		Pupae weight
	Larvae	pupae	larvae	Pupae	(mg)
Control	91.7 ± 4.8	95.5 ± 2.2	23.4 ± 0.3	12.3 ± 0.2	246.3 ± 3.9
16	91.7 ± 8.3	95.5 ± 2.2	23.4 ± 0.3	12.3 ± 0.2	245.3 ± 4.8
56	87.5 ± 8.0	90.5 ± 3.2	23.5 ± 0.3	12.3 ± 0.2	242.8 ± 4.6
120	83.3 ± 9.6	90.1 ± 3.8	24.0 ± 0.4	12.4 ± 0.2	239.1 ± 4.3
160	70.8 ± 11.2	88.2 ± 4.2	26.5 ± 0.4*	12.7 ± 0.3	235.6 ± 5.2
400	62.5 ± 14.2*	80 ± 5.0*	27.4 ± 0.5*	12.8 ± 0.3	222.5 ± 6.3*
960	54.2 ± 8.0*	76.9 ± 5.3*	29.2 ± 0.8*	13.1 ± 0.5*	201.7 ± 8.4*
1,600	25 ± 10.8*	50 ± 11.5*	32.7 ± 0.8*	13.3 ± 0.5*	124.8 ± 9.2*
LV_{50}	0.857×10^3 ppm				
VC	21.3	10.4	11.6	8.8	8.1

Results are the mean of at least 24 determinations ± standard error; VC= Variability Coefficient; * = significant different with the control at $P < 0.05$.

hexane leaf extract of *R. communis* that exhibited low insectistatic and insecticidal activities, because the activity from larval viability started at 9600 ppm, the pupal viability at 24000 ppm, the larval duration at 1600 ppm, the pupal duration at 9600 ppm and pupal weight at 9600 ppm.

The activity of both acids on *S. frugiperda* was almost equal, the LV_{50} was 849 ppm for linolenic acid and 857 ppm for linoleic acid, and this fact might be due that both acids have similar structure besides them have 18 carbon atoms, and similar molecular weight linolenic acid (278.43) and linoleic acid (280.45).

These results show that linolenic acid and linoleic acid at low concentrations had insectistatic and insecticidal activities against *S. frugiperda*.

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