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Authors: Valladares-Cisneros, María Guadalupe, Rios-Gomez, Maria Yolanda, Aldana-Llanos, Lucila, Valdes-Estrada, Ma. Elena, and Ochoa, Mirna Gutierrez

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BIOLOGICAL ACTIVITY OF *CRESCENTIA ALATA* (LAMIALES: BIGNONIACEAE) FRACTIONS ON LARVAE OF *SPODOPTERA FRUGIPERDA* (LEPIDOPTERA: NOCTUIDAE)

María Guadalupe Valladares-Cisneros[1], Maria Yolanda Rios-Gomez[2], Lucila Aldana-Llanos[3,*], Ma. Elena Valdes-Estrada[3] and Mirna Gutierrez Ochoa[3]

[1]Laboratorio de Principios Fitoquímicos Bioactivos de la Facultad de Ciencias Químicas e Ingeniería

[2]Laboratorio 10 del Centro de Investigaciones Químicas, Universidad Autónoma del Estado de Morelos, México

[3]Centro de Desarrollo de Productos Bióticos del Instituto Politécnico Nacional. Carretera Yautepec-Jojutla Km. 6, AP 24, 62731 San Isidro, Yautepec, Morelos, México

*Corresponding author; E-mail: laldana@ipn.mx

Abstract

The need for new bioinsecticidal compounds motivates the study of natural products. Therefore, we studied the activity of *Crescentia alata* Kuth (Lamiales: Bignoniaceae) against *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae). We showed that *C. alata* has bioinsectidal activity. After 7 days of exposure to *C. alata* fractions in the diet at 200 ppm, fractions 3, 4 and 7 caused 90.7% weight loss in the larvae, and at 100 ppm, fractions 2, 4, 7 and 8 caused 90.1% weight loss with respect to the control. After 14 days of exposure to fractions 4 and 7 at 200, 100, and 50 ppm, the larvae had lost 94% of their weight compared to the control. There were large differences in larval mortalities between treatments, and fractions 5 and 6 at 200, 100, and 50 ppm induced the highest mortalities, which ranged from 65 to 80%. Possibly the iridoids identified from the *C. alata* fruit fractions are responsible for the antifeedant activity and mortality of *S. frugiperda*. This is the first report of *C. alata* fractions being evaluated as biocides of *S. frugiperda*.

Key Words: Iridoids, bioinsecticidal activity, mortality of larvae, antifeedant

Resumen

La búsqueda de nuevos compuestos bioinsecticidas motiva el estudio de los productos naturales. *Crescentia alata* fue estudiada en contra de *Spodoptera frugiperda*. En este estudio se muestra la actividad bioinsectida de *C. alata.* Después de siete días de exposición de las fracciones en la dieta a 200 ppm las fracciones: 3, 4 y 7; indujeron un 90.76 % de reducción del peso, a 100 ppm las fracciones 2, 4, 7 y 8 redujeron el peso larval en un 90.1 % con respecto al testigo. Después de 14 días de exposición las fracciones 4 y 7 indujeron una reducción del peso larval en un 94 % con respecto del testigo. En la mortalidad larval existió una gran diferencia entre los tratamientos, la fracción 5 y 6 a 200, 100 y 50 ppm produjeron una alta mortalidad (65 a 80 %). Los iridoides identificados en las fracciones activas de *C. alata*, sean posiblemente los responsables de la actividad antialimentaria y mortalidad en *S. frugiperda*.

Palabras Clave: Iridoides, actividad bioinsecticida, mortalidad larval, antialimentario

Corn, (*Zea mays* L.; Poales: Poaceae), a cereal grain is susceptible to attack by various insect pests including the fall armyworm, *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae). Throughout Mexico, *S. frugiperda* is the most important insect pest of corn (Figueroa 2011). Moreover, *S. frugiperda* is a polyphagous pest, damaging almost 80 plant species including wheat, sorghum, rice, sugar cane, cotton, soy, alfalfa, trefoil, oats, peanut, barley, tobacco and some floral and fruit crops (Potter 2008; Machado et al. 2008; Capinera 2011). The fall armyworm affects the quality of plants because the larvae feed on their leaves and reduce plant vigor, and chemical insecticides have been used to protect crops against the fall armyworm for many years. The indiscriminate and excessive use of chemical control has induced resistance in *S. frugiperda* to pyrethroid, organophosphate, and carbamate compounds (Shad et al. 2012).

The imperative of maintaining ecological balance must give rise to research to identify and

develop more selective and ecofriendly crop protection compounds. Thus, many have proposed a search for control alternatives based on the use of natural products (Bahena et al. 2003; Isman 2006; Kamel 2010). Some plants have evaluated against *S. frugiperda*, as powders or organic extracts (Bahena et al. 2003; Pavela & Chermemskaya 2004; Silva et al. 2005; Ballesta-Acosta et al. 2008). Both powders and organic extracts have been considered good tools as new control alternatives for use in integrated pest management (IPM) programs (Drury 2012). The trumpet flower, *Crescentia alata* Kuth (Lamiales: Bignoniaceae), is traditionally known in Mexico as *cuatecomate* or *cirian*. This spice plant is endemic to Mexico and its distribution extends throughout Central America (Von Poser 2000; Argueta et al. 1994). In traditional medicine, *C. alata* is used to decrease various respiratory afflictions including asthma, bronchitis, and cough, and also is used to treat skin disorders and internal inflammations (Argueta et al. 1994; Monroy & Castillo 2001; Solares et al. 2004). The effects of *C. alata* powder against *S. frugiperda* have were studied by Aldana et al. (1993), who incorporated it into the diet and showed reductions in growth and weight of *S. frugiperda* larvae. Another study showed that *C. alata* has antimicrobial activity against *Staphylococcus aureus* (Rojas et al. 2001). *Crescentia alata* fruits were found to contain major quantities of triacylglycerides, 3β-sitosterol palmitate, stigmast-4-en-3-one, stigmast-4, 22-dien-3-one, sucrose, glycerol, 4 new iridoids (1-4) and ningpogenine (5), (Kaneko et al. 1997; Valladares & Rios 2007), which are shown in Fig. 1.

The iridoids are monoterpene compounds. They have been identified as key molecules in plant/insect and insect/insect predator interactions. Some iridoids have been reported to be deterrents to a variety of generalist insects, and in this sense they may act as plant defenses against herbivorous insects (Hix et al. 2008).

Some Bignoniaceae species have been evaluated for *in vitro* antiplasmodial activity against *Plasmodium falciparum* (Pillaya et al. 2008; Kumarasamyraja et al. 2012), larvicidal activity against *Anopheles stephensi* Liston (Diptera: Culicidae) (Silva et al. 2007; Kaushik & Saini 2008), antioxidant and antimicrobial activity (Salem et al. 2013) and anticancer activity (Thirumal et al. 2012), and other more bioactivities. However the insecticidal activity of *C. alata* has scarcely been investigated and the objective of this study was to elucidate the biocidal activity of *C. alata* against *S. frugiperda*.

Materials and Methods

Collection of Plant Material

The plant material was collected in summer 2012 at Federal Highway Cuernavaca to Taxco, in Xochitepec (N 18° 47' 35.6" -W 99° 14' 31.6"), State of Morelos, Mexico. The identity of the plant material was authenticated by Biol. Juan Carlos Juárez from Centro de Investigación en Biodiversidad y Conservación, UAEM. A voucher specimen has been deposited (voucher number 14111) in the University Morelos Herbarium.

Plant Extraction

The fruits were collected and dried at room temperature (26-28 °C) in the dark for 2 months. The fruit extracts were obtained by maceration of the dried fruit pulp (1,000 g) with methanol 5 L. (purity 99.8%; J. T. Baker) as the solvent to extract the bio-active compounds (Kuklinski 2000). The extracted materials were held in an amber glass container ware and kept for 3 days at room temperature in the darkness. Each extraction was repeated 3 times over a period of 3 days each time. After each extraction the macerated pulp was filtered and the soluble components transfered to a 1,000-mL round bottom flask and the solvent was removed by reduced pressure distillation in a Buchi 205 rotary evaporator. The crude extract was weighed, the yield was 5% and stored at 4 °C in the previously used amber glass flask.

Chromatographic Separation of *Crescentia alata* Extract

The methanol extract was fractionated by gravity column chromatography in a column packed with 500 g of silica gel 60 (70-230 mesh, Merck, Darmstadt, Germany) and eluted with hexane-methanol J.T. Baker (100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 0:100). Fractions 1 to 8 collected and met by like spots on thin layer chromatography (TLC) and weighed. TLC was carried out on percolated Kieselgel 60 F_{254} (0.25 mm thick, Merck, Darmstadt, Germany) plates. The elution pattern of each fraction was determined using hexane-methanol (80:20, 60:40, 0:100) as mobile phase and the spots were visualized by a UV lamp (280 and 360 nm UV) and after spraying with a 1% solution of $(NH_4)_4Ce\text{-}(SO_4)_4 \cdot H_2O$ in 2N H_2SO_4 (J. T Baker) solution followed by heating. A total of 8 fractions were met and numbered according to their increased polarity elution.

Analysis

The fractions 1 to 8, the 3β-sitosterol palmitate and iridoid 1 to 6 as references (Valladares & Rios, 2007) were co-spotted on thin layer chromatography plates (10.0 × 20.0 cm, percolated Kieselgel 60 F_{254} (0.25 mm thick, Merck, Darmstadt, Germany) plates. The plates were developed with acetone in hexane at different percentages as eluants. The eluted plates were dried and first observed under

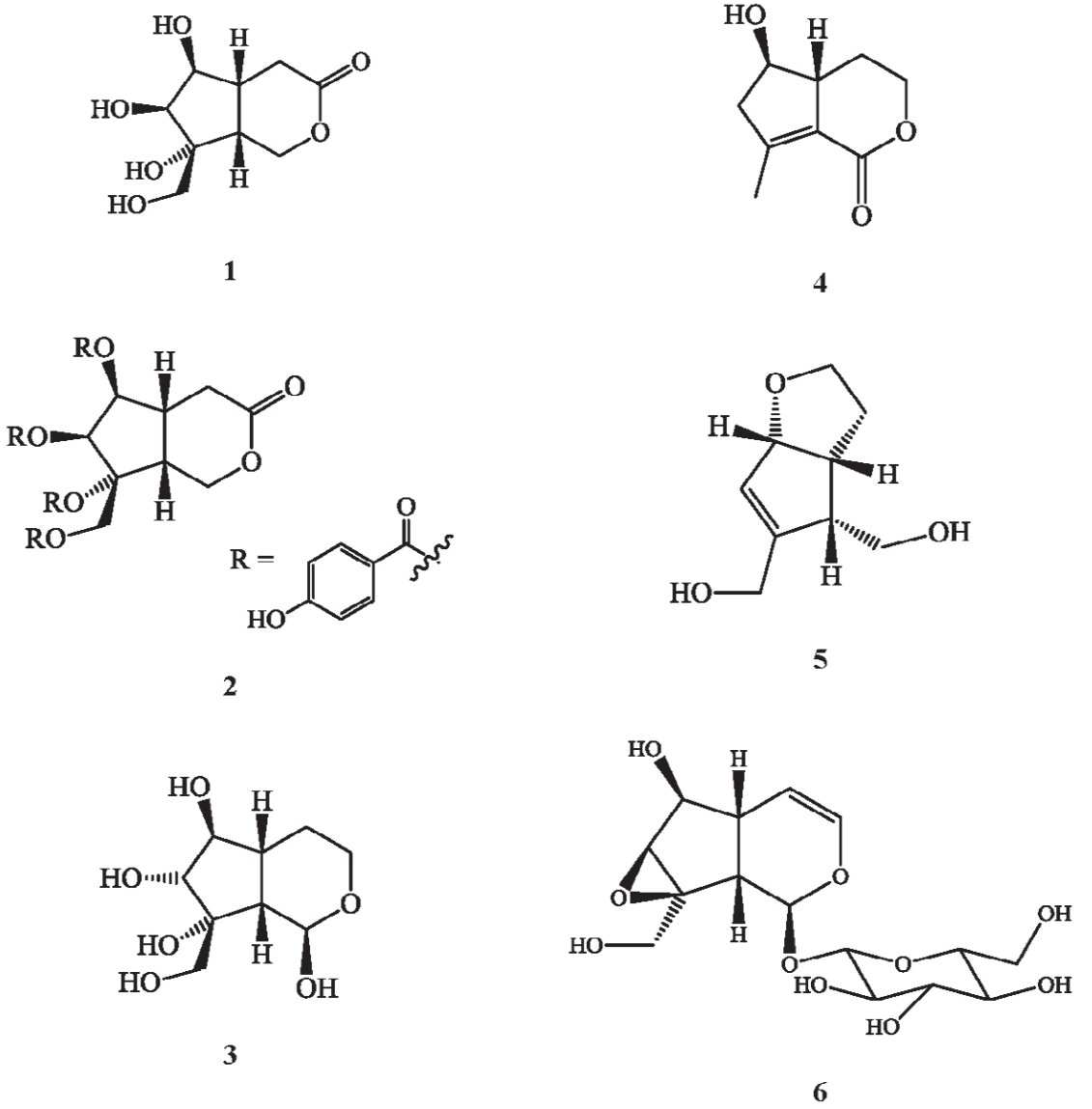

Fig. 1. Iridoids isolated from *Crescentia alata* fruits.

280 nm and 360 nm UV light after spraying with a 1% solution of $(NH_4)_4Ce$-$(SO_4)_4 \cdot H_2O$ in 2N H_2SO_4 (J. T. Baker) solution followed by heating.

The spectroscopy data of the pure compounds were obtained for Infrared spectrum on Fourier-Transform Infrared Spectrometer (FTIR) of Nicolet Series II Magna-IR System 750 and the UV spectra were obtained in UV-VIS Spectrophotometer Beckman 640, in solution with chloroform.

The major compounds presents in bioactive fractions were authenticated with authentic samples isolated previously on TLC and the IR spectroscopy data were compared with reported data on the literature (Valladares & Rios 2007).

Insects - *Spodoptera frugiperda*

Fall armyworm larvae were obtained from a colony maintained at the laboratory of the Entomology Department, Biotic Products Development Center of the National Polytechnic Institute (CEPROBI-IPN) Morelos, Mexico. The larvae were maintained in a Precision model 818 incubation chamber at 27 ± 1 °C, 60-70% RH and 12:12 h L:D, and reared on a meridic diet (Burton & Perkins 1987). Second-generation (F_2) larvae were used for all experiments with 4 replicates, and 100 neonate larvae in each treatment.

Bioassays to Assess the Toxicity of the Fractions

Only one fraction of *C. alata* (Ca) was incorporated into the above meridic diet at final concentrations of 50, 100 and 200 ppm and the effects of these preparations on the development and survival of *S. frugiperda* larvae were evaluated. A total of 8 fractions were assayed in this way. The control diet was prepared with 1 mL of methanol (J.T. Baker; 99%). Diet ingredients and the fractions were mixed following the protocol of Franco et al. (2006), and the prepared mixture was dispensed at 15 mL per container into cylindrical plastic containers (3 cm high × 3.5 cm diam). Once the diet had cooled and solidified, 1 neonate larva was placed in each container with the aid of a fine camel hair brush. Each treatment was performed in 4 replicates with a total of 100 neonate larvae. The containers were randomly arranged in a climatic chamber under the same conditions as used for rearing the laboratory colony of *S. frugiperda*. They were checked every day and the numbers of living and dead larvae were recorded.

The experimental design was completely randomized with 9 treatments and 4 replications (n = 100 larvae/treatment). Response variables were as follows: larval weights at 7 and 14 days, larval development, and larval mortality. Percent mortality was calculated by means of Abbot's formula (Abbot 1925). The statistical analyses carried out were analysis of variance (ANOVA). and mean comparison, i.e., mean ± standard deviation (MSD) (P = 0.5). Prior to ANOVA, the normality and homoscedasticity of the data was verified by the Shapiro-Wilk and Levene tests, respectively (SigmaPlot12.5).

Results and Discussion

Bioassay results of the effects of the *C. alata* fractions on weight loss of *S. frugiperda* larvae after 7 days of exposure to the fractions in the diet are shown in Table 1. Significant differences among the treated larvae and the control were observed (F = 21.984; P = < 0.001). Seven days of the exposure to *C. alata* fractions 3, 4 and 7 in the diet each at 200 ppm caused 90.7% weight loss in the larvae compared to the control. At 100 ppm, fractions 2, 4, 7 and 8 caused 90.1% weight loss with respect the control; and at 50 ppm, fractions 3 and 4 caused 89% loss in weight with respect to the control. Fractions 3 and 4 were effective at 50 ppm and higher concentrations, i.e., 100 or 200 ppm, did not cause even greater weight losses. Fractions 2, 7 and 8 may be effective only at higher levels in the diet.

Fourteen day bioassay results (Table 2) of the *C. alata* fractions in the diet on weight loss of *S. frugiperda* larvae indicate that the various *C. alata* fractions had differential effects (F = 50.682; P = < 0.001). After 14 days of the exposure to fractions 1 to 8 at 200, 100 and 50 ppm respectively, only fractions 4 and 7 induced profound weight reduction of larvae compared with the control, i.e., 96 % and 94 %, respectively.

Thus Tables 1 and 2 indicate significant losses of weight were observed in surviving larvae fed fractions 2, 3, 4, 7 and 8 at 7 and 14 days. Our results suggested that these fractions possess great antifeedant activity against larvae of *S. frugiperda*.

Figure 3 shows the observed percentages of mortality of *S. frugiperda* larvae treated with the various *C. alata* fractions and again indicate large differences between treatments. Fraction 5 induced 65 to 70% of mortality larvae. Fraction 6 caused 60 to 80% of mortality. On the other hand, fraction 4 did not produce significant mortality (> 5%), and fractions 1 and 2 produced only 5 to 15% larval mortality.

Fraction 4 was of interest because it had antifeedant activity and produced less than 5% larval

Table 1. Weights of surviving *Spodoptera frugiperda* larvae after seven days of treatment with *Crescentia alata* fractions incorporated into a Meridic diet.

Treatments *C. alata* fraction	Weight (mg) of larvae after 7 days		
	200 ppm	100 ppm	50 ppm
Ca1	6.41 ± 2.71 b	5.74 ± 2.05 bc	6.45 ± 1.87 b
Ca2	4.92 ± 2.05 bcd	2.19 ± 1.27 d	3.33 ± 1.87 bcd
Ca3	2.63 ± 1.02 d	3.95 ± 2.54 bcd	2.46 ± 0.547 d
Ca4	1.66 ± 0.695 d	1.78 ± 0.646 d	1.97 ± 0.669 d
Ca5	2.84 ± 1.85 bcd	4.35 ± 3.59 bcd	4.14 ± 2.56 bcd
Ca6	4.06 ± 2.88 bcd	3.43 ± 2.30 bcd	3.72 ± 3.54 bcd
Ca7	1.99 ± 0.847 d	2.43 ± 0.825 d	2.79 ± 1.03 cd
Ca8	3.14 ± 4.33 bcd	2.27 ± 1.10 d	2.36 ± 1.48 cd
Control	18.0 ± 4.72 a	18.0 ± 4.72 a	18.0 ± 4.72 a

*ANOVA, Standard deviation (SD), and MSD (P = 0.5) tests were carried out. Means followed by the same letter in each column are not significantly different.

TABLE 2. WEIGHTS OF SURVIVING *SPODOPTERA FRUGIPERDA* LARVAE AFTER FOURTEEN DAYS OF TREATMENT WITH *CRESCENTIA ALATA* FRACTIONS INCORPORATED INTO A MERIDIC DIET. SURVIVING WEIGTH OF *SPODOPTERA FRUGIPERDA* LARVAE.

	Weight (mg) of larvae after 14 days		
Treatments *C. alata* fraction	200 ppm	100 ppm	50 ppm
Ca1	123.0 ± 43.0 bc	139.0 ± 36.7 bc	149.0 ± 45.9 b
Ca2	33.7 ± 27.7 def	22.1 ± 17.4 ef	33.4 ± 29.0 def
Ca3	97.1 ± 53.4 cd	114.0 ± 77.8 bc	76.0 ± 41.1 cd
Ca4	10.8 ± 10.6 ef	12.0 ± 8.81 ef	15.0 ± 9.85 ef
Ca5	49.1 ± 16.9 def	40.0 ± 17.4 def	72.0 ± 28.0 def
Ca6	25.6 ± 9.73 def	38.6 ± 11.4def	58.2 ± 18.3 def
Ca7	15.8 ± 9.94 ef	14.8 ± 11.5 ef	21.0 ± 12.5 ef
Ca8	23.2 ± 18.0 def	18.6 ± 12.5 ef	23.4 ± 20.6 def
Control	283 ± 56.2 a	283 ± 56.2 a	283 ± 56.2 a

*ANOVA, Standard deviation (SD), and MSD ($P = 0.5$) tests were carried out. Means followed by the same letter in each column are not significantly different.

mortality. On the other hand, fraction 5 lacked antifeedant activity but produced close to 70% larval mortality.

According to the criterion proposed by Silva et al. (2005), plants and/or extracts with promise as bioinsecticides are those that cause not less than 40% mortality. Based on this criterion, *C. alata* fractions 3, 5, 6, and 7 have the best bioinsecticidal activity, because they produced mortalities of 40 to 80 percent. Therefore, *C. alata* has the potential to be considered as a source of bioinsecticides to control the fall armyworm.

Table 3 shows the effects of the fractions of *C. alata* on the duration of *S. frugiperda* larval period. All the fractions increased the larval period compared to the control (17 days). The duration of the larval stage was prolonged the most by the fractions 2 and 4 treatment (23 days) followed by the fractions 3 and 8 (22 days). These results agree with those reported by Aldana et al. (1993), who found that 15 percent *C. alata* powder added to a meridic diet caused an effect on the development and the larval weight of *S. frugiperda*. Based on similar assays Figueroa (2002) reported that this plant species has antifeedant effects on first instars of *S. frugiperda*.

Phytochemicals and bioactivities of *C. alata* fruits are summarized in Fig. 2, which shows the bioactive fraction and the most prevalent compound in each fraction. The phytochemical analyses of the fractions with antifeedant activity of *C. alata* fruits were carried out by direct comparison on TLC by the co-elution of one sample of authentic pure compound—previously isolated (Valladares & Rios 2007)—with fractions 1 to 8.

In fraction 2, a mixture of fatty acids was found to be present as the major constituent. In both fractions 3 and 4 the major compound in the eluted spot corresponded to 3β-sitosterol palmitate. The retention factor (Rf) for 3β-sitosterol palmitate was 0.86 in 15% ethyl acetate and 85% hexane as the solvent in the fractions 3 and 4 and in

TABLE 3. EFFECT OF *CRESCENTIA ALATA* FRACTIONS INCORPORATED INTO A MERIDIC DIET ON THE DURATION (DAYS) OF DEVELOPMENT OF *SPODOPTERA FRUGIPERDA* LARVAE.

	Mean durations of larval stadia (days) prior to pupation		
Treatment *C. alata* fraction	200 ppm	100 ppm	50 ppm
Ca1	21 ± 2.8	21 ± 2.8	21 ± 2.8
Ca2	23 ± 4.2	23 ± 4.2	23 ± 4.2
Ca3	22 ± 2.1	22 ± 2.1	22 ± 2.1
Ca4	23 ± 3.3	23 ± 3.3	23 ± 3.3
Ca5	22 ± 5.7	19 ± 3.9	19 ± 3.9
Ca6	22 ± 2.1	22 ± 2.1	19 ± 2.1
Ca7	21 ± 2.9	21 ± 2.9	20 ± 1.8
Ca8	22 ± 1.6	22 ± 1.6	21 ±1.2
Control	17 ± 0.1	17 ± 0.1	17 ± 0.1

*ANOVA, Standard deviation (SD), and MSD ($P = 0.5$) tests were carried out. Means followed by the same letter in each column are not significantly different.

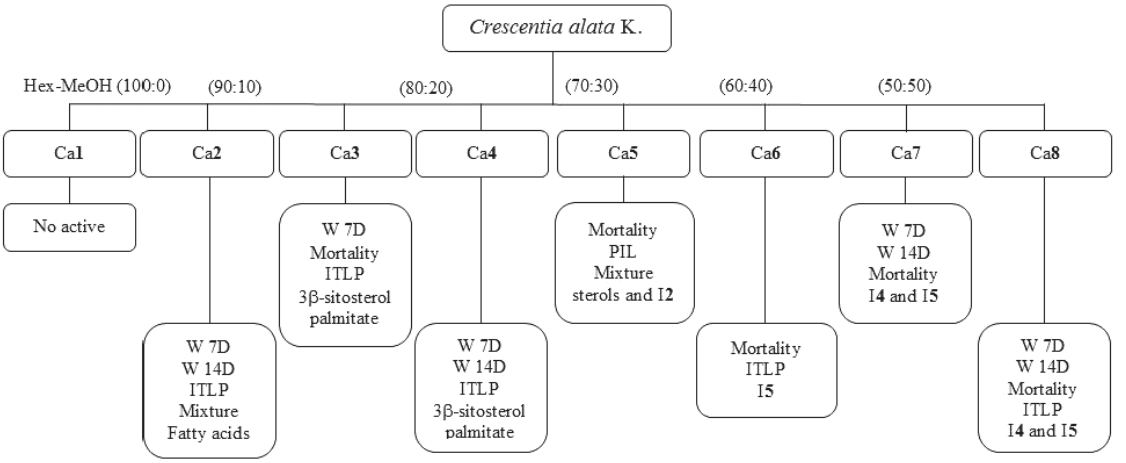

Fig. 2. Phytochemical and bioactive fractions of *Crescentia alata* fruits. W 7D = Weight reduction of *S. frugiperda* after 7 days. W 14D = Weight reduction of *S. frugiperda* after 14 days. Mortality = Mortality percent produce against *S. frugiperda* larvae. ITLP = Increase the time duration of the *S. frugiperda* larval stadia prior to pupation. I2, I4, I5 Corresponding iridoids depicted in Fig. 1. Hex-MeOH = Hexane-Methanol elution system and the number into parenthesis corresponds to the percentages of each of these 2 solvents in the elution system.

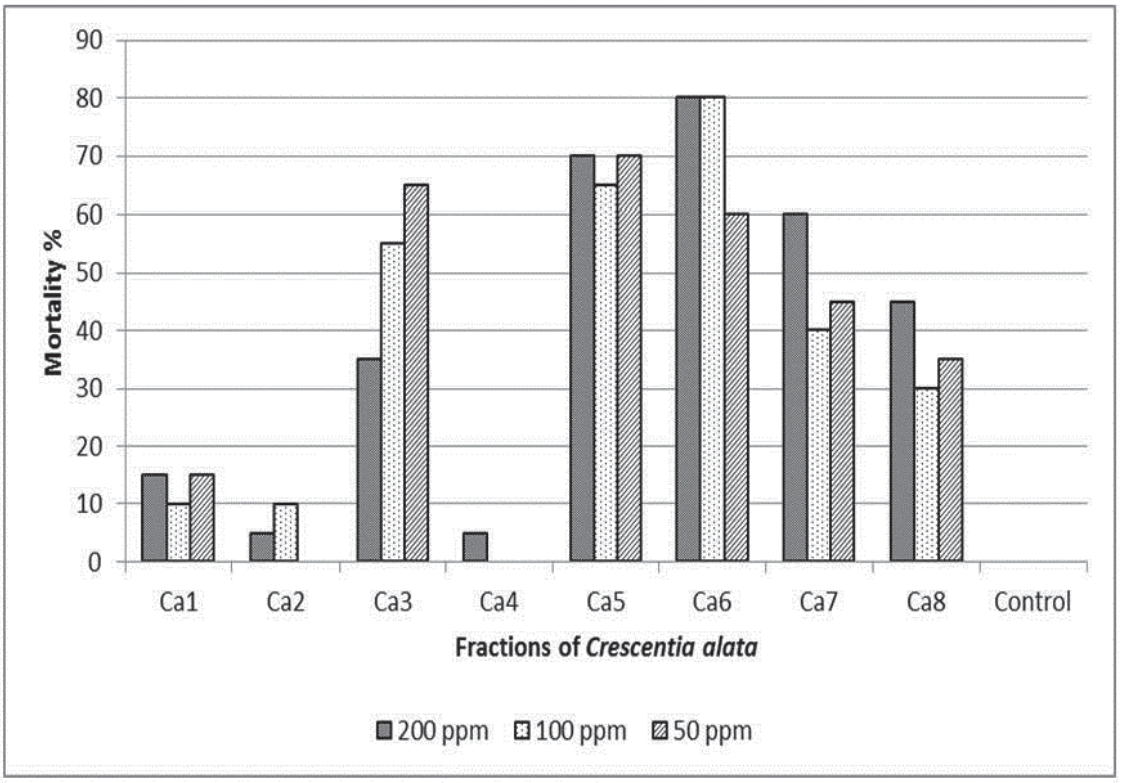

Fig. 3. Percent mortalities of *Spodoptera frugiperda* larvae caused by *Crescentia alata* fractions incorporated into a meridic diet. Fraction 5 caused 65 to 70% of larval mortality, fraction 6 caused 60 to 80% mortality. Fraction 4 did not cause significant mortality and fractions 1 and 2 produced only 5 to 15% larval mortality.

the reference. In fraction 5, the major compounds detected were 3β-sitosterol (Rf = 0.75), stigmasterol (Rf = 0.73, TLC elution in 20% acetone in hexane) and iridoid **2** (Rf = 0.6, in 25% acetone in hexane).

Kamel (2010) concluded that it was possible to use Moringa oil as a botanical insecticide against *S. frugiperda*. The major constituent of Moringa oil was β-sitosterol. In this work fraction 2 contained β-sitosterol, and this fraction showed antifeedant activity.

In fraction 6, the major component corresponded to ningpogenin (**5**) (Rf = 0.62, in 20% acetone in hexane). The iridoid, ningpogenin, was previously reported by Kaneko et al. 1997 as a secondary metabolite in *Crescentia cujete* L. Fractions 7 and 8 contained the iridoids **5** and **4**, and the major compound was iridoid **4** (Rf = 0.54, in 20% acetone in hexane).

Only the report by Pungitore et al. (2004) designated catalpol (**6**, iridoid glycoside) as a toxic iridoid that produced very great mortality during larval development of *Tribolium castaneum* (Herbst; Coleoptera: Tenebrionidae), and exhibited antifeedant activity against the adults. Catalpol by topical application (60 mg/mL) produced a series of morphological abnormalities. Pungitore et al. (2004) presented the chemical structure of this iridoid glycoside, and suggested that it is a strong inhibitor of DNA polymerase.

Conclusions

Crescentia alata fruit have antifeedant activity and cause mortality in treated *Spodoptera frugiperda* larvae. These activities may be attributed to iridoids, which are major constituents of *C. alata*. It necessary to isolate pure iridoids from *C. alata* and evaluate them on *Spodoptera frugiperda* larvae. This is the first report of *C. alata* fractions being evaluated as biocidal compounds against *S. frugiperda*.

Acknowledgments

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