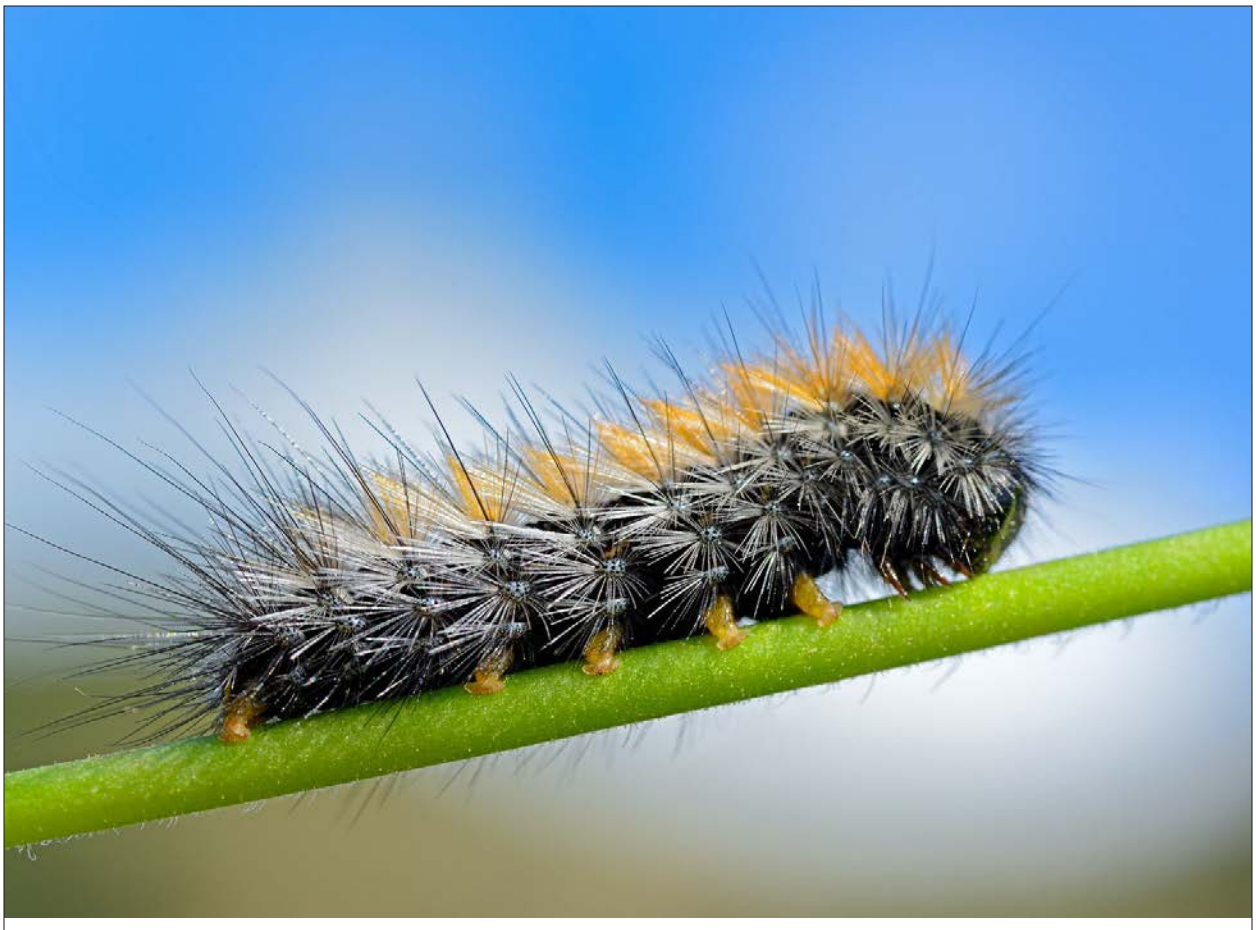

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Biological Activity of Methanolic Extracts of *Ipomoea murucoides* Roem et Schult on *Spodoptera frugiperda* J. E. Smith

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Abstract: This study was carried out to assess the biological activity of methanolic extracts and fractions from *Ipomoea murucoides* [Roem et Schult (Convolvulaceae)] on *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae). The extracts were incorporated into a meridic diet in 2 mg mL^{-1} and fed to 1st instars larvae of *S. frugiperda* and incubated at 27°C with a photoperiod of 16:8 (L:D) h. After 7 days, surviving larvae were counted and weighed; surviving pupae were incubated until moths emerged, sexed and females allowed to lay eggs on paper foil. Fecundity was measured. The crude leaf extracts produced up to 46.16% mortality with effects on development, reduction in larval weight to 42.26 mg in 3rd instar and 59.6% in 5th instar, increased time for pupation and in reaching the adult stage; there was no effect on number eggs laid. The LC_{50} calculated for methanolic leaf extract had a value of 2.692 mg mL^{-1}. The partially purified fractions showed no toxicity toward *S. frugiperda*, but had the highest effect on larval weight reduction, to 76.3% in 3rd instar and 74.6% in 5th instar, increased time for pupation and time to reach adult stage and had an effect on number eggs laid. These results indicate that the tested compounds delayed larval development.

Key words: Cazahuate, botanical extracts, resin glycoside, secondary compounds

INTRODUCTION

Myriad of secondary compounds that have toxic, growth reducing and antifeedant properties against insects (Scott *et al*., 2003) has been discovered. Typically, plants contain a mixture of biologically active compounds offering the potential for the development of botanical pesticides and synthetic analogs (Ishaaya *et al*., 2007). The compounds of plant origin typically have environmental persistence and usually a wide safety margin for non-targeted animals, including humans, domestic animals, birds, fish, amphibians and reptiles (Scott *et al*., 2005).

Peterson *et al*. (1998) demonstrated that glycosidic compounds of *Ipomoea batatas* (L.) were toxic to second instars of the diamondback moth, *Plutella xyllostella*. Subsequently, Jackson and Peterson (2000) proved toxicity of the same compounds to *P. xyllostella* 1st instars, resulting in highly significant negative correlations between resin glycoside level and survival, larval weight and life time fecundity, at sublethal doses (2 mg mL^{-1}).

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Recently, a program to test native plants of *Ipomoea* sp. for potential insecticidal activity was initiated at the Universidad Autónoma del Estado de Morelos (UAEM); including *Ipomoea murucoides* Roem et Schult (Convolvulaceae), a tree with a white bark and white flowers that grows in the south of Mexico. This specie is known as Cazahuate. Some communities use the smoke from the burned tree against mosquitoes and aqueous infusions of the leaves, bark and flowers are used as an anti-inflammatory aid and against scorpion bites (Monroy and Castillo, 2000). *Ipomoea murucoides* is part of the vegetation of the biosphere reserves Chamela-Cuixmala (Kobelkowsky, 2003) and Sierra Gorda in Guanajuato (Luegue *et al*., 2005).

Some studies with botanical extracts of *I. murucoides* on *Spodoptera frugiperda* (J. E. Smith) demonstrated that methanolic extracts induced a high percentage of mortality (95%) in neonate larvae (Vera Curzio *et al*., 2003). The main objective of this study was to assess the biological activity of natural compounds and the partially purified fractions produced by wild *I. murucoides* on *S. frugiperda*.

MATERIALS AND METHODS

Chemical and Solvents

All used reagents were commercially available. Thiamine, sorbet, methyl-paraben, ascorbate, acetic acid, choline-chloride, calcium pantothenate, niacinamide, riboflavin, folic acid, biotin and Vitamin B-12 were purchased from Sigma Chemical Co., Methanol and ethyl acetate were purchased from Merck.

Insect Rearing

Spodoptera frugiperda larvae were collected in Yautepec, State of Morelos, Mexico, in 2004. This colony was reared continuously thereafter on a premixed and modified meridic diet (Mihn, 1984). The colonies were kept in individual petri dishes (60 mm diameter) that were placed in a biological incubator at a photoperiod of 16:8 (L:D), 27°C (±1°C) and ≈50% Relative Humidity (RH). Adults were fed on 10% sucrose solution administered through a saturated cotton roll (2 cm diameter). Females oviposited on foil paper arranged around the cage. Eggs were placed in petri dishes (60 mm diameter) with a cotton roll saturated with distilled water for two or three days until occlusion of larvae.

Plant Material

Samples of *I. murucoides* were collected in the state of Morelos, Mexico (18°58′59″ N and 99°14′27″ W). Botanical classification was carried out by the Facultad de Ciencias Biológicas, UAEM and a voucher specimen (No. 22444) has been deposited at the Herbarium of the Centro de Educación Ambiental, Sierra de Huautla (CEAMISH), UAEM.

General Experimental Procedures

Silica gel (70-230 mesh, Merck, Darmstadt, Germany) was used for column chromatography. HPLC was performed using a system comprised of an Varian 9010 ternary pump, a varian variable-wavelength UV-vis 9050 detector and a rhoeadine injector.

Extraction and Isolation of Fractions

The biological material was dried at room temperature and ground, leaves, flowers and sprouts were macerated (shaking for 5 min) with methanol, at room temperature, after 3 days in the dark, the extract was pour off and add fresh methanol. The same material was extracted 3 times. The combined extracts were separated on a gravity chromatography column over silica gel (50 g) using an ethyl

acetate/methanol gradient (AcOEt/MeOH) (0:1 to 2:3), leading to 6 fractions with two being resinous. Resinous material was also collected directly from wild plants, deposited the sap fresh at vials and was dried at room temperature. Purification of the resinous fractions was performed through preparative HPLC using an MCH-10 column (10 mm i.d.× 300 mm, 5 µm, varian), eluting with a mixture of acetonitrile/water (CH_3CN/H_2O) (7:3), at a 1 mL min^{-1} flow rate at 25°C and UV detection at 215 nm. For the bioassay, from the crude extracts some fractions were selected according to their degree of elution, denominated as 100 (1), 95:5 (2), 80:20 (3), 85:15 (4); from the resinous material, 2 compounds denominated 5 and 6 were selected.

Mortality with Crude Extracts

For this bioassay, the crude extracts of *I. murucoides* were incorporated in to the diet, at a concentration of 2 mg mL^{-1} for each extract (leaves, flowers and sprouts) with 6 repetitions and 2 controls, a negative (solvent) and a commercial insecticide as positive (Fosdrim®); this concentration was chosen based on their effects in previous studies (Jackson and Peterson, 2000). The bioassays were carried out with a meridic diet placed in polystyrene plates (Cell wells, Corning, No. 25820) with 24 wells, the wells were filled with 1 mL of hot food mixed with the extract and was allowed to solidify at room temperature. When the food was cool, two neonatal larvae were placed into each well. On the 3 day only 1 larva remained. The plates with larvae were held at constant temperature and relative humidity (27°C±1.5°C and 60%) and a 16:8 h (L:D) photoperiod for 7 days. During this time, the plates were checked daily and live and dead larvae were counted. At 8th day the surviving larvae were fed with food without extract and their weight was determined at 3rd and 5th instars, to determine secondary effects of extracts on larvae.

Larvae were fed with diet free extract until pupation and the date of pupation and pupal weight were determined for each individual. Emerging males and females were placed in plastic vessels (1 L) covered with foil paper and left until the male died. Emergence date and date of mortality from natural causes were recorded for each adult. The foil paper was collected daily and the number of eggs per female was recorded.

The chromatographic factions were used for the bioassay following the same procedure described for mortality with crude extracts experiment, but using a concentration of 1 mg mL^{-1} and only 3 replications.

Median Lethal Concentration (LC_{50})

To determine the LC_{50}, the extract showing the highest mortality was selected and concentrations of leaf extracts used for the treatments were, as follows: 1.5, 2.0, 2.5, 3.0, 3.5 and 4 mg mL^{-1} with a negative control (solvent), following a randomized experimental outline with three replications.

Statistical Analysis

For the mortality bioassay and to compare 3rd and 5th instar weights, were subjected to HOVTEST _ LEVENE option of SAS to account for homogeneity of variance and normality (SAS Institute, 2002-2008) and means were separated using the Duncan's Multiple range test at 5%, before normalizing the data (angular transformation). Probit analysis was performed to determine LC_{50} using the SAS program.

RESULTS

Biological Activity of Methanolic Extracts

All tested methanolic extracts from *I. murucoides* (leaves, flowers and sprouts) induced mortality in neonate larvae, as follows: leaf extract, 49.16%; flower extract, 35.49% and sprouts extract, 26.25% (Table 1). These values are low compared to control positive (100%), however the mortality percentages in treatments of leaf and flower extracts were statistically different from the control ($p = 0.0001$). Sprouts extracts were statistically equal to the control.

Table 1: Mortality of *S. frugiperda* neonate larvae fed crude methanolic extracts (2 mg mL^{-1}) of intact *I. murucoides* incorporated into meridic diet for 7 days

Treatments	Mortality (%)
Control+(fosdrim)	100.00±1.93a
Leaf	49.16±2.39b
Flower	35.49±4.09b
Sprout	26.25±4.57c
Control-(solvent)	1.04±0c

$p<0.01$; F-value = 12.56; df = 4. Mean values followed by the same letter(s) are not significantly different (Tukey = 0.05) Data is expressed as Mean±SE

Table 2: Larval weight (mg) and percent weight reduction (%) of *S. frugiperda* 3rd and 5th instars treated with crude extracts (2 mg mL^{-1}) of intact *I. murucoides* incorporated into meridic diet

Treatments	n	3rd instar weight	Percentage	5th instar weight	n	Percentage
Control-(solvent)	134	74.78±2.57a	0.0	239.7±8.81a	120	0.0
Sprout	132	74.43±0.619a	1.0	232.2±2.57a	122	3.2
Leaf	70	30.26±1.41b	59.6	132.2±8.26b	62	44.9
Flower	95	42.26±3.46b	43.5	135.7±5.65b	90	43.4

3rd instars: $p<0.01$; F = 21.94; df = 3; 5th instars: $p<0.05$; F-values = 4.84; df = 3. Mean values followed by the same letter(s) are not significantly different (Tukey = 0.05). Data is expressed as Mean±SE

Fig. 1: (a) *Spodopterda frugiperda* larvae fed with methanolic extracts of *Ipomoea murucoides* and (b) larvae controls. Larvae are 7 days old

There was a significant reduction in surviving 3rd instar larval weight from feeding on leaf and flower treatments ($p<0.01$), with an average of 30.26 and 42.26 mg, respectively (Fig. 1a, b). This resulted in a 59.6% (leaf extract) and of 43.5% (flower extract) weight reduction compared to the sprout extract and the control. For surviving 5th instar larvae, the leaf (132.2 mg) and flower (135.7 mg) extracts showed a reduction in weight of 45 and 44%, respectively, in comparison to the positive control (239.7 mg) (Table 2).

Median Lethal Concentration

The LC_{50} calculated for methanolic leaf extract had a value of 2.692 mg mL^{-1} with a 95% confidence limit ranging between 2.397 and 3.038 ($\chi^2 = 0.955$).

Developing larvae required extra days to reach pupation when fed leaf (22.16 days) or flower (21.33 days) extracts compared to the control (18.16 days) and sprout extracts (16.76 days) (Table 3). This same trend was found with developing larvae reaching the adult stage. Larvae fed leaf (15.94 days) or flower (14.55 days) extracts took longer than those fed sprout (14.33 days) extracts or those in the control treatment (13.72 days) (Table 3). Therefore, the period of maturation was longer when extracts were applied in the diet.

Table 3: Days required to reach pupation◇ and pupae to reach the adult+ stage of *S. frugiperda* larvae fed crude extracts of *I. murucoides* incorporated into meridic diet

Treatments	n	Days◇	n	Days+
Leaf	70	22.16±0.10a	70	15.94±0.02a
Flower	95	21.33±0.21ab	95	14.55±0.03b
Control-(solvent)	134	18.16±0.10bc	134	13.72±0.05c
Sprout	132	16.76±0.13c	132	14.33±0.006bc

p<0.05; F-values = 4.5; df = 3. Mean values followed by the same letter(s) are not significantly different (Tukey = 0.05). Data is expressed as Mean±SE

Table 4: Larval weight (mg) and percent weight reduction (%) of 3rd and 5th instar *S. frugiperda* fed active fractions (1 mg mL^{-1}) of leaf methanolic extracts of *I. murucoides*

Treatments	n	3rd instar weight	Percentage	5th instar weight	n	Presentage
Control-(solvent)	35	38.5±1.18a	0.0	401.8±9.5ab	35	0
2	36	36.3±2.46a	5.8	421.5±13.2a	21	-4
1	36	27.2±2.6ab	29.3	407.6±12.6a	30	-1
5	35	17.6±2.11b	54.3	303.0±24.02c	29	24.6
6	36	15.3±1.39bc	60.3	321.5±19.63bc	34	20.0
3	36	15.2±1.60c	60.6	280.4±20.2abc	36	30.3
4	33	90.1±0.82c	76.3	102.3±19.5d	24	74.6

Third instars: p<0.01; F-values = 8.41; df = 6; Fifth instars: p<0.01; F-values = 14.22; df = 6. Mean values followed by the same letter(s) are not significantly different (Tukey = 0.05). Data is expressed as Mean±SE

Table 5: Days required for *S. frugiperda* larvae to reach pupation◇ and adult+ stage after feeding on active fractions of *I. murucoides*

Treatments	n	Days◇	n	Days+
4	18	24.1±0.14a	21	14.9±0.13a
3	36	21.3±0.08b	31	11.3±0.42b
5	31	20.8±0.10bc	31	10.1±0.28bc
6	35	20.4±0.05cd	32	10.1±0.15bcd
2	34	19.7±0.03de	28	7.9±0.22e
1	35	19.5±0.03e	34	8.1±0.15cd
Control-(solvent)	34	18.7±0.07f	33	9.0±0.08cd

p<0.01; F-values = 46.94; df = 6. Means values followed by the same letter(s) are not significantly different (Tukey = 0.05). Data is expressed as Mean±SE

The addition of extracts to the diet did not have an effect on the proportion of females with 48% to males 50%, nor on oviposition and number of eggs deposited, 268 in average.

Biological Activity of Fractions

The chromatographic fractions did not produce higher mortality, as fraction 4 induced only 8.21% mortality at a 1 mg mL^{-1} concentration. On the other hand, treated larvae were weighed at 3rd and 5th instars, the results showed a high percentage of weight reduction (Table 4) with factions 6 (60.3%), 3 (60.6%) and 4 (76.3%) at third instar, however, at fifth instar, only fraction 4 decreased larval weight (74.6%), this should by the larvae have detoxified the compounds. In this experiment, the weight diminution only was maintained until the fifth instar with fraction 4, in contrast to the crude extracts.

In the development of larvae fed active fractions, the pupal period was extended 5 days with faction 4 (24.1 days), 2.6 day with 3 (21.3 days), 2.1 day with 5 (20.8 days) and 1.7 days with fraction 6 (20.4 days), compared to the control (18.7 days) (Table 5).

The active fractions affected the days needed to reach the adult stage, with the more active factions 4 (14.9 days) and 3 (11.3 days) prolonged for 5.9 and 2.3 days, respectively. Larvae fed the control diet needed only 9 days to become adults (Table 5). Moreover, it is worthwhile mentioning that treatments 1 and 2 led to the adult stage before the control. Larvae feeding on the active fractions did not have an effect on the proportion of females 45% or males 59%.

Table 6: Average female oviposition of *S. frugiperda* after feeding on active fractions of *I. murucoides*

Treatments	n	Oviposition
Control-(solvent)	16	274.6±37.4a
5	17	198.8±42.6a
1	12	193.2±20.6a
6	23	179.3±36.5b
2	15	134.7±39.0b
3	14	116.9±17.5c
4	7	105.1±3.3c

$p<0.01$; $F = 5.72$; $df = 6$. Mean values followed by the same letter(s) are not significantly different (Tukey = 0.05), Data is expressed as Mean±SE

In contrast to the crude extracts, the fractions had an effect on oviposition (Table 6). The concentration of fractions (1 mg mL^{-1}) affected the number of eggs produced by females in treatments 6, 2, 3 and 4; the average of eggs produced with these treatments was 179.3, 134.7, 116.9 and 105.1, respectively, compared to the control (274.6 eggs).

The partially purified chromatographic fractions showed more activity than the crude extracts on both weight and larval development, since the decrease in weight was greater with fractions (74.6%) than with crude extracts (43%). The fractions also extended the larval period and affected oviposition. The chemical analysis performed to date, indicate the biological activity owing to pentasaccharide glycosides, which was not fully identified because it was doing structural elucidation.

DISCUSSION

It was tested the biological activity of extracts and chromatographic fractions of *I. murucoides* on *S. frugiperda*. The highest mortality percentage obtained with crude extracts (2 mg mL^{-1}) was produced by the leaf extract (49.16%); in a similar study (Jackson and Peterson, 2000) using a glycosides resin of *I. batatas* on first instar *P. xylostella* obtained 90% mortality the same concentration as in this experiment. The difference between percentages could be, the extract employed in present experiment is a more complex mixture of compounds than the purified resin used by them. In another study, Ver a Curzio *et al*. (2003) obtained 95% mortality of *S. frugiperda* larvae using a methanolic extract from calli of *I. murucoides* (2, 4-13.57 μM, 90 day). The difference in mortality can be explained by the fact that the leaf extract contains a large amount of chlorophyll (absent in calli), which could mask the compounds and therefore diminish the activity. On the other hand, in comparison with the percentage obtained for the control (100% mortality), which is a commercial insecticide, the percentage in this experiment represents a potential for further studies.

Moreover, of mortality provoked by *I. murucoides* extracts tested, we observed other effects on larval growth such as a reduction of larval weights in 3rd and 5th instars. This effect was also noted by Jackson and Peterson (2000) with *I. batatas* resin glycosides that reduced by 50% the larval weight of *P. xyllostella*. Apparently, the compounds tested have both a toxic activity, since we observed larvae mortality and development inhibition, showed for diminution in larval weight in 3rd and 5th instars, an extended larval period and increased time to reach the adult stage. This development inhibition has been observed also in other studies (Alvarenga *et al*., 2001; Koul *et al*., 2005; Jackson and Peterson, 2000). However, we did not discard possibility compounds which could be acting as antifeedants (Rostás, 2007).

With respect to LC_{50}, using methanolic extracts we found a value 3 times greater than that obtain with resin glycosides of *I. batatas* (0.90 mg mL^{-1}) (Jackson and Peterson, 2000). However, in other studies, the LC_{50} was 13.0 mg mL^{-1} with 20-α-hydroxytingenone in larvae of other lepidopterans (*Cydia pomonella*) (Alvarenga *et al*., 2001).

The crude extracts are a complex mixture of compounds and while the chromatographic fractions did not increase mortality, there was a reduction of 3rd and 5th instars larval weights. Kubo (1991)

reported similar results with an ethyl acetate extract of *Podocarpus gracilior* in the diet, the extracts were toxic to *Pectinophora gossypiella* (Gelechiidae) and *Heliothis virescens* (Noctuidae) larvae, but, when subjected to column chromatography, the fractions caused a non-toxic growth inhibition. This effect is consistent with our results, not only diminishing larval weight, but maintaining diminution until the 5th instar. Céspedes *et al*. (2000) described the insect growth regulatory activity of a photogedunin epimeric mixture against *S. frugiperda*, this compounds tested inhibited each larval stage, when incorporated compounds into diets at ca. 52 ppm. On the other hand, the addition of fractions to the diet also extended the larval period and the time required to reach the adult stage. Present results are similar to those presented by Calderón *et al*. (2001), as they tested oxyflavones of *Gutierrezia microcephala* over *S. frugiperda*, to probed 7.5 ppm was increased the development time of surviving larvae and a significant delay in time to pupation and adult emergence.

The results showed that the differences in biological activity between crude extracts and partially purified fractions are due to the fact that the mixture of compounds (mainly chlorophyll content) in crude extracts, apparently covers active fractions, hence, crude extracts depict less activity since, they did not have an effect on female oviposition of *S. frugiperda*, whereas partially purified fractions showed the highest activity on larval development and on oviposition.

In conclusion, *I. murucoides* synthesizes secondary metabolites apparently acting as an inhibitor of larval development in *S. frugiperda*. However, therefore, it is necessary to perform more experiments that would explain its physiological effects. We consider that *I. murucoides* is a potential plant to obtain a compounds with new biological activities and be included in integrated pest management at a regional level in Mexico.

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