

TOXICITY OF *Argemone mexicana* (L.) EXTRACTS AND IMIDACLOPRID ON *Chrysoperla carnea* (Stephens)

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ABSTRACT

In sorghum (*Sorghum bicolor* L. Moench) cultivation, the main pest is the sugarcane aphid (*Melanaphis sacchari* Zehntner (Hemiptera: Aphididae)). The insecticide imidacloprid is frequently used to control this insect, and in some cases, it is complemented with the release of lacewings of the species *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae). The insecticide has been shown to be harmful to non-target insects, such as the natural enemies of this aphid, so it is necessary to find other less harmful products. The objective of this study was to evaluate the toxicity of aqueous and oily extracts of chicalote (*Argemone mexicana* L.) on *C. carnea* and to contrast it with that of imidacloprid, with the hypothesis that extracts of *A. mexicana* applied to the developmental stages of *C. carnea* generate less mortality on the predator than imidacloprid. The aqueous and oily extracts had a concentration of 30 g L⁻¹. For imidacloprid, the recommended dose for sugarcane aphid control (0.35 g L⁻¹) was used. Mortality and the treatments effect were evaluated by exposure methods: T1) topical on larvae, T2) egg immersion, T3) treated *Sitotroga cerealella* eggs, and T4) liquid ingestion in adults. Mortality, hatching and emergence variables were analyzed with a one-way ANOVA, followed by Tukey's comparison of means ($p \leq 0.05$). Treatments were classified according to the toxicity levels proposed by the International Organization for Biological Control. The aqueous and oily extracts were in toxicity class 1 for *C. carnea*, except in eggs treated with the oily extract (class 4). Imidacloprid was classified in class 4 when applied topically and by ingestion in adults and in class 2 in ingestion of treated *S. cerealella* eggs. In general, extracts of *A. mexicana* could be used as selective insecticides, while imidacloprid can be considered incompatible with releases of this predator.

Keywords: *Melanaphis sacchari*, *Sitotroga cerealella*, *Sorghum bicolor*, side effects.

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INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the main crops in Puebla, Mexico, and a raw material for the animal feed industry (SAGARPA, 2017). Several pests affect

sorghum; however, the sugarcane aphid (*Melanaphis sacchari* Zehntner (Hemiptera: Aphididae)) is the one to which the greatest control efforts are devoted (INIFAP, 2015). One of the main insecticides used against this aphid is imidacloprid (SAGARPA, 2017). As an alternative control measure, releases of the lacewing species *Chrysoperla carnea* Stephens are conducted (Neuroptera: Chrysopidae), a beneficial, polyphagous and cosmopolitan insect, considered one of the most important predators of *M. sacchari* (Delgado-Ramírez *et al.*, 2016). This neuropteran is present in agricultural systems treated with synthetic chemical insecticides, which can affect its life cycle (Haramboure, 2017). However, both imidacloprid application and lacewing release represent the main recommendations of SAGARPA (2017) for the control of *M. sacchari* in Mexico. An alternative to the use of synthetic pesticides are insecticides of botanical origin that are effective against insect pests in different crops, are economical, biodegradable, have varied modes of action and low toxicity to non-target organisms (Lengai *et al.*, 2020). Chicalote (*Argemone mexicana* L.) is a species of the Papaveraceae family, widely distributed in tropical America. Its extracts are used for their toxic properties in insects due to the presence of alkaloids such as: berberine, palmatine, sanguinarine, protoberberine, benzophenanthridine, benzyloquinoline, and protopine (Singh *et al.*, 2010; Brahmachari *et al.*, 2013) and other secondary metabolites such as glycosides, tannins, and saponins (Upreti *et al.*, 1991). Prior to the incorporation of a pesticide, either synthetic or of plant origin, into an integrated pest management program, it is necessary to determine its secondary effects on non-target insects in order to generate the guidelines to ensure the conservation of natural enemies (Alegre *et al.*, 2017b). The objective of this research was to compare the toxicity of *A. mexicana* aqueous and oily extracts on different stages of *C. carnea* under laboratory conditions to the toxicity of imidacloprid. The hypothesis was that extracts of *A. mexicana* applied to different stages of development of *C. carnea* would result in lower mortality than the insecticide imidacloprid.

MATERIALS AND METHODS

Biological material

Adults of *C. carnea* collected in horticultural plots in the municipality of Los Reyes de Juárez, state of Puebla, Mexico (18° 55' 36" N, 97° 47' 54" W, 2197 m mean altitude), were transferred to the Entomology Laboratory of the Colegio de Postgraduados, Campus Puebla, to establish a brood stock. Once oviposition was initiated, eggs were extracted and larvae were individualized in brood units. The food supplied to the larvae consisted of *Sitotroga cerealella* (Olivier) eggs *ad libitum*, biological material provided by the Saltillo Reproduction Center of Beneficial Organisms of the State Plant Health Committee of Coahuila, Mexico. Adult *C. carnea* were fed with artificial diet proposed by Vogt *et al.* (2000), which contains 15 mL condensed milk, one egg, 30 g honey, 20 g fructose, 50 g wheat germ, 30 g brewer's yeast, and 45 mL distilled water. Furthermore, they were provided with drinking water in a 10 mL beaker and a cotton

swab was placed on the surface to prevent drowning of the individuals. Insects were maintained in a bioclimatic chamber at a temperature of 25 ± 2 °C, relative humidity of 70 ± 10 %, and 12 h light/dark photoperiod.

Treatments

Plant collection and extract production

In spring 2019, at the locality of Libertad Tecola, Puebla ($18^{\circ} 52' 53.86''$ N, $98^{\circ} 10' 56.29''$ W, 2145 m altitude), whole plants of *A. mexicana* in flowering stage were collected. The plant stems, flowers and seeds were used, according to the methodology proposed by Pérez-Torres *et al.* (2017). Each plant was washed several times to remove dust particles, and then dried in the shade at room temperature (27 ± 2 °C) for 20 days. Once dried, these parts were cut into small pieces, mechanically pulverized and sieved through a plastic strainer to obtain a fine powder. The seeds were crushed using a mortar and pestle. To obtain the aqueous extract, 30 g of crushed dry matter were mixed in 1 L of distilled water; the mixture was left to stand for 24 h and filtered through filter paper. The same procedure was followed to obtain the oily extract, but using Maxima Premium® edible vegetable oil instead of water. Both extractions were performed in the shade at room temperature according to the protocol described by Aragón-García *et al.* (2012). In this study, it was decided to use both aqueous and oily extracts to determine any difference when testing the two solvents.

Insecticide formulation and dosage

A third treatment was based on imidacloprid (Dynasty 350 SC®, 350 g i. a. L⁻¹) at a dose of 0.35 g L⁻¹ equivalent to 200 mL of commercial product in 200 L of water per ha. This concentration corresponds to that recommended by SAGARPA (2017) for the control of *M. sacchari* in sorghum crop.

In each of the bioassays, a control with potable water application was included.

Application Methods

Topical application

Using a 0.1–2.5 µL Research® manual micropipette (Eppendorf, Hamburg, Germany), 0.5 µL of the previously described treatments were applied to third stage (L3) larvae of *C. carnea*. To avoid cannibalism, treated larvae were individually placed in 3 cm diameter and 1 cm height plastic boxes with perforated lids (1 cm diameter) and sealed with Micropore® tape for ventilation. Each treatment consisted of 10 larvae with four replicates. Mortality and absence of body movement of larvae when stimulated with an entomological pin for 15 s were recorded at 48 and 72 h after treatment application (Alegre *et al.*, 2017a). Likewise, the percentage of treated larvae that pupated and the number of adults that emerged was quantified.

Egg immersion in treatments

Segments of cloth gauze containing sixty 24 h-old *C. carnea* eggs were immersed for 10 s in 30 mL of the treatment to be evaluated, contained in a glass Petri dish (90 mm diameter). Subsequently, the gauze with the eggs was placed on absorbent paper for 10 min to remove excess solution and facilitate drying at room temperature. Once the gauze with the *C. carnea* eggs had dried, they were placed in Petri dishes and kept in the bioclimatic chamber under the same conditions. Each fabric gauze segment was considered one of four replicates. The percentage of larval hatching was recorded at 48, 72, and 96 h after the treatments were applied. The larvae were fed *S. cerealella* eggs *ad libitum*. To evaluate the sublethal effect, the percentage of adult emergence was recorded.

Ingestion in larvae

The 24 h-old eggs of *Sitotroga cerealella* used in this test were obtained from a laboratory-kept brood of the insect; 3 g of this material were placed in a fine cloth mesh and immersed for 10 s in 30 mL of the treatments. Treated eggs were dried on filter paper and subsequently fed to newly emerged *C. carnea* larvae until pupation. To avoid degradation of the active principles of the extracts, the treated food was replaced every 48 h. To prevent cannibalism, the larvae were placed individually in plastic boxes 3 cm in diameter and 1 cm high. The treatments consisted of 15 *C. carnea* first instar larvae (L1) with five replicates. The plastic boxes were checked every 48 h to record the mortality obtained at each stage. Mortality was determined by observing exuviae using a Motic® microscope model SMZ 168 (Vancouver, Canada); once the change was detected, dead individuals per stage were counted.

Ingestion in adults of *Chrysoperla carnea*

A pair of predator adults less than 24 h old were placed in a plastic container 7.5 cm high and 11.5 cm in diameter, with a 10 cm diameter opening covered with cloth mesh. The concentration of the treatments was 30 g L⁻¹ of water for *A. mexicana* extracts and 0.35 g L⁻¹ for imidacloprid. Adult drinkers were 20 mL plastic containers with a sponge cloth segment inserted in the center of the lid, allowing the insects access to the treatment. Furthermore, they were fed the artificial adult diet proposed by Vogt *et al.* (2000), which was placed on the side of the containers with a spatula. Each plastic container with a pair of individuals was considered an experimental unit and each treatment consisted of eight replicates. Mortality was assessed at 24, 48 and 72 h after initiation of the trial.

The toxicity of the treatments on *C. carnea* was classified according to the recommended scale of the International Organization for Biological Control, West Palearctic Regional Section (IOBC) for tests carried out under laboratory conditions (Hassan, 1992): toxicity class 1, the treatment was considered harmless if it caused less than 30 % mortality; toxicity class 2, slightly harmful when causing 30 to 79 % mortality; toxicity class 3, moderately harmful, with 80 to 99 % mortality; and toxicity class 4, harmful, when causing more than 99 % mortality.

Statistical analysis

In all cases, mortality and larval hatching and adult emergence data at 48, 72 or 96 h after application of treatments as appropriate were analyzed with a one-way ANOVA, followed by Tukey's comparison of means ($p \leq 0.05$). All analyses were performed with Statgraphics Centurion XVI statistical software (Statgraphics Technologies, 2009).

RESULTS AND DISCUSSION

Topical application

Mortality of third stage larvae (L3) of *C. carnea* recorded at 48 and 72 h did not differ between individuals treated with aqueous and oily extract of *A. mexicana* and the control (Table 1). In contrast, the imidacloprid-based treatment showed higher mortality than the control and the rest of the treatments from 48 h of evaluation and reached 100 % mortality at 72 h of exposure. The percentage of adult emergence from topically treated larvae was not different between the aqueous and oily *A. mexicana* extracts and the control.

Table 1. Mortality of third stage larvae of *Chrysoperla carnea* treated with *Argemone mexicana* extracts and imidacloprid by topical application.

Treatment	Mortality in larvae (% \pm SE) at 48 and 72 h		Adult emergence (% \pm EE)
	48 h	72 h	
Control	0.0 \pm 0.0 a	0.0 \pm 0.0 a	90.0 \pm 8.1 a
Aqueous extract	0.0 \pm 0.0 a	5.0 \pm 5.7 a	72.5 \pm 15.0 a
Oily extract	0.0 \pm 0.0 a	0.0 \pm 0.0 a	65.0 \pm 33.1 a
Imidacloprid	90.0 \pm 4.0 b	100.0 \pm 0.0 b	0.0 \pm 0.0 b
Statistics	F = 486.0, gl = 3, p = 0.00		F = 17.79, gl = 3, p = 0.00

Means with different literals indicate significant statistical differences according to the Tukey test ($p \leq 0.05$). EE: Standard error.

A high percentage of alkaloids and other secondary metabolites with toxic effects has been documented in *A. mexicana* (Brahmachari *et al.*, 2013). Extracts based on this plant have shown insecticidal effect on adults and nymphs of *Bemisia tabaci* Gennadius (Martínez-Tomás *et al.*, 2015) as well as a larvicidal and developmental inhibitory effect on *Culex quinquefasciatus* Say (Granados-Echegoyen *et al.*, 2016), and antifeedant on *Sphenarium purpurascens* Charpentier (Aragón-García *et al.*, 2019). However, in our experiment, mortality of L3 larvae treated with aqueous and oily extracts of *Argemone mexicana* was less than 5 %. Adult emergence percentages of 72.5 and 65 %, respectively, were observed. Similar mortality (3 %) of *C. carnea* larvae was reported by direct contact with the botanical insecticide Biodie®, formulated from plant extracts of *A. mexicana*, *Berberis* sp., *Ricinus communis* L., and terthienyl (Luna-Cruz *et al.*, 2018), indicating that lacewing larvae may present some tolerance to chicalote-based treatments.

The mortality recorded in third stage larvae of *C. carnea* (100 %) treated with imidacloprid, showed its toxicity in this neuropteran's stage of development, due to which it was not possible to evaluate the percentage of adult emergence. This same effect was reported by Huerta *et al.* (2003), who found that imidacloprid is highly toxic to third stage *C. carnea* and inhibits adult emergence.

Egg immersion in treatments

The hatching percentage of *Chrysoperla carnea* larvae in the control group and the aqueous extract of *A. mexicana* was not different (Table 2), suggesting that the extracts have no toxic effect at this early stage of development. The eggs of the control that did not hatch showed natural mortality, as they remained green and did not develop the embryo, which is a common phenomenon given the experience obtained in rearing the predator in the laboratory. The effect of exposure of eggs of individuals of the genus *Chrysoperla* to plant extracts based on mullein (*Lonchocarpus nicou* (Aubl.) D.C.) and neem (*Azadirachta indica* A. Juss.) has been reported by authors such as Iannacone and Lamas (2002), who found that the two botanical products did not affect the percentage of egg hatching and that at all doses evaluated this was higher than 70 %. This fact could be attributed to the natural tolerance of the species that confers resistance to botanical insecticides (Grafton-Cardwell and Hoy, 1987).

Table 2. Hatching percentage of *Chrysoperla carnea* eggs treated with *Argemone mexicana* extracts and imidacloprid by immersion.

Treatment	Hatching at 72 and 96 h (% ± EE)	
	72 h	96 h
Control	57.1 ± 3.3 a	81.6 ± 3.3 a
Aqueous extract	59.5 ± 4.4 a	72.1 ± 7.3 a
Oily extract	0.0 ± 0.0 c	0.0 ± 0.0 c
Imidacloprid	22.9 ± 9.3 b	26.6 ± 7.9 b
Statistics	F=486.0, gl= 3, p= 0.00	F=146.0, gl= 3, p= 0.00

Means with different literals indicate significant statistical differences according to the Tukey test ($p \leq 0.05$). EE: Standard error.

The data show that the oil extract clearly affected the hatching of *C. carnea* eggs and was statistically different from the rest of the treatments. After the application of this treatment, the presence of a film that permanently and completely covered the eggs was observed. Although there is evidence that the presence of secondary metabolites such as lipophilic saponins in *A. mexicana* (Dénou *et al.*, 2020) have the capacity to affect embryogenesis by modifying cell permeability, inhibiting membrane proteins, and inducing apoptosis (Korchowiec *et al.*, 2015). In the present study, due to the absence of an additional control in which only oil without plant extract was applied, it was not possible to attribute the negative effect on embryogenesis solely to secondary metabolites without ruling out a possible effect of the physicochemical properties of the oil.

Imidacloprid was also found to be toxic to *Chrysoperla carnea* eggs, with a lower cumulative hatching percentage (26.6 %) than the control and the aqueous extract of *A. mexicana*, both at 72 and 96 h after immersion. A similar result of the toxic effect of this insecticide on *C. carnea* eggs has been reported by Ayubi *et al.* (2013), indicating that at a dose of 3.87 µg i. a. L⁻¹ imidacloprid has a lethal effect on this stage of lacewing development.

Ingestion in larvae

No differences were observed in the mortality of larvae of stages L₁ and L₂ of *C. carnea* that consumed eggs of *S. cerealella* treated with *A. mexicana*-based extracts compared to the control, in all cases mortality is less than 11 % except for that recorded in larvae of stage L₃ (Table 3). The effect on mortality of chrysopid larvae exposed to continuous consumption of feed treated with botanical compounds has already been reported. For example, no mortality was found in larvae of *Chrysoperla externa* (Schneider) fed with *S. cerealella* eggs treated with rothion extracted from barbasco and neem roots at concentrations of 800 and 40 mg i. a. L⁻¹ (Iannacone and Lamas, 2002). In second stage larvae of *C. carnea*, mortality levels of 20 and 35 % were reported in bioassays of consumption of *Planococcus citri* Risso individuals treated with citrus oil (*Citrus* spp.) and neem oil at a sublethal concentration (Bibi *et al.*, 2021).

Table 3. Cumulative mortality in larval stages and percentage of pupation of *Chrysoperla carnea* treated by ingestion of food contaminated with *Argemone mexicana* and imidacloprid extracts.

Treatment	n	Cumulative mortality by stage (% ± S.E)					Pupation (% ± EE)
		L ₁	n	L ₂	n	L ₃	
Control	75	5.3 ± 2.4 a	71	2.6 ± 2.6 a	69	0.0 ± 0.0 a	91.9 ± 4.9 A
Aqueous extract		10.6 ± 3.4 a	67	3.9 ± 1.6 a	64	1.3 ± 1.3 a	83.9 ± 4.5 a
Oily extract		7.9 ± 3.8 a	69	1.3 ± 1.3 a	68	13.3 ± 2.1 b	77.3 ± 4.9 a
Imidacloprid		49.3 ± 3.4 b	38	9.3 ± 4.5 a	31	19.9 ± 4.2 b	21.3 ± 2.4 b
Statistics		F = 38.8, gl = 3, p = 0.00		F = 1.54, gl = 3, p = 0.24		F = 15.46, gl = 3, p = 0.00	F = 54.70, gl = 3, p = 0.00

Means with different literals indicate statistical differences according to the Tukey test ($p \leq 0.05$). EE: standard error.

The mortality percentages of the different larval stages of *C. carnea* that ingested feed treated with imidacloprid differed from those observed in larvae that consumed feed treated with aqueous extract of *A. mexicana* and the control, but not in L₃ larvae that ingested feed treated with oily extract. Although lacewings have developed resistance to a wide range of insecticides (Nordlund *et al.*, 2001), L₃ larvae of lacewings have a higher tolerance to insecticides than L₁ larvae (Liu and Chen, 2000), which differs from the results shown here, since the mortality recorded in the first larval stage was higher (49.3 %) than that observed in the third (19.9 %).

The high susceptibility of lacewing larvae to imidacloprid significantly reduced the percentage of pupation (21.3 %), which is not entirely unexpected. Although neonicotinoid insecticides have been considered as biorational insecticides, this toxicological categorization is highly disputed, mainly due to the high mortality recorded in natural enemies such as *C. carnea* (Cerna *et al.*, 2012).

Ingestion in adults

Mortality rate of *C. carnea* adults that ingested the aqueous extract of *A. mexicana* was not significantly different from that of the control after 72 h of exposure. However, neither treatment differed from the mortality observed in adults ingesting the oily extract, although it cannot be ruled out that mortality occurred as a result of a possible dietary effect. The time of exposure, however, is a critical point to explain this fact. According to observations made during predator rearing, the predator can survive up to 6 days without water by consuming only diet (Table 4).

Table 4. Mortality of adults of *Chrysoperla carnea* treated by ingestion of *Argemone mexicana* and imidacloprid extracts.

Treatment	Cumulative mortality at 24, 48 and 72 h (% \pm SE)		
	24 h	48 h	72 h
Control	0.0 \pm 0.0 a	12.5 \pm 8.1 a	12.5 \pm 8.1 a
Aqueous extract	0.0 \pm 0.0 a	6.2 \pm 6.2 a	12.5 \pm 8.1 a
Oily extract	0.0 \pm 0.0 a	12.5 \pm 8.1 a	43.7 \pm 11.3 a
Imidacloprid	75.0 \pm 13.3 b	100.0 \pm 0.0 b	100.0 \pm 0.0 b
Statistics	= 31.50, gl = 3 p = 0.000	= 46.59, gl = 3 p = 0.000	= 25.96, gl = 3 p = 0.000

Means with different literals indicate significant statistical differences according to the Tukey test ($p \leq 0.05$). EE: Standard error.

The effects of botanical compounds on adults of *C. carnea* have been reported by other authors. Medina *et al.* (2005) evaluated the effect of consumption of aqueous extracts of plum (*Trichilia havanensis* Jacq.) at a concentration of 1000 mg i. a. L⁻¹, which showed no toxicity after continuous exposure via ingestion. Similar to this study, *C. carnea* was fed botanical extracts without apparently achieving any effect. The species *A. mexicana* contains a high percentage of alkaloids (Brahmachari *et al.*, 2013), some of them which are benzylisoquinoline alkaloids (ABI), which show toxicity and affect the central nervous system of insects, with a high potential for use as pesticides (Ziegler and Facchini, 2008; Juárez-García *et al.*, 2020). These metabolites have a greater impact on *C. carnea* individuals when they are obtained in an oily form rather than when their base is water.

In adults, the ingestion of aqueous extract of *A. mexicana* caused a mortality similar to that obtained in the control, though both cases were less than 13 %. Although the oil extract of *Argemone mexicana* showed a higher percentage of mortality (43.7 %),

it did not differ significantly from the other botanical treatments, but it did differ significantly from imidacloprid.

The results show that imidacloprid ingestion has a lethal effect on adults of *C. carnea*. In this study, 100 % mortality was recorded after 48 h of exposure. The harmful effect of imidacloprid on other adults of the genus *Chrysoperla* has been reported by authors such as Silva *et al.* (2017) at a dose of 1.05 g L⁻¹ water.

Toxicity of treatments according to IOBC classification under laboratory conditions

The mortality percentage of *A. mexicana* aqueous extract in the different bioassays performed was less than 30 %, so it was classified as harmless (class 1 IOBC). The mortality recorded in the bioassays in which oily extract of *A. mexicana* was applied shows a similar pattern, except for the harmful effect observed in the ingestion of this treatment in adults of *C. carnea* at 72 h of exposure (class 2), and in eggs, where this treatment produces total mortality of this stage of development (class 4) and in adults. Imidacloprid was classified as harmful (class 4) after 72 h when applied topically to L₃ larvae and when consumed by adults. The present study shows that the effect of this neonicotinoid varies depending on the developmental stages of the predator *C. carnea*, as it was less susceptible when applied to eggs (71.9 % mortality, class 2) and when ingested by larvae (78.7 % cumulative mortality, class 2).

CONCLUSIONS

The aqueous extract of *Argemone mexicana* was innocuous for eggs, larvae and adults of *Chrysoperla carnea*. The results suggest that this treatment could be included as part of an integrated pest management (IPM) program.

The oily extract of *A. mexicana* had a significant effect on egg mortality (100 %), so it was deemed harmful for this stage. However, additional bioassays are needed to rule out the possibility that these effects are the result of the activity of secondary metabolites associated with this plant, rather than the effect of the physical properties of the oil *per se* with which this treatment was developed. Additionally, a slightly harmful effect (43.7 % mortality, toxicity class 2 according to IOBC) was observed in adults after 72 h of treatment application. When applied topically and via ingestion on larvae, it was placed in class 1 IOBC as it presented a mortality rate of less than 30 %.

The imidacloprid concentration recommended for sugarcane aphid control in sorghum, was harmful for L₃ larvae and adults 72 h after topical application. Although this same treatment was classified as slightly harmful in eggs and via ingestion in larvae, the recorded mortality percentages of 71.9 and 78.5 %, respectively, suggest a low compatibility of this product with eggs, larvae and adults of the predator.

If the application of imidacloprid and the release of *C. carnea* remain as the first recommendations for the control of *M. sacchari* in sorghum, more bioassays under semi-field and field conditions, as well as tests of sublethal effects, are recommended to determine concentrations of this product that will provide effective control of the aphid with minimal effects on beneficial organisms such as lacewings.

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