

## EFFECT OF AN INSECTICIDE FORMULATION ON *Hippodamia convergens* (GUÉRIN) AND NYMPHS OF *Aphis craccivora* (KOCH) IN THE LABORATORY

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### RESUMEN

Se evaluó la toxicidad de una formulación insecticida con triazamato y alfa-cipermetrina (120 y 38,4 g i.a. L<sup>-1</sup>, respectivamente) sobre *Hippodamia convergens* (Guérin), y su presa, el áfido *Aphis craccivora* (Koch). Ambos insectos se colectaron en alfalfa *Medicago sativa* L. y se criaron sobre plantas de haba (*Vicia faba* L.) La formulación se asperjó en una torre Potter sobre folíolos de alfalfa a la dosis máxima recomendada (400 mL ha<sup>-1</sup>) y al 50, 25, 15, 10, 5 y 1% de esa dosis. Los huevos y pupas de *H. convergens* se dejaron sobre los folíolos después de tratarlos. La mortalidad de los estados de desarrollo de *H. convergens* y de los áfidos adultos se evaluó a las 24 y 48 h. La CL<sub>50</sub> para ambos insectos se determinó mediante análisis Probit. La dosis máxima fue moderadamente tóxica (mortalidad de 80 a 99%) para los adultos, pupas, y huevos de *H. convergens*, pero tóxica (> 99%) para *A. craccivora* y las larvas del depredador. Las CL<sub>50</sub> para los adultos, huevos, larvas, y pupas de *H. convergens* fueron 0,405; 341,2; 0,163; y 1,057 mL L<sup>-1</sup>, respectivamente, y 0,013 mL L<sup>-1</sup> para *A. craccivora*. La dosis menor (1% de la máxima) causó 93% de mortalidad del áfido, lo que indica que dosis bajas son suficientes para controlar esta plaga por aspersión. Así, la dosis comercial se podría disminuir al mínimo para satisfacer las necesidades de manejo de *A. craccivora*, pues el plaguicida aún controlaría la plaga y tendría menos efectos sobre *H. convergens*.

Palabras clave: CL<sub>50</sub>, chinita, toxicidad insecticida.

### ABSTRACT

The toxicity of an insecticide formulation containing triazamate and alpha-cypermethrin (120 and 38.4 g L<sup>-1</sup>, respectively) was evaluated on *Hippodamia convergens* (Guérin), and its prey, the aphid *Aphis craccivora* (Koch). Both insects were collected on alfalfa, *Medicago sativa* L., and reared on faba bean (*Vicia faba* L.). The formulation was sprayed on alfalfa leaflets in a Potter tower at the maximum dose recommended (400 mL ha<sup>-1</sup>) and at 50, 25, 15, 10, 5 and 1% of that dose. The eggs and pupae of *H. convergens* were left on the leaflets after treatment. The mobile stages of both insects were set on the leaflets once they had dried at room temperature to prevent them from escaping. Mortality during the growth stages of *H. convergens* and adult aphids was evaluated 24 and 48 h after treatment application. The LC<sub>50</sub> for both insects was determined by Probit analysis. The maximum dose was moderately toxic (mortality from 80 to 99%) for adults, pupae, and eggs of *H. convergens*, but toxic (> 99%) for *A. craccivora* and the ladybird larvae. The LC<sub>50</sub>s for adults, eggs, larvae, and pupae of *H. convergens* were 0.405; 341.2; 0.163; and 1.057 mL L<sup>-1</sup>, respectively, and 0.013 mL L<sup>-1</sup> for *A. craccivora*. The lowest dose (1% of the maximum) caused 93% aphid mortality, indicating that low doses are sufficient to control this pest by spraying. Therefore, the commercial dose could be reduced to this amount and still control *A. craccivora*, resulting in lesser effects on *H. convergens*.

Key words: CL<sub>50</sub>, insecticide toxicity, ladybird.

**INTRODUCTION**

The ~6000 world ladybirds (Virteiu et al., 2015) are among the most common natural enemies of many pest insects. Most adults and larvae of ladybirds have a beneficial action and feed mainly on mites, aphids and other insects, contributing to their natural and biological control. If their prey are at low density, the adults and larvae of ladybirds also feed on moth and beetle eggs, mites, thrips, and other small arthropods, as well as pollen and nectar.

*Hippodamia convergens* (Guérin) is a widely known and distributed ladybird species of the genus, and probably the most abundant of the family, in México (Gibson and Carrillo, 1959), Argentina (Cichón et al., 1996), and particularly in the USA, where it is an important aphid predator (De Bach, 1987; Davison and Lyon, 1992). In Chile, it was introduced in 1903 (Durán and Cortés, 1941), and it has also been recognized as a predator species of diverse aphids by Aguilera (1970), Aguilera et al. (1981), Prado (1991), Artigas (1994), and Rebolledo et al. (2002).

*Aphis craccivora* (Koch) is an important vegetable pest that can have many host plants (Prado, 1991). This aphid is 1.5 to 2.2 mm long and it is mainly dark in color. Its feeding produces abundant honeydew, which promotes the growth of black soot, reducing photosynthesis.

Pest control with insecticides is widely used in the world to avoid pest damage to crops (Aguilera, 1989). However, the use of conventional insecticides also affects natural enemies and their populations, particularly ladybirds (Obrycki and Kring, 1998), although this can result in variable effects (Hassan, 1985; Kaakeh et al., 1996). In the short term, the intensive and careless use of insecticides has produced biological imbalance and increased environmental contamination (Saini, 1983). The market offers products to control aphids, like Strike® 158.4 EW, containing triazamate and alpha-cypermethrin. This insecticide has been withdrawn from the market, but it was registered in Chile when the study was conducted. It was recommended for the control of thrips, caterpillars, meloid beetles, aphids, and others that have developed resistance to other

insecticides (AFIPA, 2003). This study validates a method to evaluate the effect of insecticides on both the aphid and the ladybird.

The IOBC (International Organization for Biological Control) has developed standard methods to evaluate the side effects of pesticides (Viñuela, 1996). The sequential scheme of this process tries to achieve the least number of products before proceeding to field trials, as these are the most difficult to establish. The initial tests are designed to evaluate the effects of only one type of pesticide exposure, by contact, while beneficial organisms have three sources of exposure in the field: direct sprays, persistent residues on surfaces, and oral intake of contaminated food (Longley and Stark, 1996). Based on these tests, the IOBC has classified pesticides in four toxicity categories depending on the degree of mortality they cause to beneficial organisms (Hassan, 1992), as summarized in Table 1.

The objective of this study was to validate a methodology to determine in the laboratory the LC<sub>50</sub> of a commercial formulation of an insecticide containing triazamate + alpha-cypermethrin on the growth stages of *H. convergens* and to evaluate its effect on *A. craccivora*.

**MATERIALS AND METHODS**

This study was conducted in the Toxicology Laboratory, Department of Crop Protection, College of Agronomic Sciences, University of Chile, Santiago, Chile.

**Rearing methods.** Adults of both the ladybird *H. convergens* and the aphid *A. craccivora*, were collected with a sweeping net and a hand aspirator in never sprayed alfalfa fields, in the Antumapu Campus of the University of Chile (33°34'11"S 70°37'50"W). These insects were reared separately in the laboratory in Flanders batteries and on *Vicia faba* L. potted plants covered with acetate cylinders with a cloth screen lid. Both species were used to evaluate the effect of an insecticide formulation (Strike®) containing triazamate + alpha-cypermethrin on the growth stages of the ladybird, and also on the aphid; triazamate is a systemic aphicide of the carbamoil triazol

**Table 1. Categories of toxicity and selectivity of pesticides according to the IOBC (Hassan, 1992)**

Toxicity levels	Toxicity categories	Mortality in the laboratory (%)
1	Inocuous	< 30
2	Slightly toxic	30-79
3	Moderately toxic	80-99
4	Toxic	> 99

chemical group, while alpha-cypermethrin is a pyrethroid. The treatments (several doses of the insecticide formulation) were applied to alfalfa leaflets in Petri dishes using a Potter ST4 tower, starting with the maximum commercial dose (120 g triazamate + 38.4 g alpha-cypermethrin L<sup>-1</sup>) recommended for tomato, cauliflower, beans, and sugar beet crops. A volume of 200 L water ha<sup>-1</sup> was used for calculations (400 mL insecticide/ha/200 L water/ha = 2 mL insecticide/L water). The amount of insecticide mixture was calculated by surface based on the recommended dose to apply in the field. The maximum dose of the insecticide formulation was diluted in lower doses to obtain mortality rates that ranged from 20 to 80%. The treatments were applied by spraying 0.13 mL of the insecticide mix in the Potter tower per replicate.

Treatments for the eggs and pupae of *H. convergens* were applied directly on the pestfleets on the Petri dishes. Treatments for mobile stages (larvae and adults) were applied to alfalfa leaflets, and the insects were placed on them when the residues had dried in order to prevent the individuals from escaping during sprays. The individuals of each stage (n = 20) were exposed to dry residues at room temperature for 30 min. The sprayed/exposed ladybird specimens were 2 d old (eggs), 5-6 d old (larvae), 3 d old (pupae), and 2<sup>nd</sup> wk old (adults) from the beginning of the stages, while the aphids were 5-7 d old adults. The dish covers had vent holes covered with cloth to avoid a lethal chamber inside. Mortality of the predator by contact with insecticide residues was recorded 24 and 48 h after treatment application (both larvae and pupae alive moved when touched slightly). The same insecticide doses were tested on the aphid, while the control insects were sprayed with distilled water (Schiess, 2006).

#### Experimental design and statistical analysis.

A randomized complete block design was used, with 4 replicates for treatment (5), plus a water control. The experiment unit was a Petri dish with 20 individuals. Mortality (%) at 24 and 48 h was corrected with Abbott's formula (1925). The results corresponding to the dose applied

were adjusted to a Probit model (Busvine, 1980), which allowed estimating LC<sub>50</sub> values to evaluate susceptibility of *H. convergens* instars and the aphid to the insecticide.

## RESULTS AND DISCUSSION

**Results of the bioassays.** The concentration used on each *H. convergens* instar and the aphid caused 20-80% mortality so that Probit analyses were adequately carried out. From those tests, then LC<sub>50</sub> values were significantly different for each growth stage of both the ladybird and the aphid (Table 2), which resulted very susceptible to the insecticide formulation. The ladybird eggs exhibited the lowest level of susceptibility. This result is in agreement with Croft (1990), who states that the eggs of predators are immune even to direct applications; the tolerance of a particular stage can be due to physiological, behavioral, and chemical factors. The pupae stage (not mobile) followed the egg stage in susceptibility, which is also in agreement with Liu and Stansly (1996). The Chi<sup>2</sup> values obtained indicate that the mortality rate fitted the insecticide mortality-dose curve (Ulloa, 2005).

Mortality rates for the four growth stages of *H. convergens* and *A. craccivora* appear in Table 3.

**Results for adults of *H. convergens*.** The insecticide formulation at the commercial and 50% dose by direct spray (topic) was classified as moderately toxic for adult ladybirds, according to Hassan's (1992) toxicity table, since cumulative mortality did not exceed 83% after 48 h from application. Treatments at 25, 15, 5, and 1% of the commercial dose were slightly toxic. After a time period of 24 h, the highest mortality was 78% with the commercial dose. The lowest dose (1% of the commercial one) produced only 15% mortality, while the rate did not exceed 47% after 48 h (Table 3). The LC<sub>50</sub> for adults was 0.405 mL L<sup>-1</sup> (Table 3).

Molina (1999) conducted laboratory studies with cypermethrin sprayed on *H. convergens*, and obtained 100% mortality so that this insecticide was classified as toxic for the predator. In

**Table 2. Probit analysis parameters for each growth stage of *H. convergens* and *A. craccivora* adults at 24 h.**

Stages of <i>H. convergens</i> and the aphid	LC <sub>50</sub> mL L <sup>-1</sup>	Confidence intervals	χ <sup>2</sup> (P)
Adults	0.405 c	0.315-0.525	3.079 (0.545)
Eggs	341.2 a	292.37-347.25	0.816 (0.936)
Larvae	0.163 d	0.127-0.202	8.136 (0.087)
Pupae	1.057 b	0.954-1.185	4.874 (0.310)
<i>Aphis craccivora</i>	0.013 e	0.0047-0.0243	1.314 (0.859)

**Table 3. Total mortality levels [% values corrected with Abbott's (1925) formula] for adults, eggs, larvae, and pupae of *H. convergens*, and adults of *A. craccivora* 24 h after treatment of several doses of a commercial insecticide containing triazamate 120 g L<sup>-1</sup> + alpha-cypermethrin 38.4 g L<sup>-1</sup> (T+A).**

Doses (mL L <sup>-1</sup> )	Adults	Eggs	Larvae	Pupae	<i>A. craccivora</i>
T+A 2.0	78	11	94	94	98
T+A 1.0	68	10	90	30	94
T+A 0.5	50	8	61	13	89
T+A 0.3	39	6	57	4	86
T+A 0.1	29	4	42	1	79
T+A 0.03	15	1	20	0	62
Control <sup>1</sup>	6	0	10	0	1

<sup>1</sup> Only water applied.

greenhouse tests, cypermethrin caused also 100% mortality. In a study by Peckman and Wilde (1993), 95.6% of *H. convergens* females exposed to permethrin residues on alfalfa presented initial paralysis symptoms in 12 h. However, 61% of the females recovered when the residues were taken out, with no negative effects in the long term or in later cycles. Inglesfield (1985) evaluated alpha-cypermethrin on beneficial arthropods, including coccinellids. Those results agree with ours, as the lowest dose applied caused 15% mortality after 24 h, indicating a low susceptibility of the specimens to those treatments. Studies on fenvalerate, which is another pyrethroid, conducted by Wilkinson et al. (1975), and Yokoyama et al. (1984) reported average mortality levels for adults of *H. convergens*, while Kaakeh et al. (1996) found a high toxicity. Mizell and Schiffhauer (1990) found high toxicity of fenvalerate to *Cycloneda sanguinea* L., *H. convergens*, and *H. olla v-nigrum* (Say).

**Results for eggs of *H. convergens*.** A time period of 24 h after treatment was insufficient to detect effects (Table 3), as mortality did not reach 20%. In addition, these eggs produced larvae that continued living 48 h later. For more accurate results, the eggs were defined as not viable when they presented dehydration and a dark shell. These effects were observed 48 h from the spray.

Mortality rates of the eggs exposed to the commercial and lower doses 48 h after treatment reached values of 93 and 35%, respectively. The LC<sub>50</sub> was 341.2 mL L<sup>-1</sup>. This indicates that the commercial dose can be classified as moderately toxic, while the treatments at 50, 25, 15, 5, and 1% of the commercial dose can be classified as slightly toxic according to Hassan's (1992) scale (Table 1).

**Results for larvae of *H. convergens*.** The highest mortality occurred in larvae compared to the other growth stages of *H. convergens*. According

to Hassan's (1992) scale, the commercial dose can be classified as toxic; 50% of that dose is was moderately toxic; doses at 25, 15, and 5% are slightly toxic; and 1% corresponded to innocuous. The commercial dose caused 95% mortality after 24 h from application, while the lowest dose resulted in 22%. Similarly, values observed after 48 h reached 100% and 23% for the commercial and lowest doses, respectively. The LD<sub>50</sub> obtained was 0.163 mL L<sup>-1</sup>.

To the effect of an insecticide, that of cannibalism observed between larvae must be added, despite that the Petri dishes were provided abundant aphids for the predators to have more than sufficient prey. This behavior was observed 24 and 48 h after treatment. It has been described for many coccinellids (Hodek, 1967, Obrycki and Kring, 1998), and allows the survival of some larvae when the aphid density is low (Sundby, 1966). Mass rearing of *H. convergens* in the laboratory is difficult by this high larval cannibalism, which herein would have been due to a competition for space rather than food. Polis (1981) states the cannibalism is a mechanism for reducing the number of potential competitors. Therefore, larvae should be separated individually after sprays.

**Results for pupae of *H. convergens*.** Mortality of pupae was defined as follows: if pupae moved the abdomen slightly when touched with a hair brush they were considered alive; no movement indicated they were dead.

At 24 h from application, the commercial dose affected 94% of pupae, but when the dose was at 50%, mortality reached levels close to 50%. The treatment with 25% of the commercial dose obtained 13% mortality, while the lowest dose evaluated (1% the commercial dose) did not cause mortality on pupae. At 48 h, the commercial dose caused 96% mortality. When reducing

the dose to 50% the commercial dose, mortality rate reached 54%, while the lowest dose did not produce mortality (Table 3). Based on these results, the commercial dose can be classified as moderately toxic in Hassan's (1992) scale; 50% of the commercial dose as slightly toxic; and 25, 15, 5, and 1% as innocuous for pupae. The  $LC_{50}$  obtained was 1.057 mL L<sup>-1</sup>.

Some pupae survived and produced adults that were observed 48 h after application. However, those adults developed only the characteristic color of the species but later died.

**Results for adult aphids.** The lowest dose caused 93% mortality 48 h from application (Table 3). According to Hassan's (1988, 1992) scale, the commercial dose and the dose at 50% should be classified as toxic, while doses at 25, 15, 5, and 1% were considered moderately toxic. The evaluation of the commercial dose at 24 h yielded 98% mortality, and the lowest dose (1% of that commercial) reached 62%. The  $LC_{50}$  was 0.013 mL L<sup>-1</sup>.

Inglesfield (1985) observed that alpha-cypermethrin at 20 g i.a. ha<sup>-1</sup> applied on corn in France caused a delay in the population recovery of aphids. Wiles et al. (1995) conducted field trials of integrated management of the Russian wheat aphid, *Diuraphis noxia* (Mordwilko), and determined that deltamethrin was effective to control *Sitobion avenae* F. in winter wheat at doses of 6.25, 3.13 and 1.56 g a.i. ha<sup>-1</sup>. However, they suggested that a reduction in dose-rate by as much as three quarters of the recommended application rate in UK cereals may be necessary to preserve approximately 60% of adult *Coccinella septempunctata* (L.) over 10 d after a deltamethrin spray application.

## CONCLUSIONS

The egg stage was less susceptible to the insecticide formulation than the other growth stages of *H. convergens*, while a time period of 48 h was required to observe mortality. Further studies are required to evaluate longer time periods.

Larvae were the most susceptible in case of *H. convergens*. Thus, when they predominate in the field, lower doses of the insecticide should be used.

Aphids of *A. craccivora* were the most susceptible target at small doses when compared to the growth stages of *H. convergens*. This would allow applying the insecticide in at reduced doses in integrated management programs against *A. craccivora*, and possibly other aphids.

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