TOXICITY OF CYPERMETHRIN ON THE NEOTROPICAL LACEWING *CHRYSOPERLA EXTERNA* (NEUROPTERA: CHRYSOPIDAE)

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SUMMARY

The generalist predator *Chrysoperla externa* (Neuroptera: Chrysopidae) is distributed in the Neotropical Region and presents a strong potential as a biological control agent due to its easily rearing, high voracity and tolerance to several pesticides. This species feeds on relevant pests such as aphids, whiteflies, trips and various lepidopterans. In Argentina, an indiscriminate chemical control with broad spectrum pesticides is still the first option for controlling pests causing resistance problems and reduction of beneficial organisms. Therefore, it would be desirable that predators or parasitoids develop tolerance or resistance as phytophagous pests for their maintenance in the agricultural ecosystems. The objective of this work was to evaluate the tolerance of *C. externa* to the pyrethroid cypermethrin by topical exposure in laboratory conditions. The symptoms evaluated were tremors, movement, knockdown, inability to walk due to being glued. Recovery from poisoning symptoms was also evaluated. Six different concentrations of the pesticide, including the full field one, were tested. The exposure method was by topical applications on third larval instar. A low toxicity on larvae at 24 h post-treatment was observed, even at the highest concentration evaluated. Most of treated individuals looked normal at 24 h post-treatment; those who were knocked down showed a complete recovery between 48 and 72 h post-treatment. *C. externa* demonstrated a high tolerance to pyrethroids. This could be due to the activity involved in pyrethroids biotransformation (for example, esterases and mixed-function oxidases), or to a low sensitivity of the molecular target. Future studies should be addressed at biochemical and molecular levels to complete our knowledge about insecticide effects on this predator.

INTRODUCTION

In Argentina, an indiscriminate chemical control with conventional pesticides is the main strategy for managing agricultural pests. Besides causing pest resistance due to repetitive use of insecticides with the same mode of action and indiscriminate use of them, this type of control causes reductions of natural enemies' populations (Rimoldi et al., 2008) too. Integrated Pest Management (IPM) has to confront the difficulties of biological and chemical control owing to the higher susceptibility that natural enemies often show to pesticides (Pathan et al., 2008). However, it would be desirable that predators or parasitoids could develop resistance or tolerance as phytophagous pests do for their maintenance in an agricultural ecosystem (Pree et al., 1989) mainly in those where the spraying with pesticides is frequent.
Chrysoperla externa (Neuroptera: Chrysopidae) is a generalist predator that feeds on, aphids, whiteflies, trips and various lepidopterans even spider mites (Godoy et al, 2004; Silva et al., 2005; Gonçalves Silva et al., 2004). This species is distributed throughout the Neotropical Region (Albuquerque et al., 1994). It presents a strong potential as a biological control agent due

to its high voracity, easily rearing, and low susceptibility to several pesticides. Thus, this predator could be included in IPM systems which might prevent pest resurgences and secondary pest outbreaks (Pree et al., 1989).

Cypermethrin is a broad spectrum pesticide commonly used in Argentina for agricultural pest control. It interferes with ionic conductance in the nervous system, specifically acting on the sodium channel and causing repeated impulses and paralysis (Stenersen, 2004).

The objective of this work was to evaluate the susceptibility of *C. externa* to the pyrethroid cypermethrin by topical exposure in laboratory conditions. Symptoms of intoxication and recovery capacity were quantified in exposed individuals.

MATERIALS AND METHODS

Insects

Individuals of *C. externa* were collected from a field without any history of pesticide applications and reared in the laboratory from 2009. Every year, new insects obtained from the field were incorporated to the colony in order to maintain the genetic variability. The rearing was under controlled conditions: 25 ± 0,5°C temperature, 75 ± 5% RH and 16:8 LD of photoperiod. Adults were fed on artificial diet according to Núñez et al. (2008). Larvae were maintained on a *Rhopalosiphum padi* (Homoptera: Aphididae) colony as prey. Futhermore, an artificial diet for this stage was developed in our laboratory.

Bioassays

Six concentrations of Glextrin 25 (25% a.i. Cypermethrin; Gleba, Argentina) were evaluated including the full field one. The solutions were prepared using acetone (analytical grade) as dissolvent and the exposure method was by topical application of 1 µl of solution on the dorsal part of third larval instar. The following doses were applied: 7.5; 10; 12.5; 37.5; and 50 ng/larva.

Larvae were considered affected after 24 h if they did not walk after being gently touched with a brush. The symptoms evaluated were tremors, movement, knockdown (paralysis due to pesticide intoxication) and individuals glued to the substrate by the anal region. Recovery was also evaluated at 24, 48, 72 and 96 h. Twenty five to 35 independent replicates of each experiment were performed.

Data of symptoms of intoxication were recorded in a contingency table and analyzed by Chi Square Test. Recovery capacity was analyzed by regression.

RESULTS

Figure 1 shows the percentages of normal and affected *C. externa* larvae treated with different doses of cypermethrin. Individuals treated with 7.5 or 10 ng/larvae did not show any symptom of intoxication. Percentage of affected larvae varied between 25% (for 12.5 ng/larvae and 42% (for 50 ng/larvae). These doses produced a significant effect compared to controls (where 0% of affected individuals was observed) (X^2 = 39,452; GDL = 6; P ≤ 0,001).

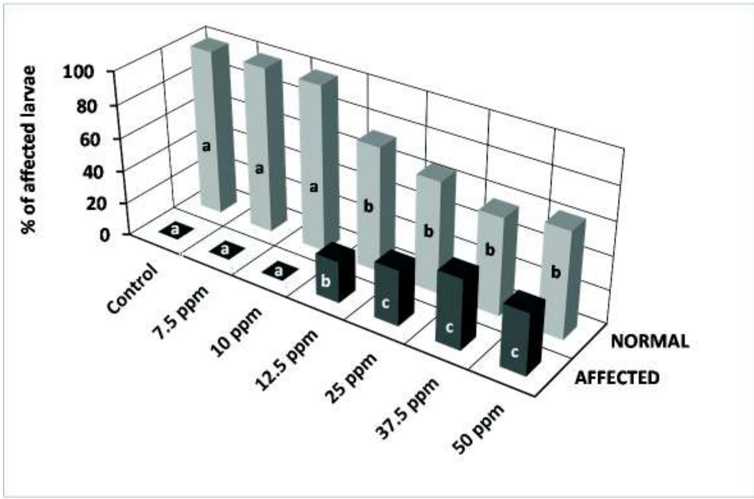

Figure 1. Percentage of normal and affected individuals at 24 h post-treatment. Each bar is the mean of 25-35 replicates. Bars marked with the same letter are not significantly different.

All concentrations of cypermethrin produced tremors (Figure 2). The percentage of individuals which presented this symptom increased as the dose increased. Only doses in the range 12.5 – 50 ng/larvae produced an effect significantly different from control (X^2 = 68,189; GDL = 6; P ≤ 0,0001).

Figure 3 shows the percentage of larvae who presented slow movement compared to control. Individuals treated with 7.5 or 10 ng/larvae presented this symptom, but it was not significantly different from control. The remained doses produced this symptom in a dose-dependent way and their effect was significantly different from control (X^2 = 47,337; GDL = 6; P ≤ 0,0001).

Knockdown results are showed in Figure 4. Only doses equal or higher than 12.5 hg/larvae produced knockdown significantly different from control (X^2 = 39,580; GDL = 6; P ≤ 0,001).

As observed when other effects were evaluated, no larvae glued to the substrate were obtained when 7.5 or 10 ng/larvae were applied (Figure 5). The effect produced by the remain doses were significantly different form control (X^2 = 16,029; GDL = 6; P ≤ 0,014), but no differences between doses were observed.

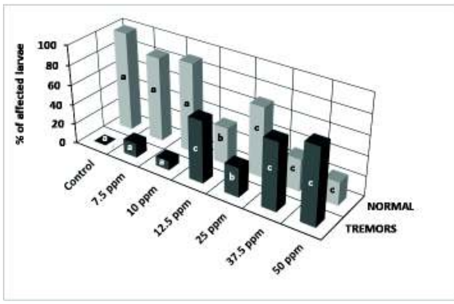

Figure 2. Percentage of individuals with tremors

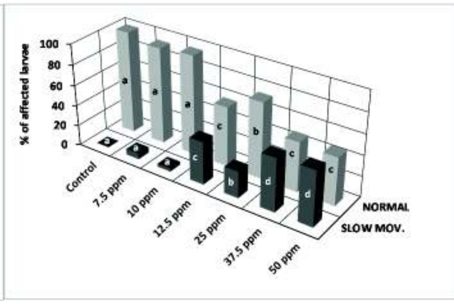

Figure 3. Percentage of individuals with slow movement compared to control

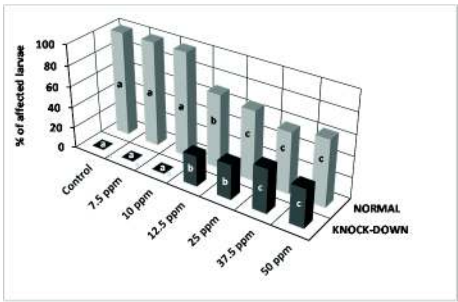

Figure 4. Percentage of knocked down individuals

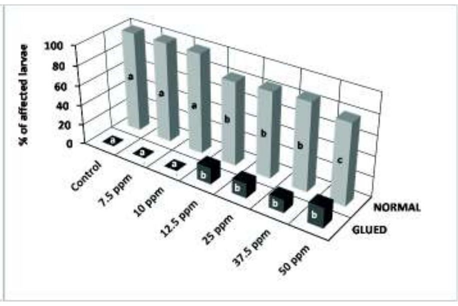

Figure 5. Percentage of individuals glued to the substrate

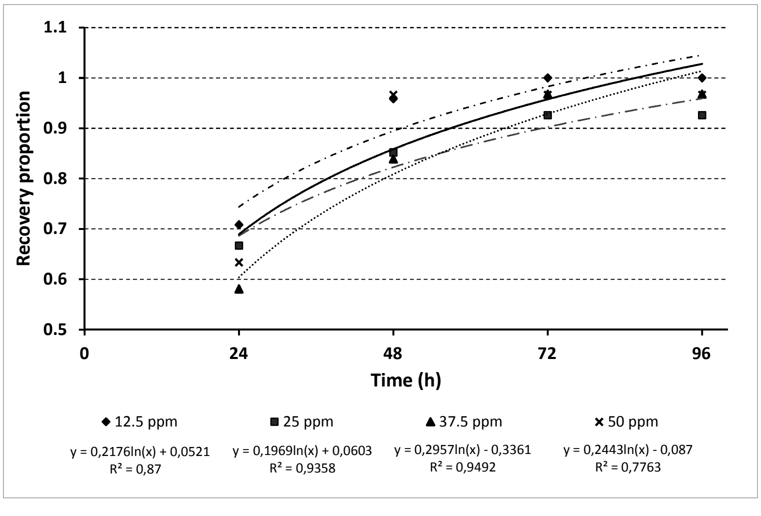

Figure 6. Recovery proportion of individuals evaluated during four days after insecticide application. Each value is the mean of 25-35 replicates.

DISCUSSION AND CONCLUSIONS

Within 24 h post-treatment, cypermethrin resulted toxic for third larval instar of *C. externa* from 12.5 ng/larvae. The symptoms observed are usual in insects intoxicated with pyrethroids (Naumann, 1990). Hyperactivity was reported as the first visible symptom of poisoning with pyrethroids (Gammon, 1978; Toth and Sparks, 1990; Alzogaray et al., 1997), however we did not observed hyperactivity in larvae of *C. externa*.

All concentrations tested produced tremors and slow movement, but knockdown and the insect glued to substrate were noticeable from 12,5ppm, which corresponds to the half the full-field recommended concentration.

In the present work, some treated larvae were glued to the substrate by the anal region due to a secretion-like substance. Regurgitation and increased excretion were observed in pyrethroid treated insect as a result of a general excitement of the nervous system (Naumann, 1990). The secretion-like substance could also be the defensive fluid that lacewings exude by the anus (Canard and Volkovich, 2001).

The symptoms of pyrethroid poisoning are reversible and recovery is a typical characteristic of the effects of these insecticides. In *Musca domestica*, recovery began 1 h after treatment with pyrethroids and it reached a plateau near 24 h later (Scott and Georghiu, 1984). In *Triatoma infestans*, recovery began two days after exposition to pyrethroids and it continued at least one week (Alzogaray and Zerba 1997). In our work, recovery started 24 h after treatment and it continued at 96 h even at the highest concentration tested, which corresponds to the double recommended for use in the field. Recovery has been attributed to detoxification processes catalyzed by microsomal mixed-function oxidases. When these enzymes were inhibited by application of piperonyl butoxide, recovery was not observed (Sawicki, 1982; Alzogaray and Zerba, 1977).

C. externa demonstrated a high tolerance to pyrethroids. This could be due to enzymatic activities involved in pyrethroids biotransformation (for example, esterases and mixed-function oxidases), or to a low sensitivity of the molecular target. Future studies should be addressed at biochemical and molecular levels to complete our knowledge about insecticides effects on this predator. Identification of the physiological mechanisms that confer to *C. externa* its capacity to survive from cypermethrin intoxication could help us to use this specie in IPM programs or in crops where the sprayings with broad spectrum insecticides is still used.

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