

Lethal and Sublethal Effects of Insecticides Used in Cotton on *Harmonia Axyridis*

Dyrson O. A. Neto

Universidade Federal de Lavras

Pablo C. Gontijo

Instituto Federal Goiano

Geraldo Andrade CARVALHO (✉ gacarval@ufla.br)

Universidade Federal de Lavras <https://orcid.org/0000-0002-8379-8008>

Research Article

Keywords: Gossypium spp., Pests, esticides, Predator, Selectivity, Biological, control.

Posted Date: August 17th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-756382/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

The agriculture industry seeks to reconcile control tactics for pest management, necessitating studies of lethal and sublethal effects of insecticides on natural enemies. The effect of six insecticides with diverse modes of action—ethiprole, etofenprox, lambda-cyhalothrin, lambda-cyhalothrin + chlorantraniliprole, pymetrozine, and thiamethoxam + chlorantraniliprole—were evaluated after spraying on eggs, third-instar larvae, pupae, and adults of *Harmonia axyridis*, a generalist predator in cotton crops. Ethiprole was the only one that had no lethal effect on the predator at any stage of its development; however, it caused sublethal effects when applied to eggs (shortening of the larval phase and lower reproduction). Lambda-cyhalothrin caused high predator mortality after exposure at all stages. Due to the high compatibility between the ladybug *H. axyridis* and ethiprole, this compound is recommended for use in cotton integrated pest management programs. The other insecticides were harmful in lethal or sublethal tests, and further work in semifield and field conditions is required to confirm if they reduce *H. axyridis* populations.

Highlights

- The lethal and sublethal effects of six insecticides on *Harmonia axyridis* were evaluated.
- Pupae of *H. axyridis* showed greater tolerance than other stages to the insecticides tested.
- Lambda-cyhalothrin was highly harmful to *H. axyridis* in the egg, pupae, and adult phases.
- Ethiprole was selective to *H. axyridis*.

Introduction

Cotton crops are attractive to insect pests for many reasons, such as the long period of crop development and its cultivation in large areas (Luo et al., 2014), as well as factors of the plant itself, such as the presence of showy vegetative and reproductive structures, floral exudates, and semiochemicals (Loughrin et al., 1994). These factors stimulate the colonization not only by arthropod pests but also important natural enemies of the pests (Ali et al., 2016). Pests that attack cotton crops have been controlled mainly with chemical insecticides, which represent approximately 20% of the total production cost of cotton (Miranda and Rodrigues, 2016; Wu and Guo, 2005). These compounds usually cause environmental contamination, biological imbalances, resurgence and selection of resistant arthropod populations (Biondi et al., 2012; Carazo-Rojas et al., 2018; Köhler and Triebkorn, 2013; Moscardini et al., 2015).

The implementation of integrated pest management (IPM) programs for cotton crops aims to integrate different control tactics to keep pest populations below the economic injury level using the lowest possible pesticide input. This will result in less impact of the compounds on natural enemies, which are the leading cause of pest natural mortality in agroecosystems (Bueno et al., 2017; Carvalho et al., 2019). In this context, studies on the selectivity of pesticides to natural enemies should be encouraged because they can provide the necessary information on the compatibility of chemical and biological methods within IPM programs of cotton, allowing for greater sustainability of this production sector (Gontijo et al., 2018; Santos et al., 2017). In this context, the lethal and sublethal effects of pesticides applied to this crop should be evaluated to find ones that best preserve natural enemies (Desneux et al., 2007; Passos et al., 2018).

Predatory insects act as important population regulators of herbivorous arthropods in many agroecosystems (Juen et al., 2012). Coleoptera, belonging to the family Coccinellidae, are polyphagous predators at all stages of their development that feed on various agricultural pests (Farooq et al., 2018; Hodek and Evans, 2012; Oliveira et al., 2004). Among the many species of this family, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) has stood out for its high voracity, as it can feed on approximately 77 species of prey, in addition to its wide distribution in agroecosystems, as it lives on approximately 82 plant species (Honěk, 2012; Koch, 2003). This predator feeds and reduces populations of several aphid species, especially one of the key pests of cotton crops, *Aphis gossypii* Glover (Hemiptera: Aphididae) (Seko et al., 2014; Iguchi et al., 2012; Xue et al., 2009), as well as other insect pests such as thrips (Li et al., 2020), psyllids (Michaud, 2002), eggs and juvenile stages of Curculionidae (Kalaskar and Evans, 2006; Stuart et al., 2002), and small lepidopteran larvae (Koch et al., 2003). Therefore, *H. axyridis* is arguably an important biological control agent (Riddick, 2017).

Due to the importance of preserving natural enemies in agroecosystems, the present study aimed to evaluate the lethal and sublethal effects of insecticides used on cotton crops towards *H. axyridis* to search for information that may ensure the compatibility of the chemical and biological methods with this predator in cotton IPM programs.

Materials And Methods

The rearing of *H. axyridis* and all bioassays were conducted in a climate-controlled room at $24 \pm 2^\circ\text{C}$, $60 \pm 10\%$ RH, and a 12-hour photoperiod. The experimental design used for all bioassays was completely randomized.

Insects used in the bioassays

Adult *H. axyridis* were collected from cotton plants present in the campus of the Federal University of Lavras in Lavras, Minas Gerais, Brazil (latitude: $21^\circ 14' 45''$ south, longitude: $44^\circ 59' 59''$ west). Approximately 200 adults were transported to the laboratory, where they were kept in acrylic cages (60 cm \times 30 cm \times 30 cm), with side openings lined with voile fabric to allow ventilation and prevent escape. Cabbage leaves (*Brassica oleracea* L. var. *acephala* DC.) were kept inside the cages to serve as oviposition substrates. Every 24 hours, the eggs were removed and transferred to Petri dishes 5 cm in diameter, where they remained until the larvae hatched. Larvae were transferred to 20 cm \times 10 cm \times 10 cm plastic cages and kept until they became pupae, which were placed in Petri dishes to obtain adult insects. Upon emergence, the insects were transferred to an acrylic cage, thus starting a new development cycle.

The larvae and adults of the predator were fed *ad libitum* every 48 hours with eggs of *Ephestia kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae) and *Myzus persicae* Sulzer (Homoptera: Aphididae) reared in leaves of *Nicandra physalodes* (Linnaeus) (Solanaceae). Water was provided to the insects via moistened cotton.

Compounds evaluated and their application

Six commercial insecticides that are widely used in cotton pest control were evaluated for their effects on *H. axyridis* at their maximum field concentrations recommended by the manufacturers (MAPA, 2020). The active ingredients, chemical groups according to the International Resistance Action Committee (2020), trade name, concentration used in g or mL of commercial product per hectare, and manufacturer were as follows: chlorantraniliprole + lambda-cyhalothrin (diamide and pyrethroid, Ampligo SC[®] - 400, Bayer S.A., São Paulo, SP,

Brazil); chlorantraniliprole + thiamethoxan (diamide and neonicotinoid, Voliam Flexi SC[®] - 250, Syngenta Proteção de Cultivos Ltda., São Paulo, SP, Brazil); ethiprole (phenylpyrazole, Curbix 200 SC[®] - 500, Bayer S.A., São Paulo, SP, Brazil); etofenprox (pyrethroid, Safety EC[®] - 500, Iharabras SA Indigenous Chemicals, Sorocaba, SP, Brazil); lambda-cyhalothrin (pyrethroid, Karate Zeon 250 CS[®] - 60, Syngenta Proteção de Cultivos Ltda., São Paulo, SP, Brazil); and pymetrozine (Pyridine azomethine derivative, Chess 500 WG[®] - 400, Syngenta Proteção de Cultivos Ltda., São Paulo, SP, Brazil). Distilled water was used as a control treatment.

Each product was applied via a Potter spray tower, adjusted to a pressure of 15 lb·pol⁻², enabling the application of 1.5 ± 0.5 mg of chemical solution·cm⁻², according to the method established by the International Organization for Biological and Integrated Control of Noxious Animals and Plants (van de Veire et al., 1996).

Effects of insecticides on *H. axyridis* eggs

For each treatment, 70 eggs (≤ 24 hours old) of the predator, obtained from laboratory rearing, were distributed at a ratio of 14 per 10-cm-diameter Petri dish and were treated by spraying in a Potter spray tower. The Petri dishes were then sealed with polyvinyl chloride (PVC) film with small holes to allow gas exchange.

The seven treatments evaluated were done in five replicates, each consisting of a Petri dish with 14 eggs of the predator. The evaluations began 24 hours after insecticide spraying. We recorded the duration of the embryonic period and the viability of the treated eggs. To avoid cannibalism, 48 hours after hatching, the surviving larvae were put in individual Petri dishes of 5-cm diameter that were sealed with PVC film. They were fed *ad libitum* with *E. kuehniella* eggs every 48 hours, and water was provided through moistened cotton. The following parameters were analyzed: durations of the larval and pupal stages, pupal viability, numbers of males and females for determining the sex ratio, and longevity of adult females.

To evaluate the effects of the products on reproduction, at least five pairs of surviving adults were separated per treatment (Hassan 1977), which were put individually in Petri dishes of 10-cm diameter, fed, and kept under the same conditions mentioned above. After the preoviposition period, to avoid predation of the eggs, the males were removed and weighed, and the total number of eggs (total fecundity) laid during the first five consecutive ovipositions were recorded. To evaluate viability, at least 40 eggs from the third and fourth egg lays in each replicate were put individually in Petri dishes. At the end of the evaluations of the number of eggs laid, the adult females were weighed.

Effect of insecticides on *H. axyridis* larvae

Forty third-instar larvae per treatment were treated by spraying the insecticides in a Potter spray tower and were then put individually in PVC-sealed Petri dishes 5 cm in diameter. Survival was evaluated daily up to 96 hours after spraying the insecticides, and the duration of the larval and pupal phases and pupal viability were also evaluated.

To evaluate the effects on the reproduction of adults originating from treated larvae, at least five surviving couples were separated per treatment (one couple per replicate), which were individualized into 10-cm-diameter Petri dishes. After the preoviposition period, the total number of eggs laid during the first 5 days was recorded to calculate the fecundity of the females, and then the adults were weighed. To assess egg viability, at least 40 eggs from the third and fourth ovipositions were individualized in Petri dishes.

Effects of insecticides on *H. axyridis* pupae

A total of 40 pupae aged up to 24 hours were subjected to each treatment. They were distributed at a total of five per Petri dish and treated in a Potter spray tower as above. Next, each pupa was put into its own Petri dish 5 cm in diameter, and after the emergence of the adults, they were kept and fed as described above.

The seven treatments had five replicates, and each plot consisted of eight pupae. The duration of the pupal phase, pupal viability, and morphological deformities in the wings of adult insects were evaluated. To observe the possible effects of insecticides on *H. axyridis* reproduction, at least five pairs were formed, which were allocated at one pair per 10-cm Petri dish to perform the evaluations. The biological characteristics evaluated were the same as those described in the third-instar bioassay.

Effect of insecticides on *H. axyridis* adults

For each treatment, 40 adults up to 48-hours old were distributed at five per Petri dish and treated with the products via Potter tower spraying. Soon after the application of the insecticides, the adults were transferred to new Petri dishes 10 cm in diameter, where they were kept and fed as above. Insect survival was evaluated daily up to 96 hours after application of the products. To evaluate the effects of the compounds on the reproduction of the surviving adults, at least five couples were separated per treatment (each couple represented one replicate) into 10-cm Petri dishes, and the evaluations were performed as above.

Data analysis

Data on the number and viability of eggs and pupae, duration of the larval period, larval survival, duration of the pupal period, adult weight, morphological deformities in the wings of the insects, and duration of the embryonic period were checked for homoscedasticity and normality to test the assumptions of analysis of variance (ANOVA). Next, they were subjected to one-way ANOVA with the Scott-Knott test ($p < 0.05$) (R Development Core Team, 2016). When the data did not have a normal distribution, they were fitted to generalized linear models (GLMs) using other distributions, such as Poisson and binomial, and the means were subjected to pairwise comparison (R Development Core Team, 2016). Sex ratio data were analyzed by the chi-squared test at the $p < 0.05$ level (Prop test, R Development Core Team, 2016).

The survival data of larvae and adults were subjected to survival analysis following the Weibull distribution model ('Survival', R Development Core Team, 2016). This procedure estimated survival curves using least squares estimators without censoring, generated from the proportion of surviving adults or larvae. To draw the graph, the following equation was used: $f(x) = \exp(-\mu^{-\alpha}t^{\alpha})$, where α in the software R is given by $1/\text{Scale}$, where Scale is the value returned by the command "summary", and μ is the 50%-lethal time (mean time for achieving the death of half of the treated population) in each treatment.

Results

Effects of insecticide application on *H. axyridis* eggs

When the insecticides were applied to *H. axyridis* eggs, none of them shortened the embryonic period, which averaged 2.4 to 2.8 days ($p = 0.9201$; $df = 6$; $F = 0.3216$). Lambda-cyhalothrin + chlorantraniliprole, etofenprox, and lambda-cyhalothrin reduced egg viability, differently from the other insecticides (Figures 1A and 2).

Despite the high percentage of hatching in the treatments with thiamethoxam + chlorantraniliprole, pymetrozine, and etofenprox, the survival of the larvae after 48 hours was less than 10%, making it impossible to evaluate other biological parameters. All larvae that hatched from eggs treated with lambda-cyhalothrin + chlorantraniliprole or lambda-cyhalothrin died within 48 hours after hatching. Ethiprole did not reduce larval survival, allowing evaluations of other biological parameters (Figures 1A and 2).

The larval stage was shortened in the ethiprole treatment, but this insecticide did not reduce pupal viability, adult weight (females and males), preoviposition period, total fecundity, or sex ratio. Nevertheless, ethiprole decreased egg viability of treated adults (Table 1).

Effects of insecticide application on *H. axyridis* larvae

Only ethiprole did not reduce larval survival. Thiamethoxam + chlorantraniliprole, lambda-cyhalothrin + chlorantraniliprole, and lambda-cyhalothrin were the most toxic, followed by pymetrozine and etofenprox (Figure 1B). Larvae treated with etofenprox showed “knockdown”, but most recovered within 48 hours.

For the three insecticides that caused the highest mortality, evaluations of sublethal effects were impossible. Of the evaluable insecticides, only etofenprox prolonged the larval stage (Table 2). The duration of the pupal stage, pupal survival, adult female weight, duration of the preoviposition period, and total fecundity were not reduced by any insecticide. The adult male insects that grew from larvae treated with pymetrozine or etofenprox had reduced body weight, whereas ethiprole had no such effect. Egg viability was reduced only by etofenprox (Table 2).

Effects of insecticide application on *H. axyridis* pupae

The pupal period was prolonged in the treatments with lambda-cyhalothrin + chlorantraniliprole, etofenprox, and lambda-cyhalothrin. The survival of insecticide-treated pupae was not reduced by thiamethoxam + chlorantraniliprole, pymetrozine, ethiprole, or etofenprox (Figure 1C). Lambda-cyhalothrin + chlorantraniliprole and lambda-cyhalothrin reduced pupal survival, in addition to causing morphological deformities in the elytra and difficulting exuvia remotion (Figure 1C and Figure 3). Two days after emergence, the insects of these two groups began to die, and after 5 days, fewer than 10% of them were alive, which differed from the other groups (Figure 4).

Due to the high mortality caused by lambda-cyhalothrin + chlorantraniliprole and lambda-cyhalothrin, we could not evaluate their effects on reproduction. The thiamethoxam + chlorantraniliprole treatment was the only one that prolonged the preoviposition period, while the other treatments did not affect this biological parameter. Fecundity and egg viability were reduced by thiamethoxam + chlorantraniliprole and pymetrozine, whereas ethiprole had no effect (Table 3).

Effects of the application of insecticides on *H. axyridis* adults

Lambda-cyhalothrin + chlorantraniliprole and lambda-cyhalothrin were the only insecticides toxic to *H. axyridis* adults (Figure 1D). The low number of survivors in these groups precluded the evaluation of sublethal effects. The preoviposition period ($F_{4,20} = 0.826$; $p = 0.518$) and total fecundity ($F_{4,20} = 0.881$; $p = 0.486$) were not reduced by the other insecticides: mean values for these biological traits ranged 4.1 to 5.0 days and 149.2 to 128.4 eggs, respectively. Egg viability was not reduced by thiamethoxam + chlorantraniliprole or ethiprole, while pymetrozine and etofenprox reduced this variable ($F_{4,20} = 7.219$; $p < 0.001$).

Discussion

In the present study, the insecticides affected the biological parameters of *H. axyridis* at varying degrees. Among the life stages of the predator, higher levels of toxicity were observed when eggs or third-instar larvae were exposed to insecticides, showing that these developmental stages are more sensitive to these insecticides than pupae or adults. The greater tolerance of adults to insecticides may be related to the higher sclerotization of their tegument (Nation, 2015), as greater rigidity and waxiness may reduce the penetration capacity of chemical compounds. Other important factors are the changes in the detoxification enzyme activity and sensitivity of the action site that occur over the insect life cycle. In the study by Cho et al. (2002), the concentration of an organophosphate (phosphamidon) required for acetylcholinesterase inhibition was 183.5 times higher in *H. axyridis* adults than in larvae, in addition to a 6.2-fold increase in the enzymatic activity of glutathione S-transferase. In addition, the weight of insects increased throughout development (egg to adult), reducing the proportion of insecticide by insect weight. This variation in toxicity between life stages has also been observed in *H. axyridis* in response to other insecticides and in other coccinellid species, such as *Eriopis connexa* (German) and *Adalia bipunctata* (Linnaeus) (Coleoptera: Coccinellidae) (Fogel et al., 2013; Garzón et al., 2015), where the larvae of these predators were more susceptible to insecticides than adults.

Ovicidal action occurs when the insecticide does not allow the larvae to hatch, despite the development of the embryo, which can be observed as a change in egg color from yellow to black (Koch, 2003). The physical and chemical structures of the egg chorion promote protection against the dissection and entry of hydrophilic substances during embryo development (Nation, 2015). The ability of insecticides to penetrate the chorion depends on their octanol–water partition coefficient ($\log K_{o/w}$), and those with high $\log K_{o/w}$ values more easily cross the chorion and translocate to the site of action (Hoffmann et al., 2008). In the present study, insecticides with higher $\log K_{o/w}$, such as lambda-cyhalothrin ($\log K_{o/w} = 7.0$) and etofenprox ($\log K_{o/w} = 6.9$), had an ovicidal effect. The insecticides with lower lipophilicity, pymetrozine ($\log K_{o/w} = -0.18$), thiamethoxan + chlorantraniliprole ($\log K_{o/w} = -0.13$ and 2.8), and ethiprole ($\log K_{o/w} = 2.9$), might have been blocked by the wax layer of the chorion and were therefore innocuous to the egg.

We assumed that lambda-cyhalothrin caused the ovicidal effect of the lambda-cyhalothrin + chlorantraniliprole mixture since this compound has high lipophilicity and, when evaluated alone, also caused a low hatching rate. Chlorantraniliprole belongs to the chemical group of anthranilic diamides, and due to its lower lipophilicity ($\log K_{o/w}$ of 2.8), it must have had difficulty penetrating the chorion. Yoriatti et al. (2009) found a low ovicidal effect of chlorantraniliprole when eggs of *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae) were directly exposed to its residues. The habit that coccinellid larvae have of feeding on and remaining on the chorion up to 24 hours after hatching (Nedvěd and Honěk, 2012) may have favored their contamination and consequently caused their death soon after they hatched. Similar results are reported for *H. axyridis*, in which thiamethoxam did not reduce egg viability but caused high mortality of first-instar larvae, and in which etofenprox reduced egg viability and caused high mortality among newly hatched larvae (Youn et al., 2003).

Unlike the other insecticides, ethiprole did not cause mortality of newly hatched larvae but shortened the larval period, and the adults generated laid eggs with reduced viability. Adults with inadequate nutritional status have reduced egg quality and viability (dos Santos Rodrigues et al., 2013; Shapiro and Shirk, 2010). The shortening of the larval stage may have led to malnourished adults, which resulted in reduced egg viability.

Ethiprole was innocuous to third-instar larvae, pupae, and adults of *H. axyridis*. There are few studies on the selectivity of ethiprole for coccinellids, but other phenylpyrazoles have been shown to be selective for *Cycloneda sanguinea* (Linnaeus) (Soares and Busoli, 2000) and *Serangium japonicum* Chapin. (Coleoptera: Coccinellidae) (Ozawa and Uchiyama, 2016), as well as for other natural enemies, such as *Trichogramma galloi* Zucchi, 1988 (Oliveira et al., 2013) and *Trichogramma ostrinae* (Peng & Chen) (Hymenoptera: Trichogrammatidae) (Wang et al., 2012).

Thiamethoxan + chlorantraniliprole, pymetrozine, and etofenprox caused lower mortality of third-instar *H. axyridis* larvae than first-instar larvae that hatched from treated eggs. Often, insects in their first instars are more susceptible to the effects of insecticides as a result of their lower enzymatic activity (Cho et al., 2002).

Like the chorion, the tegument of insects also has a protective wax layer that provides impermeability (Nation, 2015). Thus, the specific physicochemical characteristics of each insecticide may provide greater or lesser penetration through the tegument. Lambda-cyhalothrin, lambda-cyhalothrin + chlorantraniliprole, and etofenprox reduced the survival of *H. axyridis* larvae, probably due to their high $\log K_{o/w}$ values (> 6.9), which may have contributed to their greater penetration of the tegument and thus greater likelihood of reaching its site of action. Although etofenprox is also a pyrethroid, the results of our study show that this active ingredient is less harmful to *H. axyridis* than lambda-cyhalothrin since most of the larvae recovered from the knockdown effect and the insecticide did not cause mortality in pupae or adults of this predator. However, it lengthened the larval stage and reduced the total fecundity and viability of the eggs of the adults. It is noteworthy that the larvae and adults that suffered a knockdown effect of etofenprox recovered within 48 hours.

The different results found for the insecticides might be related to the different metabolism and/or excretion routes due to variations in the chemical structures of the insecticides, even in the case of compounds belonging to the same chemical group (Nauen et al., 2015). The main enzymes involved in pyrethroid tolerance are cytochrome P450 and esterases, as they catalyze multiple oxidative reactions due to increased gene expression of this family of enzymes (Scott and Wen, 2001). In *Propylaea japonica* (Thunberg) (Coleoptera: Coccinellidae), the increase in the activity of the enzymes glutathione S-transferase, carboxylesterase, and cytochrome P450 monooxygenase is reported to be involved in greater tolerance to insecticides (Wu et al., 2018).

The energy expenditure needed to detoxify etofenprox may have been responsible for the prolongation of the larval phase of the predator and negative effects on the nutritional status of the emerged adults, which ultimately culminated in decreased viability and fecundity of eggs. These results corroborate those of Galvan et al. (2005), who also observed a longer young stage of *H. axyridis* when the insects were treated by spraying the insecticide indoxacarb. They assumed that such an effect this was resulted from metabolism and/or energy expenditure required for the insecticide excretion. Santos et al. (2016) found that *E. connexa* ladybug recovered from the knockdown effect of pyrethroid insecticides within up to 72 hours.

The sublethal effects (reduced weight of males, reduced fertility, and reduced egg viability) caused by pymetrozine when sprayed on larvae, pupae, and adults, are related to its mode of action. This insecticide blocks the salivary gland of insects, causing the interruption of food intake mainly in sucking insects (Sechser et al., 2002). Cunha et al. (2015) found that Coleoptera were also sensitive to pymetrozine, with histological changes in the intestine. Thus, the reduced weight of adult males may have been due to these effects of the insecticide, leading to reduced food consumption of *H. axyridis*. Although only the weight of males changed, this may explain the reduction in egg viability because males with higher weight may supply a more sperm, oviposition stimulants,

and other nutrients during copulation than smaller males (Omkar and Afaq, 2013; Singh et al., 2019). According to Almasi et al. (2013), pymetrozine caused close to 40% mortality of third-instar larvae of the ladybug *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae). On the other hand, when applied to adults of *Coccinella septempunctata* Linnaeus and *H. variegata* (Coleoptera: Coccinellidae), pymetrozine was shown to be harmless (Skouras et al., 2019).

Overall, the pupal stage was the most tolerant to the insecticides in this study since only one insecticide (lambda-cyhalothrin) reduced pupa survival. This compound also led to deformities in adult elytra. In addition, when applied in pupae, thiamethoxam, pymetrozine, ethiprole and etofenprox affected the pupal period, adult fecundity and egg viability. These negative effects may be related to the interference of insecticides on the nervous system of insects, which is responsible for regulating the synthesis of neurohormones and different enzymatic processes, which affect the production of hormones related to development and metamorphosis. Li et al. (2019) found that changes in the production of the enzyme α -glucosidase-trehalose in *H. axyridis* impair the chitin synthesis pathway, hindering insect development and causing wing deformities. Corroborating the results obtained in the present work, other studies have demonstrated that *H. axyridis* pupae exposed to thiamethoxam or etofenprox had reduced survival (Youn et al., 2003). However, in *E. connexa* pupae, the application of cypermethrin significantly reduced their survival and led to the formation of adults with morphological deformities in the wings (Fogel et al., 2016). The high mortality after the emergence of the deformed insects in our study probably occurred due to dehydration and starvation since they had difficulty moving and feeding.

In the adult stage, only lambda-cyhalothrin and lambda-cyhalothrin + chlorantraniliprole were toxic to *H. axyridis*. Possibly, the high mortality caused by the lambda-cyhalothrin + chlorantraniliprole mixture is due only to the active ingredient lambda-cyhalothrin, since the numbers of dead insects with and without chlorantraniliprole were similar. In addition, studies have shown the selectivity of chlorantraniliprole for *H. axyridis* (Liu et al., 2016) and other natural enemies of the Coccinellidae family, such as *C. septempunctata* (He et al., 2019; Jiang et al., 2020). Lambda-cyhalothrin has a broad spectrum of action and low selectivity for natural enemies of the Coccinellidae family. When larvae of *H. axyridis* and *A. bipunctata* were exposed to a surface treated with lambda-cyhalothrin, the mortality was higher than 80% (Benelli et al., 2015).

In short, the early stages of development of *H. axyridis*, such as eggs and larvae, were the most susceptible to insecticides, followed by the pupal and adult stages. The present results can help IPM programs achieve compatibility between the chemical and biological methods, through the use of predator-friendly insecticides. In this context, the insecticide that showed the greatest compatibility with the predator was ethiprole, which may be recommended in IPM programs of cotton. The other insecticides were toxic and should be evaluated under semifield and field conditions to confirm their deleterious effects on *H. axyridis*.

Declarations

Acknowledgments

The authors thank the Department of Entomology at the Universidade Federal de Lavras for their technical support.

Funding

This research was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) – Financial code 306892/2017-0, and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) – Financial code CAG APQ – 02118-16.

Author contributions

DOAN and GAC conceived research. DOAN and PCG assisted in the creation and maintenance of insects, the assembly and conduct of experiments, as well as in the collection and tabulation of data. PCG, DOAN and GAC performed formal analysis, data analysis and writing of the manuscript.

Affiliations

Department of Entomology of the Federal University of Lavras – UFLA, Lavras 37200-900, Minas Gerais, Brazil

Corresponding author

Correspondence to Geraldo Andrade Carvalho

Ethics declarations

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors. The authors agree with the publication of the manuscript in this form.

References

1. Ali, A., Desneux, N., Lu, Y., Liu, B., Wu, K., 2016. Characterization of the natural enemy community attacking cotton aphid in the Bt cotton ecosystem in Northern China. *Sci. Rep.* 6, 1–9. <https://doi.org/10.1038/srep24273>
2. Almasi, A., Sabahi, Q., Talebi, K., Mardani, A., 2013. Laboratory evaluation of the toxicity of proteus, pymetrozine, deltamethrin, and pirimicarb on lady beetle *Hippodamia variegata* (Goeze) (Col.: Coccinellidae). *J. Plant Prot. Res.* 53, 143–147. <https://doi.org/10.2478/jppr-2013-0021>
3. Benelli, M., Leather, S.R., Francati, S., Marchetti, E., Dindo, M.L., 2015. Effect of two temperatures on biological traits and susceptibility to a pyrethroid insecticide in an exotic and native coccinellid species. *Bull. Insectology* 68, 23–29.
4. Biondi, A., Desneux, N., Siscaro, G., Zappalà, L., 2012. Using organic-certified rather than synthetic pesticides may not be safer for biological control agents: Selectivity and side effects of 14 pesticides on the predator *Orius laevigatus*. *Chemosphere* 87, 803–812. <https://doi.org/10.1016/j.chemosphere.2011.12.082>
5. Bueno, A. de F., Carvalho, G.A., Santos, A.C. dos, Sosa-Gómez, D.R., Silva, D.M. da, 2017. Pesticide selectivity to natural enemies: challenges and constraints for research and field recommendation. *Ciência Rural* 47. <https://doi.org/10.1590/0103-8478cr20160829>

6. Carazo-Rojas, E., Pérez-Rojas, G., Pérez-Villanueva, M., Chinchilla-Soto, C., Chin-Pampillo, J.S., Aguilar-Mora, P., Alpízar-Marín, M., Masís-Mora, M., Rodríguez-Rodríguez, C.E., Vryzas, Z., 2018. Pesticide monitoring and ecotoxicological risk assessment in surface water bodies and sediments of a tropical agro-ecosystem. *Environ. Pollut.* 241, 800–809. <https://doi.org/10.1016/J.ENVPOL.2018.06.020>
7. Cho, J.R., Kim, Y.J., Kim, H.S., Yoo, J.K., 2002. Some biochemical evidence on the selective insecticide toxicity between the two aphids, *Aphis citricola* and *Myzus malisuctus* (Homoptera: Aphididae), and their predator, *Harmonia axyridis* (Coleoptera: Coccinellidae). *J. Asia. Pac. Entomol.* 5, 49–53. [https://doi.org/10.1016/S1226-8615\(08\)60132-6](https://doi.org/10.1016/S1226-8615(08)60132-6)
8. Cunha, F.M., Wanderley-Teixeira, V., Teixeira, Á.A.C., Pereira, B.F., Caetano, F.H., Torres, J.B., Gonçalves, G.G.A., Santos, F.A.B., 2015. Effects of pymetrozine on biochemical parameters and the midgut ultrastructure of *Anthonomus grandis* Boheman (Coleoptera: Curculionidae). *Anim. Biol.* 65, 271–285. <https://doi.org/10.1163/15707563-00002476>
9. Desneux, N., Decourtye, A., Delpuech, J.-M., 2007. The sublethal effects of pesticides on beneficial arthropods. *Annu. Rev. Entomol.* 52, 81–106. <https://doi.org/10.1146/annurev.ento.52.110405.091440>
10. dos Santos Rodrigues, A.R., Spíndola, A.F., de Moraes Oliveira, J.E., Torres, J.B., 2013. Dietary effects upon biological performance and lambda-cyhalothrin susceptibility in the multicolored Asian lady beetle, *Harmonia axyridis*. *Phytoparasitica* 41, 285–294. <https://doi.org/10.1007/s12600-013-0289-x>
11. Farooq, M., Shakeel, M., Iftikhar, A., Shahid, M.R., Zhu, X., 2018. Age-stage, two-sex life tables of the lady beetle (Coleoptera: Coccinellidae) feeding on different aphid species. *J. Econ. Entomol.* 111, 575–585. <https://doi.org/10.1093/jee/toy012>
12. Fogel, M.N., Schneider, M.I., Desneux, N., González, B., Ronco, A.E., 2013. Impact of the neonicotinoid acetamiprid on immature stages of the predator *Eriopis connexa* (Coleoptera: Coccinellidae). *Ecotoxicology* 22, 1063–1071. <https://doi.org/10.1007/s10646-013-1094-5>
13. Fogel, M.N., Schneider, M.I., Rimoldi, F., Ladux, L.S., Desneux, N., Ronco, A.E., 2016. Toxicity assessment of four insecticides with different modes of action on pupae and adults of *Eriopis connexa* (Coleoptera: Coccinellidae), a relevant predator of the Neotropical Region. *Environ. Sci. Pollut. Res.* 23, 14918–14926. <https://doi.org/10.1007/s11356-016-6654-9>
14. Galvan, T.L., Koch, R.L., Hutchison, W.D., 2005. Effects of spinosad and indoxacarb on survival, development, and reproduction of the multicolored Asian lady beetle (Coleoptera: Coccinellidae). *Biol. Control* 34, 108–114. <https://doi.org/10.1016/J.BIOCONTROL.2005.04.005>
15. Garzón, A., Medina, P., Amor, F., Viñuela, E., Budia, F., 2015. Toxicity and sublethal effects of six insecticides to last instar larvae and adults of the biocontrol agents *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) and *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae). *Chemosphere* 132, 87–93. <https://doi.org/10.1016/J.CHEMOSPHERE.2015.03.016>
16. Gontijo, P.C., Abbade Neto, D.O., Oliveira, R.L., Michaud, J.P., Carvalho, G.A., 2018. Non-target impacts of soybean insecticidal seed treatments on the life history and behavior of *Podisus nigrispinus*, a predator of fall armyworm. *Chemosphere* 191, 342–349. <https://doi.org/10.1016/j.chemosphere.2017.10.062>
17. Hassan, S.A. Standardized techniques for testing side-effects of pesticides on beneficial arthropods in the laboratory. *Zeitsc. für Pflanzenk. Pflanz., Stuttgart*, v.84, n.3, p.158-163, 1977.
18. He, F., Sun, S., Tan, H., Sun, X., Shang, D., Yao, C., Qin, C., Ji, S., Li, X., Zhang, J., Jiang, X., 2019. Compatibility of chlorantraniliprole with the generalist predator *Coccinella septempunctata* L. (Coleoptera: Coccinellidae)

- based toxicity, life-cycle development and population parameters in laboratory microcosms. *Chemosphere* 225, 182–190. <https://doi.org/10.1016/J.CHEMOSPHERE.2019.03.025>
19. Hodek, I., Evans, E.W., 2012. Food Relationships. *Ecol. Behav. Ladybird Beetles* 141–274. <https://doi.org/10.1002/9781118223208.ch5>
 20. Hoffmann, E.J., Middleton, S.M., Wise, J.C., 2008. Ovicidal activity of organophosphate, oxadiazine, neonicotinoid and insect growth regulator chemistries on northern strain plum curculio, *Conotrachelus nenuphar*. *J. Insect Sci.* 8, 1–6. <https://doi.org/10.1673/031.008.2901>
 21. Honěk, A., 2012. Distribution and Habitats. *Ecol. Behav. Ladybird Beetles* 110–140. <https://doi.org/10.1002/9781118223208.ch4>
 22. Iguchi, M., Fukushima, F., Miura, K., 2012. Control of *Aphis gossypii* and *Myzus persicae* (Hemiptera: Aphididae) by a flightless strain of *Harmonia axyridis* (Coleoptera: Coccinellidae) on green pepper plants in open fields. *Entomol. Sci.* 15, 127–132. <https://doi.org/10.1111/j.1479-8298.2011.00471.x>
 23. Jiang, J., Wang, Y., Mu, W., Zhang, Z., 2020. Sublethal effects of anthranilic diamide insecticides on the demographic fitness and consumption rates of the *Coccinella septempunctata* (Coleoptera: Coccinellidae) fed on *Aphis craccivora*. *Environ. Sci. Pollut. Res.* 27, 4178–4189. <https://doi.org/10.1007/s11356-019-06993-z>
 24. Juen, A., Hogendoorn, K., Ma, G., Schmidt, O., Keller, M.A., 2012. Analysing the diets of invertebrate predators using terminal restriction fragments. *J. Pest Sci.* (2004). 85, 89–100. <https://doi.org/10.1007/s10340-011-0406-x>
 25. Kalaskar, A., Evans, E.W., 2006. Larval responses of aphidophagous lady beetles (Coleoptera: Coccinellidae) to weevil larvae versus aphids as prey. *Ann. Entomol. Soc. Am.* 94, 76–81. [https://doi.org/10.1603/0013-8746\(2001\)094\[0076:lroalb\]2.0.co;2](https://doi.org/10.1603/0013-8746(2001)094[0076:lroalb]2.0.co;2)
 26. Koch, R.L., 2003. The multicolored Asian lady beetle, *Harmonia axyridis*: A review of its biology, uses in biological control, and non-target impacts. *J. Insect Sci.* 3. <https://doi.org/10.1093/jis/3.1.32>
 27. Koch, R.L., Hutchison, W.D., Venette, R.C., Heimpel, G.E., 2003. Susceptibility of immature monarch butterfly, *Danaus plexippus* (Lepidoptera: Nymphalidae: Danainae), to predation by *Harmonia axyridis* (Coleoptera: Coccinellidae). *Biol. Control* 28, 265–270. [https://doi.org/10.1016/S1049-9644\(03\)00102-6](https://doi.org/10.1016/S1049-9644(03)00102-6)
 28. Köhler, H.R., Triebkorn, R., 2013. Wildlife ecotoxicology of pesticides: Can we track effects to the population level and beyond? *Science* 341, 759–765. <https://doi.org/10.1126/science.1237591>
 29. Li, X.-Y., Qi, P.-Y., Zhang, Q.-P., Zhang, R.-F., Chen, Z., Jin, L.-H., Song, B.-A., 2020. Introduction of two predators to control *Dendrothrips minowai* (Thysanoptera: Thripidae) in tea (*Camellia sinensis*) plantations in China. *Biocontrol Sci. Technol.* 0, 1–11. <https://doi.org/10.1080/09583157.2020.1729699>
 30. Li, Y., Chen, X., Wang, S.-S., Pan, B.-Y., Wang, S.-G., Wang, S., Tang, B., 2019. Evaluation of the expression and function of the TRE2-like and TRE2 genes in ecdysis of *Harmonia axyridis*. *Front. Physiol.* 10, 1371. <https://doi.org/10.3389/fphys.2019.01371>
 - Liu, Y., Li, X., Zhou, C., Liu, F., Mu, W., 2016. Toxicity of nine insecticides on four natural enemies of *Spodoptera exigua*. *Nat. Publ. Gr.* 1–9. <https://doi.org/10.1038/srep39060>
 31. Loughrin, J.H., Manukian, A., Heath, R.R., Turlings, T.C., Tumlinson, J.H., 1994. Diurnal cycle of emission of induced volatile terpenoids by herbivore-injured cotton plant. *Proc. Natl. Acad. Sci.* 91, 11836–11840. <https://doi.org/10.1073/PNAS.91.25.11836>

32. Luo, Q., Bange, M., Clancy, L., 2014. Cotton crop phenology in a new temperature regime. *Ecol. Modell.* 285, 22–29. <https://doi.org/10.1016/j.ecolmodel.2014.04.018>
33. MAPA (2020). Ministério da Agricultura, Pecuária e Abastecimento, Brazil. http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons
34. Michaud, J.P., 2002. Biological control of Asian citrus psyllid (Homoptera: Psyllidae) in Florida. A preliminary report. *Entomol. News.*
35. Miranda, J.E., Rodrigues, S.M.M., 2016. Manejo do bicudo-do-algodoeiro em áreas de agricultura intensiva. *EMBRAPA Algodão Circ. técnica* 140 18.
36. Moscardini, V.F., Gontijo, P.C., Michaud, J.P., Carvalho, G.A., 2015. Sublethal effects of insecticide seed treatments on two nearctic lady beetles (Coleoptera: Coccinellidae). *Ecotoxicology* 24, 1152–1161. <https://doi.org/10.1007/s10646-015-1462-4>
37. Nation, J.L., 2015. *Insect physiology and biochemistry*, Third Edit. ed. CRC Press, Boca Raton, FL - USA.
38. Nauen, R., Jeschke, P., Velten, R., Beck, M.E., Ebbinghaus-Kintscher, U., Thielert, W., Wölfel, K., Haas, M., Kunz, K., Raupach, G., 2015. Flupyradifurone: A brief profile of a new butenolide insecticide. *Pest Manag. Sci.* 71, 850–862. <https://doi.org/10.1002/ps.3932>
39. Nedvěd, O., Honěk, A., 2012. Life history and development. *Ecol. Behav. Ladybird Beetles* 54–109. <https://doi.org/10.1002/9781118223208.ch3>
40. Oliveira, N.C. De, Wilcken, C.F., Matos, C.A.O. De, 2004. Ciclo biológico e predação de três espécies de coccinélidos (Coleoptera, Coccinellidae) sobre o pulgão-gigante-do-pinus *Cinara atlantica* (Wilson) (Hemiptera, Aphididae). *Rev. Bras. Entomol.* 48, 529–533. <https://doi.org/10.1590/S0085-56262004000400016>
41. Oliveira, H.N., Antigo, M. de R., Carvalho, G.A., Glaeser, D.F., Pereira, F.F., 2013. Selectivity of inseticides used in the sugar-cane on adults of *Trichogramma galloi* Zucchi (Hymenoptera: Trichogrammatidae). *Biosci. J.* 29, 1266–1273.
42. Omkar, O., Afaq, U., 2013. Evaluation of darwin's fecundity advantage hypothesis in parthenium beetle, *Zygogramma bicolorata* Pallister. *Insect Sci.* <https://doi.org/10.1111/1744-7917.12058>
43. Ozawa, A., Uchiyama, T., 2016. Effects of pesticides on adult ladybird beetle *Serangium japonicum* (Coleoptera: Coccinellidae), a potential predator of the tea spiny whitefly *Aleurocanthus camelliae* (Hemiptera: Aleyrodidae). *Japanese J. Appl. Entomol. Zool.* 60, 45–49. <https://doi.org/10.1303/jjaez.2016.45>
44. Passos, L.C., Soares, M.A., Collares, L.J., Malagoli, I., Desneux, N., Carvalho, G.A., 2018. Lethal, sublethal and transgenerational effects of insecticides on *Macrolophus basicornis*, predator of *Tuta absoluta*. *Entomol. Gen.* 38, 127–143. <https://doi.org/10.1127/entomologia/2018/0744>
45. R Development Core Team (2016) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.
46. Riddick, E.W., 2017. Spotlight on the positive effects of the ladybird *Harmonia axyridis* on agriculture. *BioControl* 62, 319–330. <https://doi.org/10.1007/s10526-016-9758-8>
47. Santos, D.S., Rodrigues, A.R.S., Torres, J.B., Lira, R., 2016. Performance of *Eriopis connexa* (Coleoptera: Coccinellidae) resistant to lambda-cyhalothrin after extended recovery from knockdown. *Neotrop. Entomol.* 45, 718–724. <https://doi.org/10.1007/s13744-016-0422-x>

48. Santos, K.F.A., Zanuzo Zanardi, O., de Morais, M.R., Jacob, C.R.O., de Oliveira, M.B., Yamamoto, P.T., 2017. The impact of six insecticides commonly used in control of agricultural pests on the generalist predator *Hippodamia convergens* (Coleoptera: Coccinellidae). *Chemosphere* 186, 218–226. <https://doi.org/10.1016/J.CHEMOSPHERE.2017.07.165>
49. Scott, J.G., Wen, Z., 2001. Cytochromes P450 of insects: The tip of the iceberg. *Pest Manag. Sci.* 57, 958–967. <https://doi.org/10.1002/ps.354>
50. Sechser, B., Reber, B., Bourgeois, F., 2002. Pymetrozine: Selectivity spectrum to beneficial arthropods and fitness for integrated pest management. *Anzeiger fur Schadlingskd.* 75, 72–77. <https://doi.org/10.1034/j.1399-5448.2002.02021.x>
51. Seko, T., Sumi, A., Nakano, A., Kameshiro, M., Kaneda, T., Miura, K., 2014. Suppression of aphids by augmentative release of larvae of flightless *Harmonia axyridis*. *J. Appl. Entomol.* 138, 326–337. <https://doi.org/10.1111/jen.12090>
52. Shapiro, J.P., Shirk, P.D., 2010. Ovarian development in predacious *Orius pumilio*: Relationship to diet, mating, and juvenile hormone. *Ann. Entomol. Soc. Am.* 103, 971–978. <https://doi.org/10.1603/AN09123>
53. Singh, P., Mishra, G., Omkar, 2019. Influence of body size and familiarity on mating and reproductive parameters in the zig-zag ladybird beetle, *Menochilus sexmaculatus* (Coleoptera: Coccinellidae). *Can. J. Zool.* 97, 453–463. <https://doi.org/10.1139/cjz-2017-0344>
54. Skouras, P.J., Stathas, G.J., Demopoulos, V., Louloudakis, G., Margaritopoulos, J.T., 2019. The effect of five insecticides on the predators *Coccinella septempunctata* and *Hippodamia variegata*. *Phytoparasitica* 47, 197–205. <https://doi.org/10.1007/s12600-019-00731-4>
55. SOARES, J.J., BUSOLI, A.C., 2000. Efeito de inseticidas em insetos predadores em culturas de algodão. *Pesqui. Agropecuária Bras.* 35, 1889–1894. <https://doi.org/10.1590/S0100-204X2000000900023>
56. Stuart, R.J., Michaud, J.P., Olsen, L., McCoy, C.W., 2002. Lady beetles as potential predators of the root weevil *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in Florida citrus. *Florida Entomol.* 85, 409–416. [https://doi.org/10.1653/0015-4040\(2002\)085\[0409:lbappo\]2.0.co;2](https://doi.org/10.1653/0015-4040(2002)085[0409:lbappo]2.0.co;2)
57. Van de Veire, M., Smagghe, G.; Degheele, D., 1996. A laboratory test method to evaluate the effect of 31 pesticides on the predatory bug, *Orius laevigatus* (Heteroptera: Anthocoridae). *Entomophaga*, v.41, p. 235–243.
58. Wang, Y., Chen, L., Yu, R., Zhao, X., Wu, C., Cang, T., Wang, Q., 2012. Insecticide toxic effects on *Trichogramma ostrinae* (Hymenoptera: Trichogrammatidae). *Pest Manag. Sci.* 68, 1564–1571. <https://doi.org/10.1002/ps.3343>
59. Wu, G., Wang, Y., Wang, J.N., Chen, X.Z., Hu, Q.X., Yang, Y.F., Liu, Q.Q., 2018. Vitality and stability of insecticide resistance in adult *Propylaea japonica* (Coleoptera: Coccinellidae). *J. Insect Sci.* 18. <https://doi.org/10.1093/jisesa/iey109>
60. Wu, K.M., Guo, Y.Y., 2005. The evolution of cotton pest management practices in China. *Annu. Rev. Entomol.* 50, 31–52. <https://doi.org/10.1146/annurev.ento.50.071803.130349>
61. Xue, Y., Bahlai, C.A., Frewin, A., Sears, M.K., Schaafsma, A.W., Hallett, R.H., 2009. Predation by *Coccinella septempunctata* and *Harmonia axyridis* (Coleoptera: Coccinellidae) on *Aphis glycines* (Homoptera: Aphididae). *Environ. Entomol.* 38, 708–714. <https://doi.org/10.1603/022.038.0322>
62. Youn, Y.N., Seo, M.J., Shin, J.G., Jang, C., Yu, Y.M., 2003. Toxicity of greenhouse pesticides to multicolored Asian lady beetles, *Harmonia axyridis* (Coleoptera: Coccinellidae). *Biol. Control* 28, 164–170.

Tables

Table 1. Duration of the larval and pupal phases (days), weight of adults formed (mg), preoviposition period (days), fecundity, and viability of the eggs of *Harmonia axyridis* adults that hatched from eggs treated with the insecticide ethiprole via a Potter spray tower.

Treatment	Larval stage (days)	Pupal stage (days)	Weight \bar{x} (mg)	Weight \bar{x} (mg)	Preoviposition period (days) *	Total fecundity *	Egg viability (%) *
Control	11.3±0.13 a	4.9±0.07	40.3±0.64	36.3±0.67	6.8±0.22	143.0±13.52	84.8±1.75 a
Ethiprole	10.4±0.16 b	4.8±0.10	42.1±0.96	35.5±0.66	6.4±0.26	153.1±10.90	73.1±2.40 b
<i>p</i> -value	<0.05	0.4484	0.1571	0.4011	0.304	0.5695	<0.01
df	7.717	7.2192	25.148	35.08	19	16.666	18.633
<i>t</i>	2.8937	0.80146	-1.4585	0.84994	1.056	-0.5803	3.9005

Means (\pm SE) followed by the same letter in a column do not differ by the *t*-test ($p < 0.05$). *At least five couples were evaluated per treatment.

Table 2. Duration of the larval and pupal stages (days), survival of pupae (%), weight of adults formed (mg), total fecundity per female, and egg viability (%) of adult *Harmonia axyridis* originated from third-instar larvae treated with the insecticides via a Potter spray tower.

Treatment	Larval stage (days) *	Pupal stage (days)	Pupal survival (%) ¹	Weight \bar{x} (mg)	Weight \bar{x} (mg)	Total fecundity	Egg viability (%)
Control	4.1±0.21 a	4.2±0.14	100.0±0.00	43.6±0.97	38.4±0.99 a	164.4±17.99	85.8±1.38 a
Pymetrozine	4.6±0.31 a	4.4±0.19	96.0±4.00	42.3±2.21	34.7±0.79 b	160.4±31.9	76.0±2.29 a
Ethiprole	4.0±0.17 a	4.3±0.61	97.5±2.50	44.7±1.20	36.8±1.22 a	144.4±10.03	82.4±3.34 a
Etofenprox	5.5±0.11 b	4.4±0.08	100.0±0.00	42.5±2.40	33.3±1.01 b	106.8±12.56	54.2±3.33 b
<i>p</i> -value	<0.001	0.4592	0.174	0.5498	<0.01	0.1885	<0.001
df	3	3	3	3	3	3	3
<i>F</i>	9.9089	0.9075	1.8778	0.6518	5.3079	1.745	14.8

Means (\pm SEM) followed by the same letter in a column do not differ. One-way ANOVA $p < 0.05$; ¹ binomial GLM, pairwise comparison, $p < 0.05$; (quasi-Poisson GLM, pairwise comparison, $p < 0.05$). * Time recorded after

treatment application. At least five pairs per treatment were evaluated on preoviposition period, fecundity, and egg viability.

Table 3. Duration of the pupal period (days), preoviposition period (days), total fecundity, and egg viability (%) of *Harmonia axyridis* adults that grew from pupae treated with insecticides via a Potter tower.

Treatment	Pupal stage (days) *	Deformities in adults (%) ¹	Preoviposition period (days)	Total fecundity ²	Egg viability (%)
Control	3.9±0.03 c	2.50±0.91 b	4.6±0.48 a	139.1±7.98 a	77.8±1.71 a
Thiamethoxam + Chlorantraniliprole	3.9±0.07 c	5.00±1.11 b	6.4±0.24 b	99.8±12.80 b	57.2±3.13 b
Lambda-cyhalothrin + Chlorantraniliprole	5.1±0.08 a	72.67±4.27 a	**	**	**
Pymetrozine	3.9±0.04 c	2.86±1.06 b	4.9±0.40 a	117.4±8.43 b	57.6±3.34 b
Ethiprole	3.9±0.03 c	2.85±1.02 b	4.4±0.30 a	159.7±7.35 a	74.4±1.02 a
Etofenprox	4.1±0.06 b	5.71±1.27 b	4.7±0.42 a	108.9±11.31 b	59.3±3.28 b
Lambda-cyhalothrin	4.9±0.06 a	62.33±4.14 a	**	**	**
<i>p</i> -value	<0.001	<0.001	<0.01	<0.001	<0.001
df	6	6	4	4	4
<i>F</i>	82.214	3.24	3.1313	6.1857	15.305

Means (± SEM) followed by the same letter in a column do not differ. One-way ANOVA ($p < 0.05$) or ¹ GLM ($p < 0.05$). * Time recorded after treatment application. At least five couples were used per treatment. ** Not enough insects were obtained to evaluate the biological parameters.

Figures

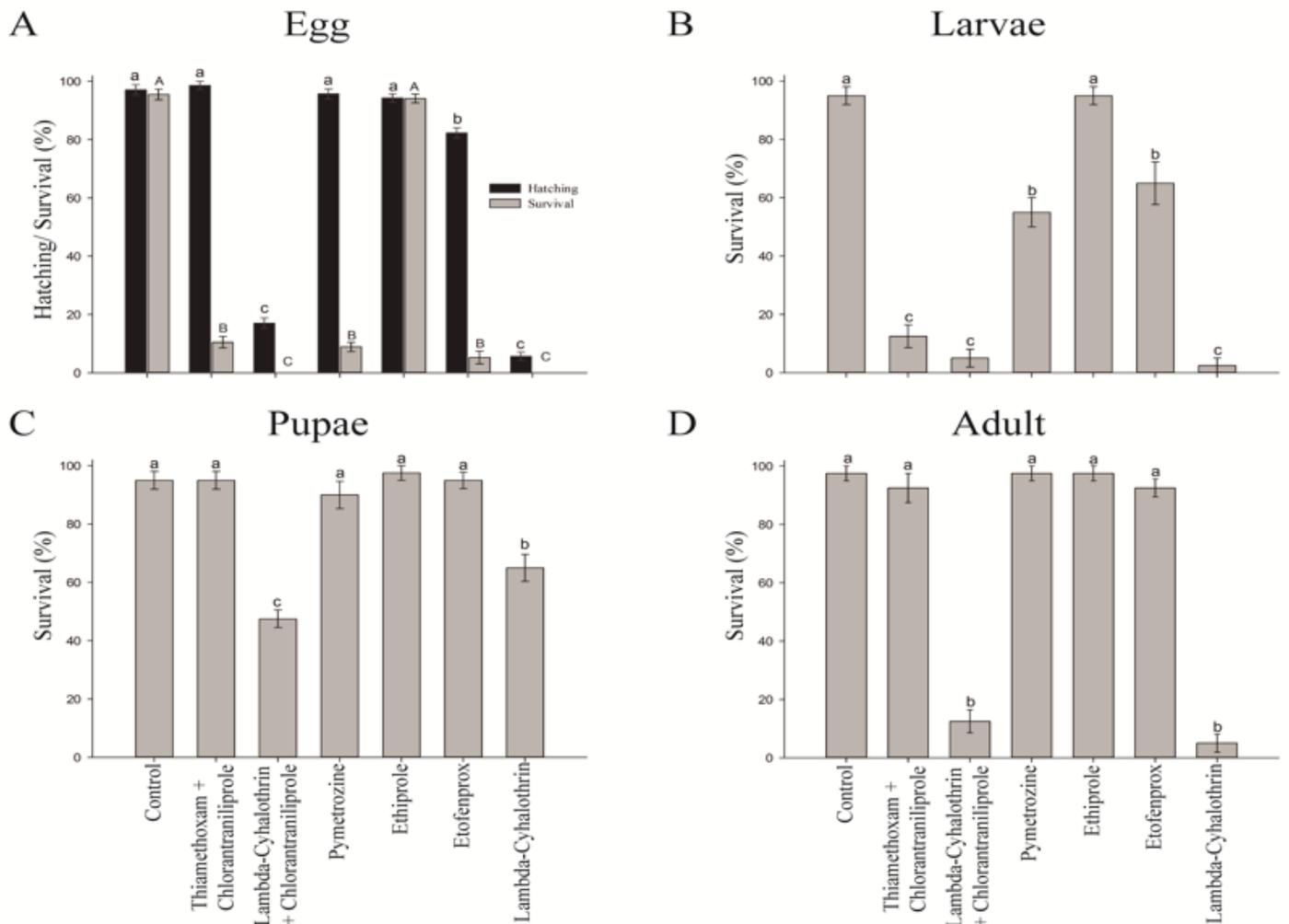


Figure 1

a) Hatching and survival (mean \pm SEM) of larvae from eggs of *Harmonia axyridis* treated with insecticides. Different capital letters indicate differences in black bars (quasi-binomial GLM, pairwise comparison, $p < 0.05$), and different lowercase letters indicate differences between gray bars (one-way ANOVA, $p < 0.05$). b) Survival (mean \pm SEM) of third-instar larvae of *H. axyridis* 96 hours after treatment with insecticides. Different letters indicate differences between treatments (one-way ANOVA, $p < 0.05$). c) Survival (mean \pm SEM) of pupae of *H. axyridis* after treatment with insecticides. Different letters indicate differences between treatments (one-way ANOVA, $p < 0.05$). d) Survival (mean \pm SEM) of pupae of *H. axyridis* after treatment with insecticides. Different letters indicate differences between treatments (quasi-binomial GLM, pairwise comparison, $p < 0.05$).



Figure 2

a) Live *Harmonia axyridis* larvae newly hatched from eggs treated with water or ethiprole. b) Dead larvae a few hours after hatching from eggs treated with thiamethoxam + chlorantraniliprole, pymetrozine, or etofenprox. 2c) Dark eggs, characterizing embryonic development, but no larvae hatched due to the effect of lambda-cyhalothrin + chlorantraniliprole or lambda-cyhalothrin. Photos taken under a stereoscopic microscope (35×).

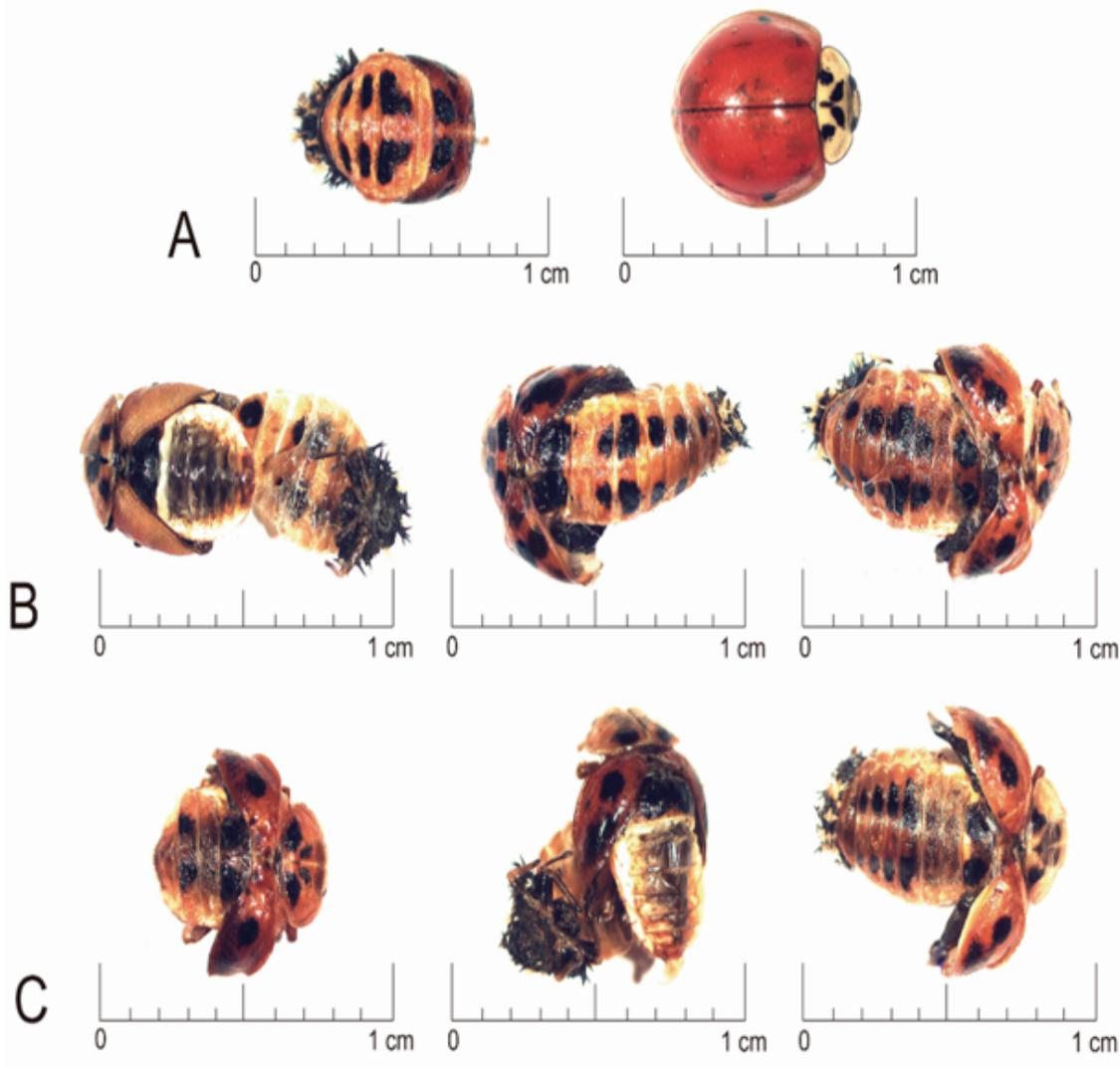


Figure 3

a) Pupa and adult *Harmonia axyridis* treated with water (control): no morphological deformity in the elytra. Adults with elytral deformity were unable to remove the exuvia in the lambda-cyhalothrin + chlorantraniliprole group (b) and lambda-cyhalothrin group (c). Photos taken under a stereoscopic microscope (25×). Scale bars: 1 cm.

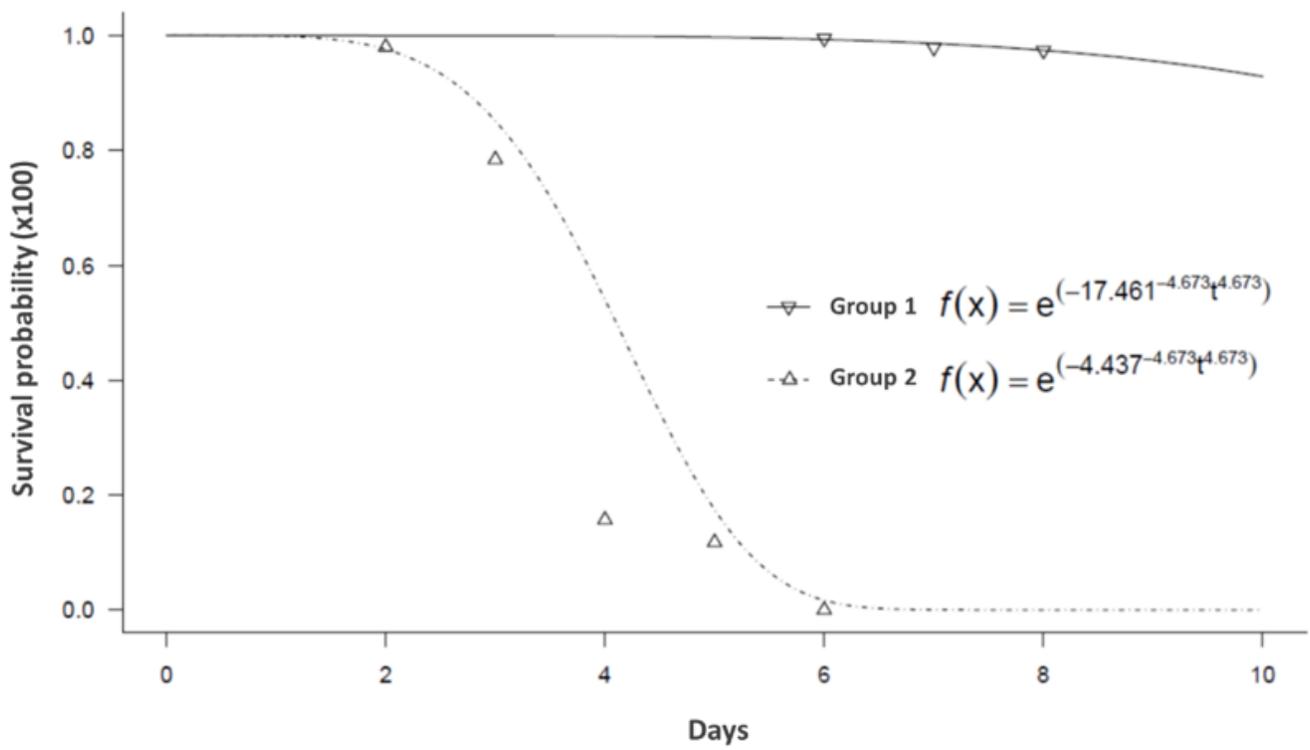


Figure 4

Survival curves (adjusted Weibull model) of adult *Harmonia axyridis* that grew from pupae treated with insecticides in a Potter spray tower. The curve of group 1 applies to the control (water), thiamethoxam + chlorantraniliprole, pymetrozine, ethiprole, and etofenprox treatments, with a half-lethal time of 17.46 days, while the curve of group 2 represents the lambda-cyhalothrin + chlorantraniliprole and lambda-cyhalothrin treatments, with a half-lethal time of 4.43 days.