



Toxicity and sublethal effects of chlorantraniliprole on the development and fecundity of a non-specific predator, the multicolored Asian lady beetle, *Harmonia axyridis* (Pallas)



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HIGHLIGHTS

- The acute toxicity of Chlorantraniliprole was determined for *H. axyridis*.
- Chlorantraniliprole increases the *H. axyridis* pre-adult developmental period.
- Chlorantraniliprole reduce the adult longevity and fecundity of *H. axyridis*.
- Chlorantraniliprole adversely affects long term life table parameters of *H. axyridis*.

ARTICLE INFO

Article history:

Received 17 December 2016
Received in revised form
17 March 2017
Accepted 21 March 2017
Available online 22 March 2017

Handling Editor: David Volz

Keywords:

Harmonia axyridis (Pallas)
Predator
Chlorantraniliprole
Sublethal effects
Two-sex life table

ABSTRACT

In order to further develop integrated pest management (IPM) approaches for controlling insect pests, it is important to estimate the effects of pesticides. In this study, the toxicity and sublethal effects of the insecticide chlorantraniliprole on a non-specific predator, the multicolored Asian lady beetle *Harmonia axyridis*, were evaluated and life table parameter data were analyzed statistically using the age-stage, two-sex life table procedure. The results of this study show that the development time of second and fourth instar larvae as well as pupa was significantly prolonged in populations treated with LC10 (2.42 mg (a.i.) L⁻¹) and LC30 (12.06 mg (a.i.) L⁻¹), while adult longevity and fecundity were both significantly reduced and the preoviposition period (POP) was significantly prolonged following treatment compared to the control. In addition, the net reproductive rate (R_0), as well as the intrinsic (r) and finite rate of increase (λ) were significantly decreased in groups treated with the insecticide. These results reveal that because sublethal concentrations of chlorantraniliprole impair the population growth of *H. axyridis*, more attention should be paid to the use of this chemical as a component of IPM strategies.

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1. Introduction

Predators are recognized as important regulators of insect pests in global agroecosystems (Juen et al., 2012; Lu et al., 2012). In particular, coccinellid beetles (Coleoptera: Coccinellidae) are important predators because of their large body sizes and polyphagous abilities (Obrycki et al., 2009; Hodek et al., 2012). *H. axyridis* (Pallas) is an important coccinellid used for insect-based IPM strategies because of its large cosmopolitan ecological

distribution and considerable ability to disturb agricultural ecosystems (Brown et al., 2011; Castro et al., 2011; Luo et al., 2014). Because it is highly polyphagous, *H. axyridis* feeds on several aphid species, as well as soft-bodied insects, and the immature stages of several coleopterans (Lundgren, 2009; Pell et al., 2008). This beetle is available commercially, is used for the control of greenhouse insect pests (Yang et al., 2014), and has been shown to be an efficient biological control agent as part of IPM strategies (Castro et al., 2011; Wang et al., 2007).

The combined use of compatible insecticides and biological control agents has been widely applied as part of IPM strategies to control pests (Elzen, 2001). Nevertheless, in some cases, the application of insecticides can lead to the development of insect

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resistance, environmental pollution, and be harmful to biological control agents (Youn et al., 2003; Garratt and Kennedy, 2006). Many pest species around the world have developed resistance to the use of insecticides (Puinean et al., 2010; Kavi et al., 2014; Bass et al., 2015; Saddiq et al., 2015), while chemical pesticides can have both acute toxicity (lethal) and sublethal effects on non-target organisms including natural predators (Galvan et al., 2005; Silva et al., 2005; Campiche et al., 2006; Castro et al., 2012).

A number of studies have addressed the adverse effects of pesticides on non-target organisms (Araya et al., 2010). However, most investigations on biological control agents have evaluated the short-term effects of insecticides (median lethal) and have ignored indirect effects such as sublethal effects that can impair important processes such as developmental time, the longevity of adults, and the fecundity of biological control agents (Saber, 2011; Stara et al., 2011). Population growth rate is one of the most important statistical parameters that can be used to provide an overall evaluation of the precise toxicity of pesticides (Kim et al., 2004); thus, the analysis of life tables is an important method for evaluating population growth as well as the sublethal effects of an insecticide on the natural enemies (Rimoldi et al., 2012). As a result, the side effects of several insecticides on insect pests and their natural enemies have been evaluated (Arno and Gabarra, 2011; Cabral et al., 2011; Rahmani and Bandani, 2013).

The insecticide chlorantraniliprole is a novel 'anthranilic diamide' that acts on insect ryanodine receptors. Specifically, chlorantraniliprole stimulates Ca^{++} depletion in the muscle cells of insects, impairing muscle contraction and leading to paralysis and death (Lahm et al., 2007). Chlorantraniliprole is considered as an efficient pesticide for use against coleopteran and lepidopteran insect pests (Bassi et al., 2009; Wang et al., 2010), and several different effects have been reported, including that this insecticide appears to be relatively benign for non-target arthropods, such as parasitic wasps and predatory insects (Brugger et al., 2010; Gontijo et al., 2015). At the same time, while harmful effects of chlorantraniliprole on *Coleomegilla maculata* and *Hippodamia convergens* (Coleoptera: Coccinellidae) have also been reported (Moscardini et al., 2015), just a handful of studies of its effects on insect pests and their natural enemies have so far been conducted (Jiang et al., 2012; Lanka et al., 2013; Moscardini et al., 2015).

The aim of this study was to evaluate the sublethal effects of the insecticide chlorantraniliprole on life table parameters of *H. axyridis*, including developmental time, survival rate, longevity, and fecundity. To do this, we applied age-stage, two-sex life table theory, which provides comprehensive information regarding the application of chlorantraniliprole insecticides in IPM and other pest management programs in both greenhouses and field crops.

2. Materials and methods

2.1. Insects and experimental design

We used the soybean aphid, *Aphis glycines* Matsumara (Hemiptera: Aphididae), to rear *H. axyridis*. Both insects were provided free-of-charge by the Key Laboratory of Hubei Insect Resources Utilization and Sustainable Pest Management, Huazhong Agricultural University, Wuhan, China. Colonies of *A. glycines* was maintained on faba bean plants (*Vicia faba* L., Fabaceae), while *H. axyridis* adults were reared in mesh-covered cages (60 cm length × 44 cm width × 34 cm height), containing plants infested with *A. glycines*.

We determined the sex of adult insects based on last abdominal segment using the methodology of McCornack et al. (2007). Newly emerged females and males were collected from rearing cages and grouped into pairs in filter paper lined petri dishes (100 mm diameter × 10 mm depth). Adult beetles were then fed with aphids

to encourage them to lay eggs. When egg clusters were found, paired adults shifted to fresh petri dishes (100 mm diameter × 10 mm depth) to allow eggs to hatch. Neonates were maintained individually in plastic petri dishes and reared with aphids until they reached the desired developmental stages for experiments. All insects were maintained at 23 ± 2 °C, with $68 \pm 5\%$ relative humidity (RH), and a photoperiod of 16:8 h (L:D).

2.2. Insecticide and short-term toxicity determination

Chlorantraniliprole (97% active ingredient, a.i.) was purchased from DuPont, Shanghai, China, and was dissolved in acetone at different concentrations for experiments. We determined lethal and sublethal concentrations of insecticide by progressively altering these until the desired mortality was achieved. The acute toxicity of this insecticide was assessed via topical application to second instar *H. axyridis* larvae. Before treatment, larvae were immobilized in a refrigerator at -4 °C for 2–3 min, and the ventral abdominal region of each was treated topically with 1 µl of chlorantraniliprole solution using a microapplicator (Burkard, England), while control larvae were treated with 1 µl acetone. Second instar larvae (<24 h) were treated with a different concentration, while 15 individuals were tested per replicate with four replications for toxicity assessment bioassays. The tested individuals from both control and treatment groups were then maintained in a climate chamber at 23 ± 2 °C, with $68 \pm 5\%$ RH, and a photoperiod of 16:8 h (L:D) with a continuous supply of live aphids. Treated larvae were observed daily until they either developed to the next stage or died, and mortality data were recorded after three days of exposure to chlorantraniliprole. Larvae that did not move when gently pushed with a fine hair brush were considered dead for the purposes of this experiment (He et al., 2012).

2.3. Evaluation of sublethal effects on second instar larvae

Approximately 400 *H. axyridis* eggs (<24 h) were collected and maintained in petri dishes (100 mm diameter × 10 mm depth) for use in our life table parameter study, following the methods outlined by Schneider et al. (2009) and Rahmani and Bandani (2013). We used two treatments (LC10 and LC30; 2.42 and 12.06 mg (a.i.) L⁻¹ chamnaeotheoantre4n6

underlying population multiplication, $R_0 = \sum l_x m_x$, and expression of population growth, including the number of female offspring per female per generation, and $r = \ln(R_0)/T$, which refers to the maximum rate of population increase, and mean generation time, $T = \sum x l_x m_x / R_0$, the average interval between the birth of one generation and the next.

2.4. Statistical analysis

We used the software PoloPlus (LeOra Software 2002, Berkeley, CA) to calculate the concentrations of chlorantraniliprole that were lethal and sublethal to second instar *H. axyridis* larvae in short-term toxicity tests. Different life stage developmental times, survival, adult longevity, and fecundity parameters were statistically analyzed using age-stage two-sex life table theory (Chi and Liu, 1985; Chi, 1988) and the TWOSEX-MSChart software downloaded from <http://140.120.197.173> (Chi, 2016). Means and standard errors (SEs) of long-term table parameters were calculated via 100,000 bootstrap replicates to obtain stable SE estimates (Huang and Chi, 2012; Akca et al., 2015). All treatments were compared using the paired bootstrap test; both bootstrap and paired bootstrap tests were computed in TWOSEX-MSChart (Chi, 2016), while the software SigmaPlot 12.0 was used to generate curves for all population parameters, including survival rate, fecundity, reproductive values, and life expectancy.

3. Results

3.1. Toxicity of chlorantraniliprole on *H. axyridis*

Lethal and sublethal toxicity results for chlorantraniliprole on second instar *H. axyridis* are shown in Table 1. These results show that three days after exposure, the LC50 value of chlorantraniliprole for *H. axyridis* second instar larvae was estimated to be 36.67 mg (a.i.) L⁻¹ (95% confidence interval: between 23.76 mg (a.i.) L⁻¹ and 54.50 mg (a.i.) L⁻¹; $\chi^2 = 0.058$), while average LC10 and LC30 values were estimated to be 2.42 mg (a.i.) L⁻¹ (between 0.85 mg (a.i.) L⁻¹ and 4.80 mg (a.i.) L⁻¹) and 12.06 mg (a.i.) L⁻¹ (between 6.43 mg (a.i.) L⁻¹ and 19.07 mg (a.i.) L⁻¹), respectively. At the same time, insect mortality in the control group was <10%.

3.2. Sublethal effects of chlorantraniliprole on *H. axyridis*

The long-term effects of chlorantraniliprole on the development times of different *H. axyridis* life stages, adult longevity, and fecundity are listed in Table 2 and Table 3. These results show that development times of second instar larvae were significantly longer when they were treated with LC10 (2.64 ± 0.07 d) and LC30 (2.85 ± 0.13 d) compared to the control (2.33 ± 0.05 d). Although the same trend in developmental time was observed for third instar larvae, the difference was not significant. Similarly, the developmental time of fourth instar larvae was significantly longer in treated populations (i.e., LC30 6.22 ± 0.10 d) compared to the control group (5.97 ± 0.08 d), while pupation duration was also significantly longer under both LC10 (6.04 ± 0.06 d) and LC30 (5.98 ± 0.05 d) treatments, respectively. Treatment with insecticide

also significantly reduced adult longevity in the LC10 (58.32 ± 1.52 d) and LC30 (52.27 ± 1.38 d) groups compared to the control (62.83 ± 0.97 d), while adult POPs (APOPs) in the former groups (LC10: 11.18 ± 0.23; LC30: 11.48 ± 0.17 d) were significantly enhanced compared to the control group (8.79 ± 0.21 d). At the same time, results show that total POPs (TPOPs) in the LC10 (36.32 ± 0.27 d) and LC30 (36.96 ± 0.39 d) groups were significantly longer compared to the control (33.93 ± 0.34 d), while in treated populations, fecundity significantly decreased with increasing sublethal concentrations of chlorantraniliprole. Results show that significantly decreased fecundity of treated insects in the LC10 and LC30 groups were 499.60 ± 22.10 and 397.00 ± 24.87 eggs per female respectively, while the control group was 707.23 ± 18.81 eggs per female (Table 3).

The effects of the insecticide chlorantraniliprole on the population growth parameters of *H. axyridis* are shown in Table 4. These results show that chlorantraniliprole treatment markedly decreased r in LC10 and LC30-treated populations (0.0974 ± 0.003 day⁻¹ and 0.0886 ± 0.004 day⁻¹, respectively) compared to the control group (0.1111 ± 0.002 day⁻¹). A similar tendency was also seen in λ (1.11 ± 0.002 day⁻¹, 1.10 ± 0.003 day⁻¹, and 1.09 ± 0.004 day⁻¹ in the control group, LC10 and LC30 treatment groups, respectively). At the same time, while results show that the R_0 was 304.01 ± 35.97 offspring per individual in the control group, this was significantly reduced in the LC10 (174.83 ± 25.16 offspring per individual) and LC30 (99.20 ± 1.38 offspring per individual) treatment groups (Table 4).

As discussed, the s_{xj} value for a *H. axyridis* population denotes the probability that a new individual will live to 'age x and stage j '. The results of this study demonstrate that different life stages clearly overlap, as the different developmental rate among individuals was used for the age-stage, two-sex life table procedure. Thus, s_{xj} values for male and female adults were negatively affected in treated populations; results show that s_{xj} reached a maximum in the control group (i.e., 0.47 for males and 0.43 for females), while this value continuously decreased in treatment groups in concert with increasing chemical concentrations (i.e., LC10: 0.40 for males and 0.35 for females; LC30: 0.30 for males and 0.25 for females). Results show that maximum mean longevities for males and females in the control group were 86.28 and 88.26 days, respectively, markedly higher than in the LC30 treatment group where these

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show that the peak V_{xj} value was seen in the control group (163.35 day^{-1}) compared to 142.61 and 135.24 day^{-1} in the LC10 and LC30 groups, respectively (Fig. 3). Similarly, data describing the sublethal effect of chlorantraniliprole on *H. axyridis* e_{xj} values are presented in Fig. 4; these results show that the lowest e_{xj} values for newly hatched *H. axyridis* eggs in the LC30 and LC10 treatment groups were 48.04 and 65.25 days, respectively, compared to 79.77 days in the control.

4. Discussion

A range of insecticides are used extensively in agriculture to control insect pests which also indirectly affect non-target organisms (Campiche et al., 2006; Dawar et al., 2016). Chlorantraniliprole is an important anthranilic diamide insecticide because it is one of

together, these results suggest that *H. axyridis* is very susceptible to chlorantraniliprole, especially during its immature life stages, more likely to be exposed to this chemical in the field.

The assessment of long-term life table parameters is one important approach for the evaluation of population growth as well as the sublethal effects of insecticides on insects (Stark and Banks, 2003; Rimoldi et al., 2012). The results of this study show that long-term population parameters, including r , λ , and R_0 , tend to be lower in chlorantraniliprole-treated populations, which indicates that sublethal concentrations of this insecticide have long-term deleterious effects on insect physiology (Lanka et al., 2013; Moscardini et al., 2015). Corroborating our results, Han et al. (2012) observed dramatically lower r , λ , and R_0 values in chlorantraniliprole-treated cohorts of *P. xylostella*, while other studies have also confirmed the adverse effects of this insecticide on long-term population parameters in *H. armigera* (Zhang et al., 2013).

Results show that two-sex life table parameters of *H. axyridis* were adversely affected by chlorantraniliprole reflecting adverse effects on population growth. Specifically, s_{xj} significantly decreased in insecticide-treated groups, use of this chemical

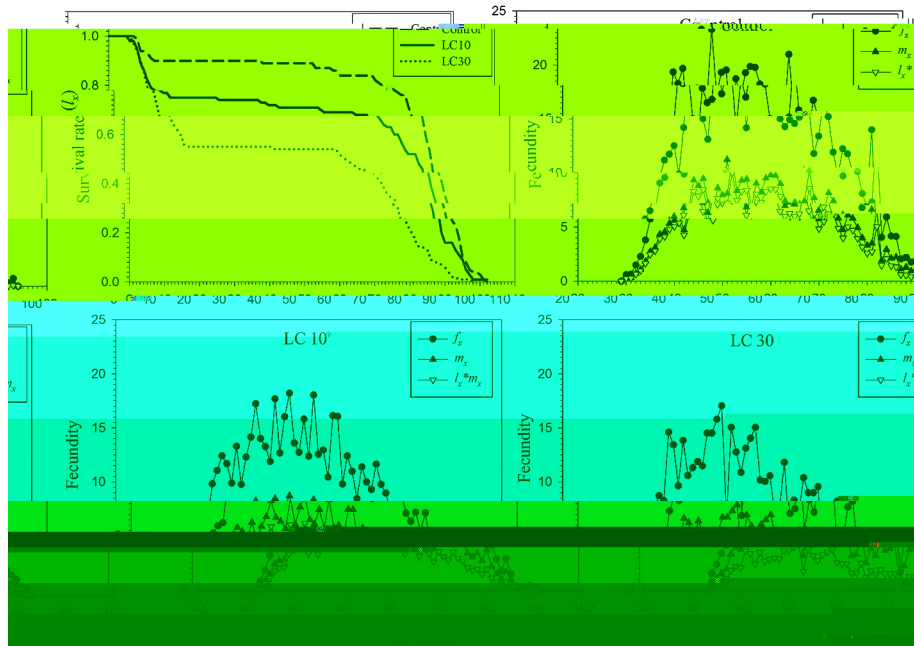


Fig. 2. Graphs showing the Age-specific survival rate, female age-specific fecundity (f_x), age-specific fecundity (m_x), and age specific maternity ($l_x m_x$) for second instar *H. axyridis* larvae exposed to sublethal chlorantraniliprole concentrations.

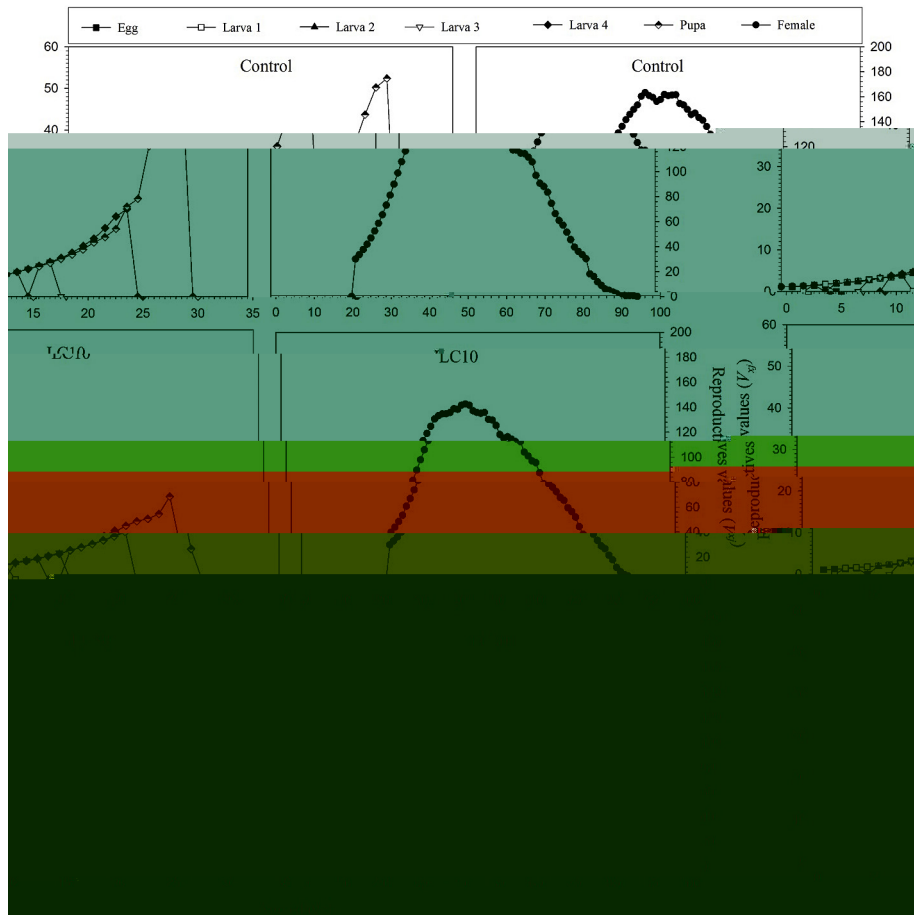


Fig. 3. Graphs showing Age-stage specific reproductive values (V_{xj}) values for second instar *H. axyridis* larvae exposed to sublethal chlorantraniliprole concentrations.

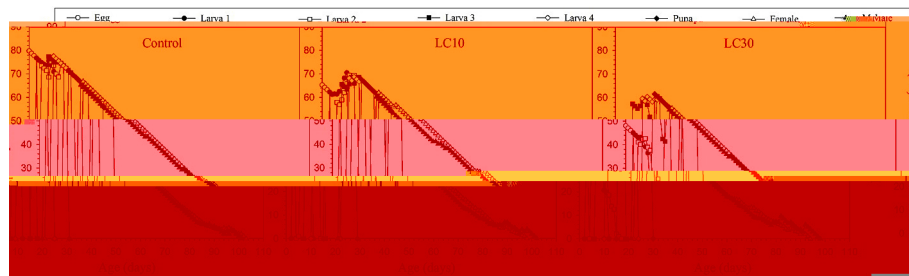


Fig. 4. Graphs showing Life expectancy (e_{xy}) values for second instar *H. axyridis* larvae exposed to sublethal chlorantraniliprole concentrations.

Acknowledgements

We are grateful to Dr. Yueping He (Huazhong Agricultural University, Wuhan, China), Dr. Hsin Chi (Department of Plant Production and Technologies, Faculty of Agricultural Sciences and Technologies, Ömer Halisdemir University, Turkey), and Miss. Jinping Liu for their technical guidance and statistical assistance with the two-sex life table theory used in this work. This research was supported by the Fundamental Research Funds for the Central Universities (Grant No. 2662015PY115).

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