



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



Muhammad Nawaz, Wanlun Cai, Zhao Jing, Xingmiao Zhou, Juma Ibrahim Mabubu, Hongxia Hua*

Hubei Insect Resources Utilization and Sustainable Pest Management Key Laboratory, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, China

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.

© 2017 Elsevier Ltd. All rights reserved.

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

* Corresponding author.

E-mail address: huahongxia@mail.hzau.edu.cn (H. Hua).

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: Aphydidae), 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 \times 44 cm width \times 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 \times 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 \times 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 μ l of chlorantraniliprole solution using a microapplicator (Burkard, England), while control larvae were treated with 1 μ l 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 \times 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} respectively) and a control (acetone only) for this experiment, in each case selecting 100 newly emerged second instar larvae, and considering each larva to be one replicate (Chi and Yang, 2003). Newly molted second instar larvae (<24 h) were treated with LC10 and LC30 following the method described above, while mortality and development time were recorded daily during the pre-adult stage. Following the emergence of adults in each treatment, females and males were paired in separate petri dishes and observed regularly to record mortality and fecundity. Data recording continued until the death of the last individual in each treatment.

In addition to life stage developmental time differences, we also recorded a number of other parameters including age-stage specific survival rate, s_{xj} , where x denotes age and j denotes stage, age-specific survival rate, l_x , a simplified version of s_{xj} that is used to describe the likelihood that a new egg will live to age x (Huang and Chi, 2012), age-stage specific fecundity, f_{xj} , that provides information about the number of eggs per female for a given number of days at age x and stage j , age-specific fecundity, m_x , which denotes the number of eggs per individual at age x , age-specific maternity, $l_x m_x$, the combination of l_x and m_x , age-stage specific reproduction, V_{xj} , a measure of the contribution of each individual to the future population, and life expectancy, e_{xj} , a measure of how long each individual can be expected to survive.

We also calculated a number of other population growth parameters, including $\lambda = \exp(r)$, an expression of the factors

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 TWISEX-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 TWISEX-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 (APOP) 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 values were 76.83 and 78.48 days, respectively (Fig. 1).

These results reveal a strong decrease in survivorship reflected in l_x with increasing chemical concentration, a simplification of s_{xj} . Graphs for f_{xj} , m_x , and $l_x m_x$ are shown in Fig. 2; these results demonstrate that the fecundity levels in treated populations were lower than controls. Indeed, the highest recorded f_{xj} peak in a control population was 23.27 eggs female⁻¹ day⁻¹ laid over 52 days, while in the LC 30 treatment group this was 17.04 eggs female⁻¹ day⁻¹ laid over 53 days. The highest recorded m_x peaks in the LC10 and LC30-treated populations were 8.71 and 7.88 eggs individual⁻¹ day⁻¹ which occurred on days 56 and 53, respectively, markedly lower than the value for the control group, 11.25 eggs individual⁻¹ day⁻¹, which occurred after 52 days (Fig. 2).

Results show that V_{xj} (Fig. 3) decreased markedly in populations treated with increasing concentrations of chlorantraniliprole. Data

Table 1
Acute toxicity of chlorantraniliprole on second instar *H. axyridis* larvae.

Insecticide	N	Concentration mg (a.i.) liter ⁻¹ (95% CL) ⁻¹				Slope ± SE	χ^2 (Degrees of freedom)
		LC10	LC30	LC50	LC90		
Chlorantraniliprole	360	2.42 (0.85–4.80)	12.06 (6.43–19.07)	36.67 (23.76–54.50)	555.08 (308.84–1331.59)	1.086 ± 0.132	0.058(3)

Abbreviation: N, number of second instar larvae treated with chlorantraniliprole.

Table 2Sublethal effects of chlorantraniliprole on the developmental time (mean \pm SE) of *H. axyridis* adults exposed to insecticide from the second instar larval stage.

Treatments	Development time of second instar larva (d)	Development time of third instar larva (d)	Development time of fourth instar larva (d)	Development time of pupa (d)
Control	2.33 \pm 0.05b	2.82 \pm 0.05a	5.97 \pm 0.08 b	5.51 \pm 0.05b
LC10	2.64 \pm 0.07a	2.76 \pm 0.07a	6.16 \pm 0.07ab	6.04 \pm 0.06a
LC30	2.85 \pm 0.13a	2.85 \pm 0.09a	6.22 \pm 0.10a	5.98 \pm 0.05a

Means followed by the same letters in the same column are not significantly different based on the paired bootstrap test at the 5% significance level. 100 insects were used for each treatment.

Table 3Sublethal effects of chlorantraniliprole on the life parameters (mean \pm SE) of *H. axyridis* adults exposed to insecticide from the second instar larval stage.

Treatments	Adult longevity (d)	Female longevity (d)	Male longevity (d)	APOP (d)	TPOP (d)	Fecundity (eggs/female)
Control	62.83 \pm 0.97a	63.72 \pm 1.13a	62.02 \pm 1.54a	8.79 \pm 0.21b	33.93 \pm 0.34b	707.23 \pm 18.81a
LC10	58.32 \pm 1.52b	59.94 \pm 2.24a	56.90 \pm 2.06b	111.18 \pm 00.23a	36.32 \pm 0.27a	499.60 \pm 22.10b
LC30	52.27 \pm 1.38c	53.00 \pm 1.84b	51.67 \pm 2.03bc	11.48 \pm 0.17a	36.96 \pm 0.39a	397.00 \pm 24.87c

Means followed by the same letters in the same column are not significantly different based on the paired bootstrap test at the 5% significance level. 100 insects were used for each treatment.

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 the fastest-acting and most effective for the management of coleopteran and lepidopteran pests (Bassi et al., 2009; Lahm et al., 2009; Cao et al., 2010). Nevertheless, while chlorantraniliprole is considered comparatively less toxic to mammals and other non-target organisms (Bassi et al., 2009; Gradish et al., 2010), lethal and sublethal effects have been reported on several non-target insects (Sabry et al., 2014; Fernandes et al., 2016), including predators (Castro et al., 2013). Thus, building on previous work, this study investigated the sublethal effects of chlorantraniliprole on the life table parameters of *H. axyridis*. Results show that LC50 (i.e., 36.67 mg (a.i.) L⁻¹) values for this insecticide on the second instar larvae of *H. axyridis* are lower than the field recommended concentrations reported in previous work (Fernandes et al., 2016), while toxicity data show that this beetle is comparatively more susceptible to this insecticide when exposed to a high concentration in the field. The results of this investigation are in agreement with those presented by Jiang et al. (2012), who showed that second instar larvae of *L. decemlineata* are also susceptible to chlorantraniliprole as their calculated LC50 value was also less than the

recommended field concentration.

The application of age-stage, two-sex life table theory in the form of life table parameters has been considered a comprehensive method for evaluating the total effects of insecticides on insect population on the basis of different life stage development times, survival, adult longevity, and fecundity (Tuan et al., 2016). The larvae and pupae developmental results presented here show that chlorantraniliprole is toxic even at low concentrations during pre-adult development. The development time for pre-adults was significantly increased in chlorantraniliprole-treated insects compared to the control group, probably because insecticide-treated larvae need to devote more of their energy to chemical detoxification (Hannig et al., 2009). A number of other studies have reported that sublethal concentrations of chlorantraniliprole and cyantraniliprole (anthranilic diamide) increase larvae and pupae development time, for example in the coleopterans *C. maculate*, *H. convergens*, and *L. oryzoophilus* as well as in the lepidopterans *O. furnacalis* and *A. ipsilon* (Lanka et al., 2013; Song et al., 2013; Moscardini et al., 2015; Xu et al., 2016).

Although the results of this study show that the POP (i.e., APOP and TPOP) was significantly prolonged by sublethal chlorantraniliprole concentrations, application of this insecticide also decreased adult male and female fecundity and longevity. Interestingly, Moscardini et al. (2015) also showed that sublethal concentrations of chlorantraniliprole increased the length of the pre-oviposition period in *C. maculata* and *H. convergens*, and a range of other studies have also reported that anthranilic diamide insecticides can sharply decrease fecundity, again in *L. oryzoophilus*, *C. maculate*, and *H. convergens* (Lanka et al., 2013; Song et al., 2013; Bielza and Guillen, 2015; Moscardini et al., 2015; Yu et al., 2015). The reduction in fecundity is probably the result of ovary deformation induced by the application of chlorantraniliprole, as this insecticide is known to adversely affect the structure of the insect reproductive system (Perveen and Miyata, 2000). Thus, taken

Table 4Sublethal effects of chlorantraniliprole on the population growth parameters (mean \pm SE) of *H. axyridis* adults exposed to insecticide from the second instar larval stage.

Treatments	Intrinsic rate of increase (r) day ⁻¹	Net reproductive rate (R_0) (offspring per individual)	Mean generation time (T) (days)	Finite rate of increase (λ) (day ⁻¹)
Control	0.1111 \pm 0.002a	304.01 \pm 35.97a	51.40 \pm 0.41b	1.11 \pm 0.002a
LC10	0.0974 \pm 0.003b	174.83 \pm 25.19b	52.91 \pm 0.41a	1.10 \pm 0.003b
LC30	0.0886 \pm 0.004bc	99.20 \pm 18.16c	51.71 \pm 0.62ab	1.09 \pm 0.004bc

Means followed by the same letters in the same column are not significantly different based on the paired bootstrap test at the 5% significance level. 100 insects were used for each treatment.

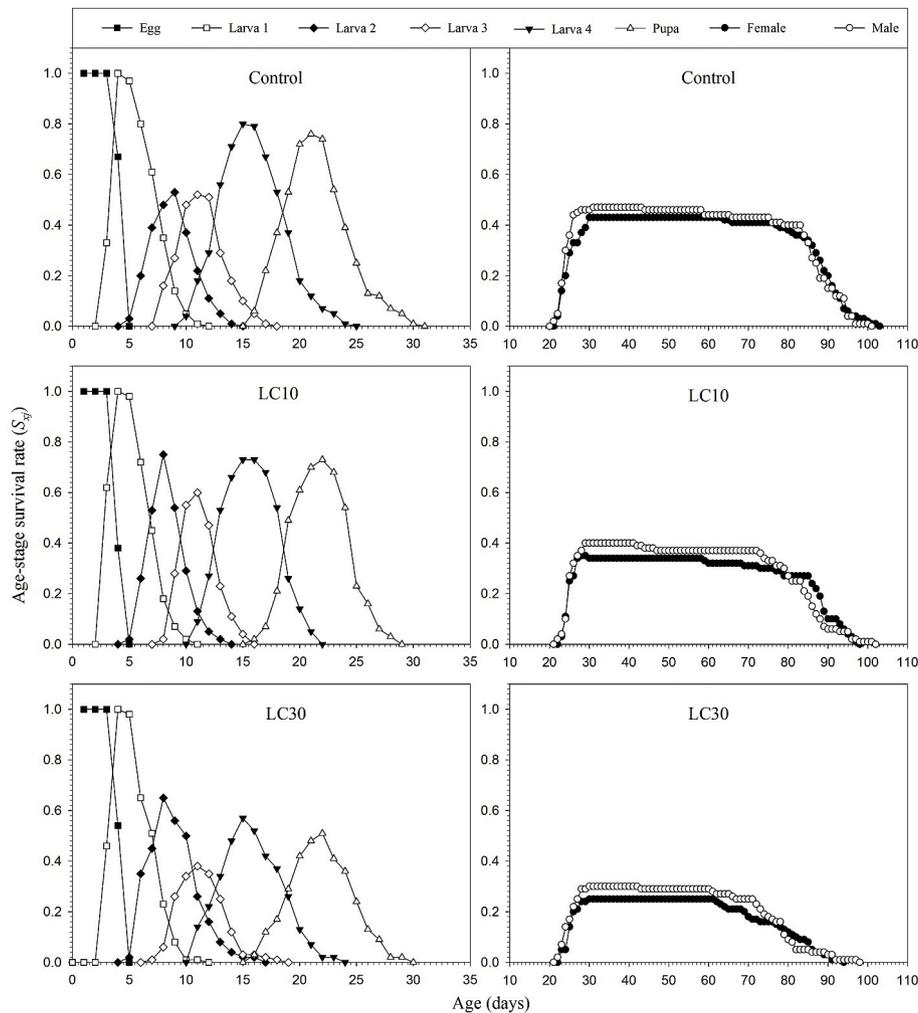


Fig. 1. Graphs showing Age-stage specific survival rate (s_{xj}) for second instar *H. axyridis* larvae exposed to sublethal chlorantraniliprole concentrations.

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 markedly reduced f_{xj} and m_x in LC10 and LC30-treated groups, and V_{xj} was significantly affected by chlorantraniliprole treatment. In addition, e_{xj} , a measure of the contribution of new individuals to future population growth, also declined sharply in second stage

larval instars within the insecticide-treated population and significantly increased subsequently. These results therefore suggest that treatment with chlorantraniliprole killed the weakest second instar individuals in the population, while stronger individuals had higher tolerance and developed to next stage. At the same time, sublethal effects also seen in the later stages; e_{xj} values for newly hatched eggs were higher in control samples compared to treatment groups due to increased chlorantraniliprole pressure. Our results indicate that the development, survival, and reproduction of *H. axyridis* are all adversely affected by the application of chlorantraniliprole, although additional work will be required to determine genetic variability in response to sublethal concentrations of this insecticide.

5. Conclusion

The results of this study show that chlorantraniliprole is toxic to *H. axyridis*, increasing the length of the pre-adult developmental time while decreasing adult longevity and fecundity. Application of this insecticide adversely affected a number of long-term life table parameters, including R_0 , r , and λ . Results suggest that the use of chlorantraniliprole in the field can impair *H. axyridis* populations, it may be used with prescription in IPM strategies.

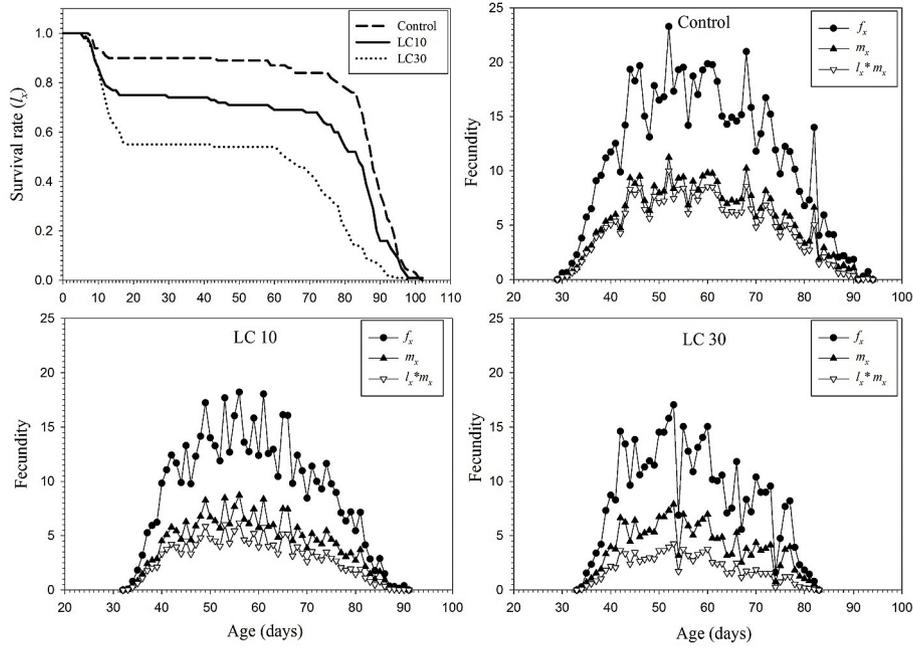


Fig. 2. Graphs showing the Age-specific survival rate, female age-specific fecundity (f_x), age-specific fecundity (m_x), and age specific maternity ($l_x \cdot m_x$) for second instar *H. axyridis* larvae exposed to sublethal chlorantraniliprole concentrations.

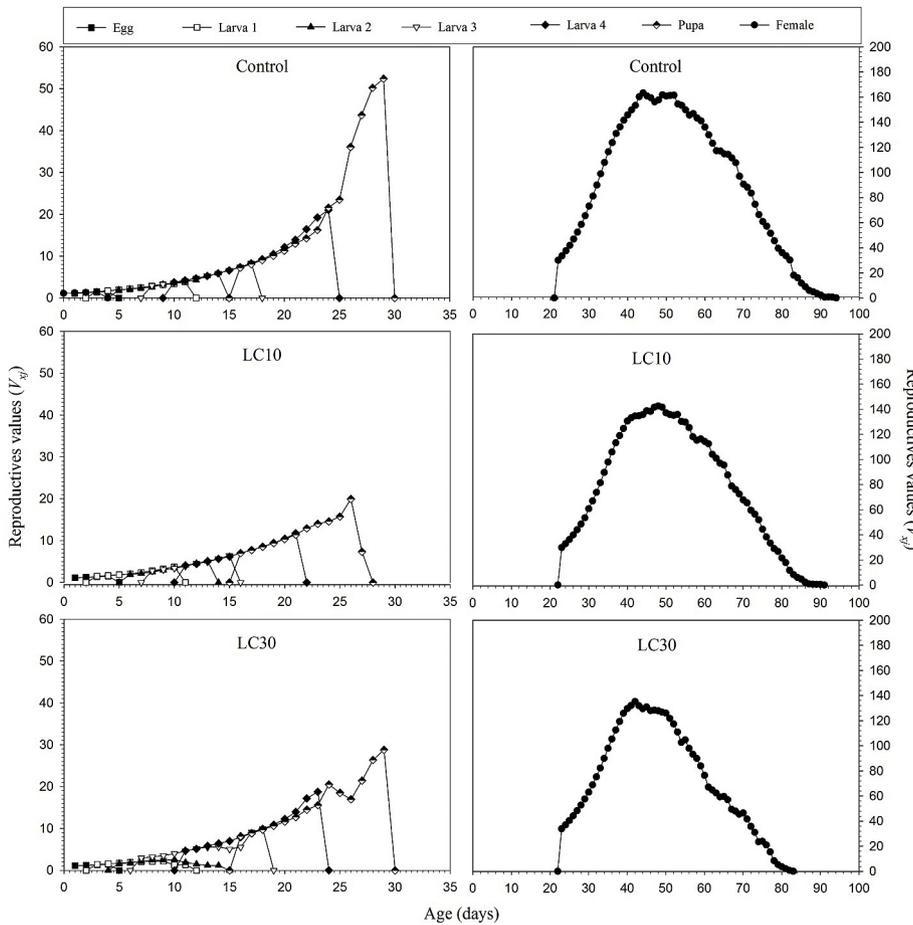


Fig. 3. Graphs showing Age-stage specific reproductive values (V_{xj}) values for second instar *H. axyridis* larvae exposed to sublethal chlorantraniliprole concentrations.

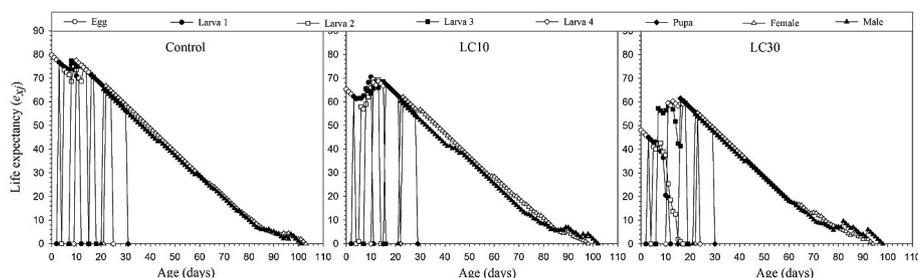


Fig. 4. Graphs showing Life expectancy (e_x) values for second instar *H. axyridis* larvae exposed to sublethal chlorantraniliprole concentrations.

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).

References

- Akca, I., Ayyavaz, T., Yazici, E., Smith, C.L., Chi, H., 2015. Demography and population projection of *Aphis fabae* (Hemiptera: Aphididae): with additional comments on life table research criteria. *J. Econ. Entomol.* 108, 1466–1478.
- Araya, J.E., Araya, M., Guerrero, M.A., 2010. Effects of some insecticides applied in sublethal concentrations on the survival and longevity of *Aphidius ervi* (Haliday) (Hymenoptera: Aphididae) adults. *Chil. J. Agric. Res.* 70, 221–227.
- Arno, J., Gabarra, R., 2011. Side effects of selected insecticides on the *Tuta absoluta* (Lepidoptera: Gelechiidae) predators *Macrolophus pygmaeus* and *Nesidiocoris tenuis* (Hemiptera: Miridae). *J. Pest Sci.* 84, 513–520.
- Bass, C., Denholm, I., Williamson, M.S., Nauen, R., 2015. The global status of insect resistance to neonicotinoid insecticides. *Pestic. Biochem. Physiol.* 121, 78–87.
- Bassi, A., Rison, J.L., Wiles, J.A., 2009. Chlorantraniliprole (DPX-E2Y45, Rynaxypyr™, Coragen™), a new diamide insecticide for control of codling moth (*Cydia pomonella*), Colorado potato beetle (*Leptinotarsa decemlineata*) and European grapevine moth (*Lobesia botrana*). *Zbornik predavanj referatov 9 slovenskega posvetovanja o varstvu rastlin z mednarodno udeležbo* 39–45.
- Bielza, P., Guillen, J., 2015. Cyantraniliprole: a valuable tool for *Frankliniella occidentalis* (Pergande) management. *Pest Manag. Sci.* 8, 1068–1074.
- Brown, P.M.J., Thomas, C.E., Lombaert, E., Jeffries, D.L., Estoup, A., Handley, L.J.L., 2011. The global spread of *Harmonia axyridis* (Coleoptera: Coccinellidae): distribution, dispersal and routes of invasion. *BioControl* 56, 623–641.
- Brugger, K.E., Cole, P.G., Newman, I.C., Parker, N., Scholz, B., Suvagia, P., Walker, G., Hammond, T.G., 2010. Selectivity of chlorantraniliprole to parasitoid wasps. *Pest Manag. Sci.* 66, 1075–1081.
- Cabral, S., Soares, A., Garcia, P., 2011. Voracity of *Coccinella undecimpunctata*: effects of insecticides when foraging in a prey/plant system. *J. Pest Sci.* 84, 373–379.
- Campiche, S., Slooten, K.B., Ridreau, C., Tarradellas, J., 2006. Effects of insect growth regulators on the nontarget soil arthropod *Folsomia candida* (Collembola). *Ecotox. Environ. Safe* 63, 216–225.
- Cao, G., Lu, Q., Zhang, L., Guo, F., Liang, G., Wu, K., Wyckhuys, K.A.G., Guo, Y., 2010. Toxicity of chlorantraniliprole to Cry1Ac-susceptible and resistant strains of *Helicoverpa armigera*. *Pestic. Biochem. Physiol.* 98, 99–103.
- Castro, C.F., Almeida, L.M., Penteado, S.R.C., 2011. The impact of temperature on biological aspects and life table of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). *Fla. Entomol.* 94, 923–932.
- Castro, A.A., Lacerda, M.C., Zanuncio, T.V., Ramalho, F.S., Polanczyk, R.A., Serrao, J.E., Zanuncio, J.C., 2012. Effect of the insect growth regulator diflubenzuron on the predator *Podisus nigrispinus* (Heteroptera: Pentatomidae). *Ecotoxicol* 21, 96–103.
- Castro, A.A., Correa, A.S., Legaspi, J.C., Guedes, R.N.C., Serrao, J.E., Zanuncio, J.C., 2013. Survival and behavior of the insecticide-exposed predators *Podisus nigrispinus* and *Suppilius cincticeps* (Heteroptera: Pentatomidae). *Chemosphere* 93, 1043–1050.
- Chi, H., 1988. Life-table analysis incorporating both sexes and variable development rates among individuals. *Environ. Entomol.* 17, 26–34.
- Chi, H., 2016. TWSEX-MSChart: a Computer Program for the Age-stage, Two-sex Life Table Analysis. National Chung Hsing University, Taichung, Taiwan. <http://140.120.197.173/Ecology/> (Accessed November 2016).
- Chi, H., Liu, H., 1985. Two new methods for the study of insect population ecology. *Bull. Inst. Zool. Acad. Sin.* 24, 225–240.
- Chi, H., Yang, T., 2003. Two-sex life table and predation rate of *Propylaea japonica* Thunberg (Coleoptera: Coccinellidae) fed on *Myzus persicae* (Sulzer) (Homoptera: Aphididae). *Environ. J. Entomol.* 32, 327–333.
- Dawar, F.U., Zuberi, A., Azizullah, A., Khan Khattak, M.N., 2016. Effects of cypermethrin on survival, morphological and biochemical aspects of rohu (*Labeo rohita*) during early development. *Chemosphere* 144, 697–705.
- Elzen, G.W., 2001. Lethal and sublethal effects of insecticide residues on *Orius insidiosus* (Hemiptera: Anthocoridae) and *Geocoris punctipes* (Hemiptera: Lygaeidae). *J. Econ. Entomol.* 94, 55–59.
- Fernandes, M.E., Alves, F.M., Pereira, R.C., Aquino, L.A., Fernandes, F.L., Zanuncio, J.C., 2016. Lethal and sublethal effects of seven insecticides on three beneficial insects in laboratory assays and field trials. *Chemosphere* 156, 45–55.
- 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.
- Garratt, J., Kennedy, A., 2006. Use of models to assess the reduction in contamination of water bodies by agricultural pesticides through the implementation of policy instruments: a case study of the Voluntary Initiative in the UK. *Pest Manag. Sci.* 62, 1138–1149.
- Gontijo, L.M., Celestino, D., Queiroz, O.S., Narciso, R., Guedes, C., Picanço, M.C., 2015. Impacts of azadirachtin and chlorantraniliprole on the developmental stages of pirate bug predators (Hemiptera: Anthocoridae) of the tomato pinworm *Tuta absoluta* (Lepidoptera: Gelechiidae). *Fla. Entomol.* 98, 59–64.
- Gradish, A.E., Scott-Dupree, C.D., Shipp, L., Harris, C.R., Ferguson, G., 2010. Effect of reduced risk pesticides for use in greenhouse vegetable production on *Bombus impatiens* (Hymenoptera: Apidae). *Pest Manag. Sci.* 66, 142–146.
- Han, W.S., Zhang, S.F., Shen, F.Y., Liu, M., Ren, C.C., Gao, X.W., 2012. Residual toxicity and sublethal effects of chlorantraniliprole on *Plutella xylostella* (Lepidoptera: Plutellidae). *Pest Manag. Sci.* 68, 1184–1190.
- Hannig, G.T., Ziegler, M., Marcon, P.G., 2009. Feeding cessation effects of chlorantraniliprole, a new anthranilic diamide insecticide, in comparison with several insecticides in distinct chemical classes and mode-of-action groups. *Pest Manag. Sci.* 65, 969–974.
- He, Y.X., Zhao, J., Zheng, Y., Zhan, Z., Desneux, N., Wu, K.M., 2012. Lethal effect of imidacloprid on the coccinellid predator *Serangium japonicum* and sublethal effects on predator voracity and on functional response to the whitefly *Bemisia tabaci*. *Ecotoxicol* 21, 1291–1300.
- Hodek, I., van Emden, H., Honk, A., 2012. Ecology and Behaviour of the Ladybird Beetles. Wiley-Blackwell, West Sussex.
- Huang, Y.B., Chi, H., 2012. Age-stage, two-sex life tables of *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) with a discussion on the problem of applying female age-specific life tables to insect populations. *Insect Sci.* 19, 263–273.
- Jiang, W.H., Lu, W.P., Guo, W.C., Xia, Z.H., Fu, W.J., Li, G.Q., 2012. Chlorantraniliprole susceptibility in *Leptinotarsa decemlineata* in the North Xinjiang Uygur autonomous region in China. *J. Econ. Entomol.* 105, 549–554.
- Juen, A., Hogendoorn, K.M.G., Schmidt, O., Keller, M.A., 2012. Analysing the diets of invertebrate predators using terminal restriction fragments. *J. Pest Sci.* 85, 89–100.
- Kavi, L.A., Kaufman, P.E., Scott, J.G., 2014. Genetics and mechanisms of imidacloprid resistance in house flies. *Pestic. Biochem. Physiol.* 109, 64–69.
- Kim, M., Shin, D., Suh, E., Cho, K., 2004. An assessment of the chronic toxicity of fenpyroximate and pyridaben to *Tetranychus urticae* using a demographic bioassay. *Appl. Entomol. Zool.* 39, 401–409.
- Lahm, G.P., Stevenson, T.M., Selby, T.P., Freudenberger, J.H., Cordova, D., Flexner, L., Bellin, C.A., Dubas, C.M., et al., 2007. Rynaxypyr™: a new insecticidal anthranilic diamide that acts as a potent and selective ryanodine receptor activator. *Bioorg. Med. Chem. Lett.* 17, 6274–6279.
- Lahm, G.P., Cordova, D., Barry, J., 2009. New and selective ryanodine receptor activators for insect control. *Bioorg. Med. Chem.* 17, 4127–4133.
- Lanka, S.K., Ottea, J.A., Beuzelin, J.M., Stout, M.J., 2013. Effects of chlorantraniliprole and thiamethoxam rice seed treatments on egg numbers and first instar survival of *Lissorhopterus tryzophilus* (Coleoptera: Curculionidae). *J. Econ. Entomol.* 106, 181–188.
- Lu, Y., Wu, K., Jiang, Y., Guo, Y., Desneux, N., 2012. Widespread adoption of Bt cotton and insecticide decrease promotes biocontrol services. *Nature* 487, 362–365.
- Lundgren, J.G., 2009. Nutritional aspects of non-prey foods in the life histories of predaceous Coccinellidae. *Biol. Control* 51, 294–305.

- Luo, S.P., Naranjo, S.E., Wu, K.M., 2014. Biological control of cotton pests in China. *Biol. Control* 68, 6–14.
- McCornack, B.P., Koch, R.L., Ragsdale, D.W., 2007. A simple method for in field sex determination of the multicolored Asian lady beetle *Harmonia axyridis*. *J. Insect Sci.* 7, 10.
- 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). *Ecotoxicol* 24, 1152–1161.
- Obrycki, J.J., Harwood, J.D., Kring, T.J., O'Neil, R.J., 2009. Aphidophagy by Coccinellidae: application of biological control in agroecosystems. *Biol. Control* 51, 244–254.
- Pell, J.K., Baverstock, J., Roy, H.E., Ware, R.L., Majerus, M.E.N., 2008. Intraguild predation involving *Harmonia axyridis*: a review of current knowledge and future perspectives. *BioControl* 53, 147–168.
- Perveen, F., Miyata, T., 2000. Effects of sublethal dose of chlorfluazuron on ovarian development and oogenesis in the common cutworm *Spodoptera litura* (Lepidoptera: Noctuidae). *Ann. Entomol. Soc. Am.* 93, 1131–1137.
- Puinean, A.M., Denholm, I., Millar, N.S., Nauen, R., Williamson, M.S., 2010. Characterisation of imidacloprid resistance mechanisms in the brown planthopper, *Nilaparvata lugens* Stal (Hemiptera: Delphacidae). *Pestic. Biochem. Physiol.* 97, 129–132.
- Rahmani, S., Bandani, A.R., 2013. Sublethal concentrations of thiamethoxam adversely affect life table parameters of the aphid predator, *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae). *Crop Prot.* 54, 168–175.
- Rimoldi, F., Schneider, M.I., Ronco, A.E., 2012. Short and Long-term effects of endosulfan, cypermethrin, spinosad, and methoxyfenozide on adults of *Chrysoperla externa* (Neuroptera: Chrysopidae). *J. Econ. Entomol.* 105, 1982–1987.
- Saber, M., 2011. Acute and population level toxicity of imidacloprid and fenpyroximate on an important egg parasitoid, *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae). *Ecotoxicol* 20, 1476–1484.
- Sabry, A.H., Hassan, K.A., Rahman, A.A., 2014. Relative toxicity of some modern insecticides against the pink bollworm, *Pectinophora gossypiella* (saunders) and their residues effects on some natural enemies. *Intern. J. Sci. Environ. Tech.* 3, 481–491.
- Saddiq, B., Shad, S.A., Aslam, M., Ijaz, M., Abbas, N., 2015. Monitoring resistance of *Phenacoccus solenopsis* Tinsley (Homoptera: pseudococcidae) to new chemical insecticides in Punjab, Pakistan. *Crop Prot.* 74, 24–29.
- Schneider, M.I., Sanchez, N., Pineda, S., Chi, H., Ronco, A., 2009. Impact of glyphosate on the development, fertility and demography of *Chrysoperla externa* (Neuroptera: Chrysopidae): ecological approach. *Chemosphere* 76, 1451–1455.
- Silva, R.A., Carvalho, G.A., Carvalho, C.F., Reis, P.R., Pereira, A.M.A.R., Cosme, L.V., 2005. Toxicidade de produtos fitossanitários utilizados na cultura do cafeeiro a larvas de *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) e efeitos sobre as fases subsequentes do desenvolvimento do predador. *Neotrop Entomol.* 34, 951–959.
- Software, LeOra, 2002. PoloPlus: Probit and Logit Analysis. LeOra Software, Berkeley, CA.
- Song, Y., Dong, J., Sun, H., 2013. Chlorantraniliprole at sublethal concentrations may reduce the population growth of the Asian corn borer, *Ostrinia furnacalis* (Lepidoptera: Pyralidae). *Acta Entomol. Sin.* 56, 446–451.
- Stara, J., Ourednickova, J., Kocourek, F., 2011. Laboratory evaluation of the side effects of insecticides on *Aphidius colemani* (Hymenoptera: Aphidiidae), *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae), and *Neoseiulus cucumeris* (Acari: Phytoseiidae). *J. Pest Sci.* 84, 25–31.
- Stark, J.D., Banks, J.E., 2003. Population-level effects of pesticides and other toxicants on arthropods. *Annu. Rev. Entomol.* 48, 505–519.
- Tuan, S.J., Yeh, C.C., Atlihan, R., Chi, H., 2016. Linking life table and predation rate for biological control: a comparative study of *Eocanthecona furcellata* (Hemiptera: Pentatomidae) fed on *Spodoptera litura* (Lepidoptera: Noctuidae) and *Plutella xylostella* (Lepidoptera: Plutellidae). *J. Econ. Entomol.* 109, 13–24.
- Wang, Y.P., Lv, F., Wang, Z.P., 2007. Progress of *Harmonia axyridis* (Pallas) utilization. *Entomol. J. East China* 18, 310–314.
- Wang, X., Li, X., Shen, A., Wu, Y., 2010. Baseline susceptibility of the diamondback moth (Lepidoptera: Plutellidae) to chlorantraniliprole in China. *J. Econ. Entomol.* 103, 843–848.
- Xu, C., Zhang, Z., Cui, K., Zhao, Y., Han, J., Liu, F., Mu, W., 2016. Effects of sublethal concentrations of cyantraniliprole on the development, fecundity and nutritional physiology of the black cutworm *Agrotis ipsilon* (Lepidoptera: Noctuidae). *PLoS One* 11, 0156555.
- Yang, N.W., Zang, L.S., Wang, S., Guo, J.Y., Xu, H.X., Zhang, F., Wan, F.H., 2014. Biological pest management by predators and parasitoids in the greenhouse vegetables in China. *Biol. Control* 68, 92–102.
- 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.
- Yu, H., Xiang, X., Yuan, G., Chen, Y., Wang, X., 2015. Effects of sublethal doses of cyantraniliprole on the growth and development and the activities of detoxifying enzymes in *Spodoptera exigua* (Lepidoptera: Noctuidae). *Acta Entomol. Sin.* 6, 007.
- Zhang, R.M., Dong, J.F., Chen, J.H., Qing, J.L., Cui, J.J., 2013. The sublethal effects of chlorantraniliprole on *Helicoverpa armigera* (Lepidoptera: Noctuidae). *J. Integ. Agric.* 12, 457–466.