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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*.

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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.

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).

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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 again 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 ± 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 ± 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} 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