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Influence of lethal and sublethal exposure to clothianidin on the seven-spotted lady beetle, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae)

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ABSTRACT

The seven-spotted ladybird beetle, *Coccinella septempunctata* L., as a dominant predator of aphids, has played a crucial role in integrated pest management (IPM) strategies in agricultural ecosystems. To study the risk of insecticides to *C. septempunctata*, the neonicotinoid clothianidin was selected for evaluation of its influence on *C. septempunctata* at lethal and sublethal doses. The LR_{50} (application rate causing 50% mortality) in the exposed larvae decreased from 19.94 to 5.91 g a.i. ha^{-1}, and the daily HQ (hazard quotient) values increased from 3.00 to 10.15, indicating potential intoxication risks. We also determined NOERs (No Observed Effect application Rates) of clothianidin on the total developmental time (10 g a.i. ha^{-1}), survival (2.5 g a.i. ha^{-1}) and pupation (5 g a.i. ha^{-1}). Moreover, clothianidin at a NOER of 2.5 g a.i. ha^{-1} did not profoundly affect adult emergence, fecundity or egg hatchability. The total effect (E) assessment also showed that clothianidin at 2.5 g a.i. ha^{-1} was slightly harmful to *C. septempunctata*. These results suggested that clothianidin would impair *C. septempunctata* when applied at over 2.5 g a.i. ha^{-1} in the field. Conservation of this biological control agent in agricultural ecosystems thus requires further measures to decrease the applied dosages of clothianidin.

1. Introduction

Within most agricultural ecosystems, insect pest management typically relies on chemical insecticidal sprays and biological control by natural enemies (Desneux et al., 2007). However, the extensive use of pesticides to control pests may seriously harm natural enemies, and pesticides have various sublethal effects on the physiological and behavioral processes in these beneficial arthropods (Desneux et al., 2007). Integrated pest management (IPM) programs suppress the pest population below the economic threshold while conserving limited biological control agents (Cook et al., 2007; Naranjo et al., 2015). Compatibility for the combination of pesticides and biological control agents, such as predators and parasitoids, meets the demands of ecologically and economically sound IPM tactics. The pesticides applied in IPM pest control strategies would be of low risk to a wide variety of natural enemies (Youn et al., 2003; He et al., 2012). Therefore, a precise preliminary assessment of the negative impacts of such pesticides on biological control agents is urgently necessary to develop sustainably effective IPM strategies (Desneux et al., 2006, 2007).

The distribution of the seven-spotted ladybird beetle, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), is generally acknowledged to be widespread over agricultural and natural habitats worldwide (Stark et al., 2007). Both the larvae and adults of *C. septempunctata* are considered highly omnivorous, feeding on many species of insect pests (e.g., Aphidoidea, Tetranychidae, Psylloidea, and Coccoidea) that infest crops in both greenhouses and fields (Volkl et al., 2007; Lu et al., 2012; Hodek and Michaud, 2013). *C. septempunctata* has thus been used as a major biological control agent for suppressing various aphids in agroecosystems (Bianchi and Werf, 2004; Landis and Fox, 2004). Furthermore, *C. septempunctata* can be an ideal model test organism for studying the side effects on non-target natural enemies as a segment of the registration process for pesticides because of the convenience and efficiency of the rearing process (MAPRC, 2017).

Biological control is the potential pillar of IPM systems, but chemical controls are still the major and most effective interventions for combating pests in China (Naranjo et al., 2015; Yao et al., 2015). In cotton and wheat crops, neonicotinoid insecticides were the most widely used for inhibiting the aphid population growth rate at seedling stages due to their systemic action, diverse application methods, high efficiency and long persistence (Jeschke et al., 2011; Zhang et al., 2017). However, the potential negative impacts of neonicotinoids on pollinators and other non-target organisms have recently led to great

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concern about their environmental and ecological impacts (Elbert et al., 2008; Cloyd and Bethke, 2011; Tirello et al., 2013). Non-target natural enemies might be negatively affected not only by direct contact with spray droplets and residues of neonicotinoid insecticides but also by ingestion of contaminated plant material and/or prey (Yao et al., 2015). Neonicotinoid exposure could entail both acute toxic effects and sublethal effects on the feeding behavior, development, longevity, and reproduction of natural enemies (Desneux et al., 2007; Cloyd and Bethke, 2011). Sublethal effects can lead to the inability of natural enemies to control pests, resulting in incompatibility between the incorporation of natural enemies and the use of insecticides in IPM strategies, causing more harm than the lethal effects on natural enemies from a demographic perspective (Desneux et al., 2007; Yao et al., 2015).

Clothianidin, as a second-generation neonicotinoid insecticide with a substituted chlorothiazolyl-methyl group, has been registered for managing a broad spectrum of insect pests (e.g., Coleoptera, Diptera, Lepidoptera and Hemiptera) on approximately 40 crops in at least 34 countries worldwide (Jeschke et al., 2011; Uneme, 2011). In China, clothianidin is registered mainly against piercing-sucking insect pests such as planthoppers, thrips, whiteflies and aphids (China Pesticide Information Network, 2017). To date, most studies have primarily evaluated the lethal effects of clothianidin on various parasitoids and predators, showing that clothianidin at the recommended label rates was directly harmful to these natural enemies (Cloyd and Dickinson, 2006; Cloyd et al., 2009; Sugiyama and Saito, 2011; Prabhaker et al., 2011). Little information is currently available on the long-term influence of clothianidin on *C. septempunctata*.

Hence, to proceed to the compatible usage of clothianidin and *C. septempunctata* for pest management under realistic field conditions, an overall risk assessment on the clothianidin exposure on *C. septempunctata* was critical and necessary. Here, we identified the LR_{50}s (application rates causing 50% mortality) and NOERs (No Observed Effect application Rates) from chronic exposure for the larvae of *C. septempunctata* in laboratory microcosms. Favorable results would promote the conservation of ladybird beetles and provide available references for optimizing the use of clothianidin as a component of effective IPM strategies in agricultural ecosystems.

2. Materials and methods

2.1. Insecticides

Technical-grade clothianidin (98.1% purity) was obtained from Veyong Bio-Chemical Co., Ltd., Hebei, China, with no greater purity available. A 100 mL stock solution (1000 mg/L) was prepared by dissolving 0.1019 g clothianidin with 100 mL HPLC-grade acetone.

2.2. Test species

The test organisms for the start of the trial were the 2nd-instar larvae of *C. septempunctata* from a laboratory-reared colony. The adults and larvae at four different stages were reared on black bean aphids, *Aphis craccivora* Koch, which were maintained on fresh seedlings of broad beans *Vicia faba* L. under laboratory conditions of 20 ± 1 °C, 50–70% relative humidity (RH) and a 16:8 (light:dark) photoperiod. The hatchability of the eggs and the growth of the first-instar larvae were evaluated based on growth in a climate-controlled incubator at 25 ± 1 °C, 70% RH and 16:8 (L:D) photoperiod.

2.3. Bioassays

The toxicity experiment was performed with a previously designed laboratory method (C. Yu et al., 2014). The six tested concentrations of clothianidin in this study were chosen on the basis of a preliminary 72 h acute toxicity experiment, (Table S1), starting with 40 mg a.i. (active ingredient) L^{-1} and decreasing by halves to the following five concentrations (20, 10, 5, 2.5, 1.25 mg a.i. L^{-1}) to mimic the half-life degradation of pesticides under actual field conditions. Acetone was used for the blank controls. A total of 580 μL of the same clothianidin solution was added to each glass culture tube (12 cm height × 1.5 cm diameter; plugged with cotton balls). Afterwards, these tubes were immediately rotated on a micro-rotator (Verly Co., Ltd., Guangzhou, China) until all the solvent evaporated to dryness. Then, thirty 2nd-instar larvae were introduced to every replicate and fed daily with 30 mixed instars of *A. craccivora*, with each treatment and blank control consisting of three replicates. The mortality of *C. septempunctata* was identified and recorded when individuals were unable to respond to a mild touch using a paint brush at daily intervals throughout the entire *C. septempunctata* generation (He et al., 2012). In the long-term experiment, the detailed periods of different life stages were also recorded at daily intervals. Emerged adults were collected and paired at a 1:1 ratio. Each pair was then removed to a 30 × 20 × 10 cm plastic cage containing sufficient *A. craccivora* as food, and the data on fecundity (i.e., the number of eggs produced) were recorded every 24 h until the death of the female parent. Dead males were removed immediately after identification. All the collected eggs in each treatment were transferred to another climate-controlled room under the conditions described above. The egg hatchability proportion was calculated by counting the number of larvae derived from each replicate group. The entire generation extended from the 2nd-instar stage of the first generation to the counterparts of the next generation. The development from egg hatching to the next 2nd-instar stage took approximately 5 days in the long-term observation.

2.4. Risk assessment

The ecological risk of clothianidin to *C. septempunctata* was determined by the hazard quotient (HQ) method. The HQ value is calculated by dividing the field-recommended label rates of clothianidin by the LR_{50} of clothianidin to *C. septempunctata* acquired from the laboratory study (Candolfi et al., 2001). Ratios of greater than or equal to 2 highlight clothianidin as a potential hazard to *C. septempunctata*. Ratios of less than 2 indicate a low intoxication risk.

2.5. Statistical analysis

The LR_{50} was determined by a log-probit regression analysis with SPSS v.17.0 (SPSS Inc., Chicago, IL). The NOER values were estimated by means of a one-way analysis of variance (ANOVA). Means were compared by Tukey's least significant difference (LSD) tests ($\boldsymbol{P} < 0.05$) (Zar, 1996). The total developmental time and survival rates over different instars were analyzed with a repeated-measures ANOVA to examine differences within the treatment groups.

The total effect (E) was estimated using the formula E (%) = 100 - (100-Mc) × ER (Overmeer and Zon, 1982), where Mc represents the corrected mortality of *C. septempunctata* in the group treated by each dosage of clothianidin; ER is the ratio of the mean weekly number of eggs laid by females in each group versus those laid by females in the control. According to the IOBC laboratory scale (Hassan, 1994) and on the basis of their total effects, the different dosages of clothianidin were classified in the following toxicity categories: (1) harmless (< 30%); (2) slightly harmful (30–79%); (3) moderately harmful (80–99%); and (4) harmful (> 99%).

3. Results

3.1. Influence of clothianidin on the survival rate of C. septempunctata

The effects of six concentrations of clothianidin on the survival percentage of larvae of *C. septempunctata* are presented in Fig. 1. The survival rate of the larvae in the control averaged 96.7% at the end of