The Toxicity Response of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) after Exposure to Sublethal Concentrations of Acetamiprid

Yong You 1,†, Zhaohua Zeng 1,†, Jie Zheng 1, Jianwei Zhao 1, Fengqiu Luo 2, Yixin Chen 1, Miao Xie 2, Xingang Liu 3,* and Hui Wei 1,*

1 Fujian Key Laboratory for Monitoring and Integrated Management of Crop Pests, Fujian Engineering Research Center for Green Pest Management, Institute of Plant Protection, Fujian Academy of Agricultural Sciences, Fuzhou 350013, China
2 College of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou 350002, China
3 State Key Laboratory for Biology of Plant Disease and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China
* Correspondence: weihui@faas.cn (H.W.); liuxingang@caas.cn (X.L.)
† These authors contributed equally to this work.

Abstract: *Coccinella septempunctata* is a nontarget beneficial arthropod and an important aphid predator in agricultural crops. In this study, the toxic effects of the neonicotinoid acetamiprid on *C. septempunctata* were investigated to determine its applicability and efficacy against the aphid predator. The results of the toxicity test showed that the second instar larvae of *C. septempunctata* were the most sensitive to acetamiprid. The LC50 values of the 1st, 2nd, 3rd, and 4th instar larvae were 15.767, 9.412, 18.850, and 25.278 mg a.i. L−1, respectively. Compared with that of the control, the predation ability of different larval instars was inhibited by sublethal concentrations of acetamiprid. The results of the predatory function test showed that sublethal concentrations of acetamiprid could reduce the consumption of aphids by fourth instar *C. septempunctata* larvae over a short duration and significantly inhibited the predatory ability of ladybird larvae. The results of the developmental test showed that sublethal concentration of acetamiprid shortened the growth duration of *C. septempunctata* larvae. Acetamiprid had considerable adverse effects on the different developmental stages of *C. septempunctata*. Together, our results provide information for implementation in biological and chemical control strategies for the integrated management of aphids.

Keywords: *Coccinella septempunctata*; acetamiprid; acute toxicity; predation capacity; development time

1. Introduction

Within most agricultural ecosystems, natural enemies and their predator/prey relationships play an important role in insect pest management [1]. The seven-spot ladybird, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), is as an important natural predator of many insect pests and has the advantages of ecological adaptability and plasticity [2,3]. Extensive research has been conducted on *C. septempunctata* to investigate artificial diets, biological characteristics, predation response, and artificial propagation [4–8]. The bean aphid (*Aphis craccivora* Koch) is a cosmopolitan pest with a high reproductive rate and short life cycle, enabling outbreaks and virus transmission among plants. *C. septempunctata* prey on aphids, which limits their population growth rate. Thus, using *C. septempunctata* to control pests in agricultural ecosystems is important for the development of integrated pest management (IPM) strategies [9].
In 1984, the first neonicotinoid insecticide imidaclopid was synthesized, and neonicotinoids have since become the most widely used class of insecticides worldwide [10,11]. Indeed, neonicotinoid insecticides are the fastest-growing and most widely used insecticides in modern crop protection. In 2007 alone, the global sales of neonicotinoids accounted for 24% of global insecticide sales for agriculture use [12]. As the third commercially developed neonicotinoid insecticide, acetamiprid has the advantages of high efficacy, long-lasting activity, good selectivity, and low toxicity to most nontarget organisms [13]. Acetamiprid plays an important role in the control of Homoptera (such as aphids, leafhoppers, and whiteflies), Lepidoptera (such as diamondback moths), and Coleoptera (such as longicorns) pests [14]. In 2014, acetamiprid experienced one of the fastest-growing market shares among all neonicotinoid insecticides [15].

However, some problems are inevitably encountered when neonicotinoid insecticides are used in large quantities [16]. Neonicotinoid insecticides such as imidacloprid can cause colony collapse disorder, hurting or killing nontarget organisms [17–19]. The effect of sublethal exposure on insect physiology or behaviors (such as the effects of predation, development, longevity, and reproduction) is more severe than that of lethal exposure [20]. Therefore, there is an urgent need to determine the sublethal effects of neonicotinoid insecticides on nontarget organisms to determine an appropriate rate of application. Acetamiprid can affect nontarget insects such as Apis mellifera, Trichogramma, Amblyseius cucumeris, and Neoseiulus fallacis [21–23]. In actual field conditions, direct residual contact or the indirect ingestion of spray can cause pest predators to suffer high levels of pesticide exposure [24,25]. Coccinella septempunctata, a predator with high mobility between agriculture land and natural habitats, is prone to discontinuous contact with insecticides [26,27]. It is thus necessary to study the sublethal effects of neonicotinoid insecticides on C. septempunctata to achieve a balance between neonicotinoid insecticides and C. septempunctata.

The lethal dose data obtained from acute toxicity tests can only partially measure harmful effects. In addition to the direct lethal effects of pesticides, the sublethal effects of pesticides on the physiology and behavior of arthropods must be considered when conducting a comprehensive analysis of their effects [28]. A previous study reported that female Euschistus heros increase their reproductive ability after sublethal exposure to imidacloprid, which may explain the recent outbreak of this neotropical brown bug E. heros in the soybean-producing regions of Brazil [29]. Half-lethal or low-dose exposure to neonicotinoids may adversely affect arthropod pest populations. Although these findings must be verified under field conditions, it could be expected that sublethal effects and hormesis of pest populations during pesticide application may occur over time in addition to the acute effects usually noted at high doses [30].

In this study, we explored the toxic effects of sublethal acetamiprid exposure on the toxicity of the predatory natural enemy C. septempunctata at different stages, as well as the sublethal effect of the neonicotinoid insecticide acetamiprid exposure on the predation effect and development time of C. septempunctata. The results not only provide meaningful data supporting the biological control effect of acetamiprid and C. septempunctata on aphids but also provide information for optimizing neonicotinoid insecticide use in IPM strategies.

2. Materials and Methods

2.1. Insecticide

Commercial formulation of acetamiprid (HengDing, 40% purity, wettable powder (WP)) was obtained from Hainan Zhengye Zhongnong High Technology Co., Ltd., Hainan, China. The WP formulation of acetamiprid was used to conduct the experiment to mimic the actual application in the field.
2.2. Test Species

A laboratory colony was established using adults collected from experimental fields at the Pesticide Environmental Safety Assessment Center, Fuzhou, Fujian Province, China. The test organisms were reared on bean aphids, *A. craccivora* Koch, that were maintained on fresh seedlings of broad beans (*Vicia faba* L.). Eggs and pupae of *C. septempunctata* were collected from the culture. Bean seedlings and *C. septempunctata* were cultivated under laboratory conditions at 25 ± 2 °C, 60–90% relative humidity (RH), and a 16:8-h (light:dark) photoperiod.

2.3. Acetamiprid Toxicity Test to *C. septempunctata* at Different Stages

The microcosm toxicity experiment was performed using the tube-drug film method. A pipette was used to accurately measure 0.7 mL of the prepared insecticide liquid in a clean finger tube (inner diameter 2.4 cm, height 4.3 cm) that was rotated quickly on a microrotator (Sitong Co., Ltd., Zhejiang, China). The solutions were evenly spread on the inner surface of the glass tube. The film was dried at 25 ± 2 °C to obtain a uniform film. The 1st and 2nd instar larvae of *C. septempunctata* were treated with 22.8, 15.2, 10.1, 6.8, and 4.5 mg a.i. L⁻¹, whereas the 3rd and 4th instar larvae were treated with 33.6, 22.4, 15, 10, 6.7, and 4.4 mg a.i. L⁻¹.

The test larvae were transferred to a drug film glass tube and provided sufficient aphids, *A. craccivora*, as food. Before feeding on new aphids, the remaining aphids were removed to ensure that the *C. septempunctata* fully contacted the drug film. Each treatment and blank control consisted of three replicates. The survival rates and symptoms of poisoning were recorded daily. The test was terminated after 48 h.

2.4. Sublethal Effects on Predatory Capacity of *C. septempunctata*

LC₅₀, LC₁₀₀, and LC₂₀₀ of acetamiprid were selected as the experimental sublethal concentrations. The 1st and 2nd instar larvae of *C. septempunctata* were starved under sublethal exposure scenarios for 12 h. The 3rd and 4th instar larvae of *C. septempunctata* were starved under sublethal exposure scenarios for 24 h. After starvation, the larvae were transferred to a clean tube covered with cotton gauze to allow air exchange. A total of 5 prey aphid densities (5, 10, 15, 20, and 25) were offered to the 1st and 2nd instar larvae of *C. septempunctata*. The 3rd and 4th instar larvae eat more and more prey. Five different prey aphid densities (30, 50, 75, 100, and 120) were offered to each 3rd instar larva, while different densities of prey aphids (50, 100, 150, 200, and 250) were offered to the 4th instar larvae. Three replicates were used for each treatment (including controls). The number of prey consumed was recorded after 24 h. All treatments were carried out under laboratory conditions of 25 ± 2 °C, 60—90% RH, and a 16:8-h (light:dark) photoperiod.

2.5. Functional Response of *C. septempunctata* to Acetamiprid

The predator–prey model with Holling type II functional response was defined for all treatments [6,31]:

\[
Na = \frac{a' \cdot TN_0}{(1 + a'T_hN_0)}
\] (1)

where *Na* is the prey quantity of *C. septempunctata*, *a'* is the instantaneous attack rate, *T* is the total time of the predatory experiment (*T* = 1 d in this study), *N₀* is the prey density, and *Tₜ* is the handling time for a predator to catch each prey.

The model of the searching efficiency in a predator–prey system is:

\[
S = \frac{a'}{(1 + a'T_hN)}
\] (2)
where \( S \) is the search efficiency, \( a' \) is the instantaneous attack rate, \( N \) is the prey density, and \( T_h \) is the handling time taken by a predator to catch each prey.

2.6. Effects of Sublethal Acetamiprid Exposure on \( C. \) septempunctata Larval Development

The larvae at 4 different instar stages were fed with \( A. \) craccivora under sublethal exposure conditions for 24 h. Each treated larva was then transferred to a new tube. Sufficient aphids were offered as food during larval instar development. The remaining aphids and molting were counted at daily intervals. Six replicates were used for each treatment and continuously observed until adult emergence.

2.7. Statistical Analysis

The LC\(_{50}\) (i.e., concentration at which 50% of the test species die) was determined by log-probit regression analysis using SPSS 25.0 (SPSS Inc., Chicago, IL, USA) [6]. Means were compared using Tukey’s least significant difference (LSD) tests \((p < 0.05)\). For each treatment group, repeated-measures analysis of variance (ANOVA) was used to analyze the total developmental duration and survival probability across the different instar stages.

3. Results

3.1. Toxicity of Acetamiprid to \( C. \) septempunctata at Different Larval Stages

The sensitivity of pest predators to pesticides varies depending on the developmental stage of the test organism. Therefore, we determined the toxicity of \( C. \) septempunctata larvae at four instar stages (Table 1). The LC\(_{50}\) values of acetamiprid for \( C. \) septempunctata, based on log-probit regression analysis, are shown in Table 1. Some larvae showed the toxic symptoms of slow movement and vomiting at 24 h after treatment. In severe cases, the larvae contracted, blackened, and died. At the end of the 48 h observation period, the survival rate of the control group was 100%. The toxicity of acetamiprid to \( C. \) septempunctata decreased with an increase in larval instars. The results showed that the second instar larvae were the most sensitive to acetamiprid.

| Larval Stage | Regression Equation | SE \(^a\) | \( X^2 \) \(^b\) | \( df \) \(^c\) | \( P \) \(^d\) | \( R^2 \) \(^e\) | LC\(_{50}\) \(^f\) (mg a.i. L\(^{-1}\)) | LC\(_5\) (mg a.i. L\(^{-1}\)) | LC\(_{10}\) (mg a.i. L\(^{-1}\)) | LC\(_{20}\) (mg a.i. L\(^{-1}\)) |
|-------------|---------------------|---------|-----------|---------|-----|-----|-----------------|-------------|-------------|-------------|
| L1          | \( y = 1.861x - 2.229 \) | 0.450   | 0.478     | 0.593   | 3   | 0.89 | 0.96 | 15.767 | 12.057–25.662 | 2.061  | 3.230  | 5.567  |
| L2          | \( y = 2.839x - 2.765 \) | 0.484   | 0.492     | 3.275   | 3   | 0.35 | 0.91 | 9.412  | 7.756–11.314  | 2.480  | 3.329  | 4.757  |
| L3          | \( y = 2.172x - 2.770 \) | 0.368   | 0.431     | 2.947   | 4   | 0.56 | 0.93 | 18.850 | 15.175–25.360 | 3.296  | 4.845  | 7.724  |
| L4          | \( y = 2.420x - 3.395 \) | 0.415   | 0.505     | 0.833   | 4   | 0.93 | 0.98 | 25.278 | 20.211–35.765 | 5.285  | 7.468  | 11.350 |

(a) Standard errors of slope and intercept, respectively. (b) Chi-square. (c) Degree of freedom. (d) \( P \)-value, probability value. (e) Coefficient of determination. (f) LC: Lethal concentration. (g) a.i. means active ingredient.
3.2. Effects of Sublethal Concentrations of Acetamiprid on Predation Capacity of C. septempunctata Larvae

When the prey density was 20, the amount of prey consumed by the 1st instar C. septempunctata larvae was significantly different between the control and sublethal acetamiprid treatment conditions (Table 2). Regardless of prey density, when the acetamiprid concentrations reached LC10, the predatory capacity of the 1st instar larvae began to decrease significantly.

Table 2. The amount of prey consumed by each instar larvae of C. septempunctata treated with the acetamiprid sublethal concentration for 24 h (Mean ± SD).

| Instar Larvae (the Number of Aphids Per Tube) | Prey Density | Control | LC5   | LC10  | LC20  | df | F    | P     |
|---------------------------------------------|-------------|---------|-------|-------|-------|----|------|-------|
| 1st                                         | 5           | 4.30 ± 0.213 a | 4.00 ± 0.333 a | 3.10 ± 0.180 b | 3.10 ± 0.146 b | 3  | 6.288 | < 0.05 |
|                                             | 10          | 7.10 ± 0.314 a | 6.80 ± 0.389 ab | 5.80 ± 0.389 bc | 5.20 ± 0.573 c  | 3  | 4.251 | < 0.05 |
|                                             | 15          | 10.30 ± 0.300 a | 10.10 ± 0.277 a | 8.90 ± 0.277 b  | 8.00 ± 0.333 c  | 3  | 13.119 | < 0.05 |
|                                             | 20          | 15.90 ± 0.407 a | 13.10 ± 0.433 b | 12.20 ± 0.490 b | 9.50 ± 0.500 c  | 3  | 33.024 | < 0.05 |
|                                             | 25          | 18.00 ± 0.558 a | 17.10 ± 0.379 a | 14.30 ± 0.396 b | 12.80 ± 0.442 c | 3  | 28.975 | < 0.05 |
|                                             | 5           | 4.50 ± 0.167 a | 3.80 ± 0.291 ab | 3.10 ± 0.277 b  | 3.30 ± 0.300 b  | 3  | 1.510  | 0.228  |
|                                             | 10          | 7.70 ± 0.300 a | 7.20 ± 0.249 a  | 6.80 ± 0.359 a  | 5.30 ± 0.300 b  | 3  | 14.666 | < 0.05 |
| 2nd                                         | 15          | 12.40 ± 0.452 a | 10.90 ± 0.277 b | 9.40 ± 0.476 c  | 8.50 ± 0.582 c  | 3  | 13.890 | < 0.05 |
|                                             | 20          | 16.00 ± 0.298 a | 15.60 ± 0.267 a | 14.20 ± 0.249 b | 12.90 ± 0.379 c | 3  | 21.839 | < 0.05 |
|                                             | 25          | 20.20 ± 0.467 a | 18.10 ± 0.433 b | 16.00 ± 0.447 c | 13.90 ± 0.458 d | 3  | 36.049 | < 0.05 |
|                                             | 30          | 28.20 ± 0.512 a | 27.50 ± 0.671 a | 27.10 ± 0.567 a | 28.00 ± 0.422 a | 3  | 0.815  | 0.494  |
|                                             | 50          | 47.20 ± 0.663 a | 45.30 ± 0.989 a | 46.30 ± 1.012 a | 41.30 ± 1.739 b | 3  | 4.954  | < 0.05 |
| 3rd                                         | 70          | 63.30 ± 1.606 a | 61.00 ± 1.229 a | 60.60 ± 1.470 a | 55.90 ± 1.767 b | 3  | 4.112  | < 0.05 |
|                                             | 100         | 94.00 ± 1.382 a | 91.80 ± 1.737 ab | 88.50 ± 1.424 bc | 86.60 ± 1.979 c | 3  | 4.028  | < 0.05 |
|                                             | 120         | 110.00 ± 2.113 a | 105.00 ± 2.290 ab | 103.00 ± 2.066 b | 94.90 ± 1.859 c | 3  | 9.057  | < 0.05 |
|                                             | 50          | 46.30 ± 1.121 a | 44.00 ± 1.300 a | 45.10 ± 1.370 a | 46.60 ± 0.859 a | 3  | 0.984  | 0.411  |
|                                             | 100         | 91.10 ± 1.278 a | 89.70 ± 1.146 a | 77.60 ± 2.001 b | 66.90 ± 2.627 c | 3  | 37.325 | < 0.05 |
| 4th                                         | 150         | 110.10 ± 3.598 a | 107.20 ± 3.359 a | 89.50 ± 3.321 b | 90.30 ± 4.585 b | 3  | 8.436  | < 0.05 |
|                                             | 200         | 139.10 ± 5.332 a | 138.40 ± 4.246 a | 129.60 ± 4.206 ab | 121.70 ± 5.428 b | 3  | 2.892  | < 0.05 |
|                                             | 250         | 197.10 ± 4.413 a | 173.00 ± 5.787 ab | 150.10 ± 3.093 b | 134.90 ± 7.155 c | 3  | 25.987 | < 0.05 |

The same column followed by different letters is significantly different based on ANOVA using Tukey’s LSD test (p < 0.05).

When the aphid densities were 15 and 25, there was a significant difference in the 2nd instar larval predatory capacity of C. septempunctata between the control and acetamiprid treatment groups (Table 2). The number of prey eaten by the 2nd instar larvae significantly decreased between the control and acetamiprid concentrations of LC5 in the different prey density groups. Predatory capacity was significantly weakened in the LC5 treatment at prey densities of 15 and 25. Table 2 shows that the lower the prey density, the lesser the effect of low acetamiprid concentrations for the 3rd instar larvae. When the aphid density was 30, there was no difference in the predation ability of the 3rd instar larvae among different acetamiprid concentrations. When the prey densities were 50 and 70, pesticide treatment at LC5 reduced the predation ability of the 3rd instar larvae. When the aphid density was ≥100, pesticide treatment at LC10 reduced the predatory ability of the 3rd instar larvae. Similar to the results of the 2nd and 3rd instars, when the prey density was the lowest, pesticide treatment had no effect on the amount of prey consumed by the 4th instar larvae (Table 2). When the aphid density was ≥100, LC20 treatment reduced the predatory ability of the 4th instar larvae. For the 1st and 2nd instar larvae, when the...
concentration was more than LC_{10}, the consumption of aphids was significantly lower than that of the control group. The LC_{5} treatment and control groups resulted in no significant differences in the 3rd and 4th instar larvae. Acetamiprid at LC_{20} had a significant effect on the 3rd instar larvae at prey densities of 50, 70, 100, and 120 (ANOVA, p < 0.05). Acetamiprid at LC_{10} and LC_{20} significantly decreased the predation by C. septempunctata at prey densities of 100, 150, and 250 in the 4th instar larvae (ANOVA, p < 0.05).

3.3. Influence of Sublethal Exposure to Acetamiprid on the Predatory Functional Response of C. septempunctata

The predation capability of C. septempunctata on A. craccivora fits a predator–prey model with a Holling type II functional response after the treatment with acetamiprid (Table 3). According to this model, the rate of successful C. septempunctata attack (a’) decreased following an increase in the acetamiprid concentration. Compared with that of the control, the handling time of C. septempunctata extended by 2–4 times after treatment with the sublethal concentrations LC_{10} and LC_{20}. Sublethal exposure to acetamiprid significantly reduced the predation capacity of C. septempunctata larvae.

Table 3. Predatory functional response model and parameters of C. septempunctata in different larval stages.

| Treatment | Equation of Predator Functional Response | R^2 | The Rate of Successful Attack (a’) | Handling Time of Predatory (T_s/d) |
|-----------|-----------------------------------------|-----|-----------------------------------|----------------------------------|
| 1st Instar Control | Na = 0.747N/(1 + 0.00060N) | 0.981 | 0.747 | 0.0008 |
| LC_5 | Na = 0.689N/(1 + 0.00096N) | 0.994 | 0.689 | 0.0014 |
| LC_{10} | Na = 0.622N/(1 + 0.00280N) | 0.996 | 0.622 | 0.0045 |
| LC_{20} | Na = 0.552N/(1 + 0.00431N) | 0.986 | 0.552 | 0.0078 |
| Control | Na = 0.817N/(1 + 0.00057N) | 0.997 | 0.817 | 0.0007 |
| 2nd Instar LC_5 | Na = 0.754N/(1 + 0.00083N) | 0.994 | 0.754 | 0.0011 |
| LC_10 | Na = 0.677N/(1 + 0.00122N) | 0.986 | 0.677 | 0.0018 |
| LC_{20} | Na = 0.606N/(1 + 0.00188N) | 0.971 | 0.606 | 0.0031 |
| Control | Na = 0.938N/(1 + 0.00019N) | 0.999 | 0.938 | 0.0002 |
| 3rd Instar LC_5 | Na = 0.916N/(1 + 0.00027N) | 0.997 | 0.916 | 0.0003 |
| LC_{10} | Na = 0.932N/(1 + 0.00065N) | 0.998 | 0.932 | 0.0007 |
| LC_{20} | Na = 0.886N/(1 + 0.00080N) | 0.988 | 0.886 | 0.0009 |
| Control | Na = 0.992N/(1 + 0.00120N) | 0.974 | 0.920 | 0.0013 |
| 4th Instar LC_5 | Na = 0.936N/(1 + 0.00159N) | 0.984 | 0.936 | 0.0017 |
| LC_{10} | Na = 0.828N/(1 + 0.00149N) | 0.971 | 0.828 | 0.0018 |
| LC_{20} | Na = 0.792N/(1 + 0.00182N) | 0.984 | 0.792 | 0.0023 |

(a) coefficient of determination.

Among all treatment conditions, the 3rd instar larvae without any treatment had the highest searching efficiency, reaching 0.934, while the 1st instar larvae treated with acetamiprid at LC_{5} had the lowest searching efficiency, which was 0.498 (Table 4). The searching efficiency of C. septempunctata on aphids decreased with the increase of prey density. Among all the larvae, the third instar ones had the highest ability to search. When the prey density remained the same, the searching efficiency decreased with an increase in the concentration of acetamiprid.
Table 4. Searching efficiency of *C. septempunctata* after exposing to acetamiprid in different larval stages.

| Developmental Time (The Number Of Aphids Per Tube) | Prey Density | Control | LC5 | LC10 | LC20 |
|---------------------------------------------------|--------------|---------|-----|------|------|
| 1st Instar                                        | 5            | 0.745   | 0.743 | 0.741 | 0.738 |
|                                                  | 10           | 0.736   | 0.686 | 0.682 | 0.679 |
|                                                  | 15           | 0.676   | 0.673 | 0.613 | 0.605 |
|                                                  | 20           | 0.597   | 0.589 | 0.581 | 0.540 |
|                                                  | 25           | 0.529   | 0.518 | 0.508 | 0.498 |
|                                                  | 5            | 0.815   | 0.813 | 0.810 | 0.808 |
|                                                  | 10           | 0.805   | 0.751 | 0.747 | 0.744 |
| 2nd Instar                                        | 15           | 0.741   | 0.738 | 0.673 | 0.669 |
|                                                  | 20           | 0.665   | 0.661 | 0.657 | 0.601 |
|                                                  | 25           | 0.595   | 0.589 | 0.584 | 0.579 |
|                                                  | 30           | 0.934   | 0.931 | 0.928 | 0.924 |
|                                                  | 50           | 0.922   | 0.908 | 0.903 | 0.898 |
| 3rd Instar                                        | 70           | 0.891   | 0.886 | 0.913 | 0.901 |
|                                                  | 100          | 0.890   | 0.873 | 0.862 | 0.865 |
|                                                  | 120          | 0.852   | 0.839 | 0.820 | 0.808 |
|                                                  | 50           | 0.868   | 0.821 | 0.779 | 0.741 |
|                                                  | 100          | 0.707   | 0.867 | 0.807 | 0.755 |
| 4th Instar                                        | 150          | 0.710   | 0.669 | 0.771 | 0.721 |
|                                                  | 200          | 0.677   | 0.638 | 0.603 | 0.727 |
|                                                  | 250          | 0.672   | 0.625 | 0.584 | 0.547 |

3.4. Effect of Acetamiprid on the Developmental Time of *C. septempunctata* at Different Larval Stages

Figures 1–4 show the effects of three sublethal concentrations of acetamiprid on the developmental duration of *C. septempunctata* at different instar larval stages.
Figure 1. Effects of acetamiprid on *C. septempunctata* developmental duration within the 1st instar larval stage (mean ± SE). The same column followed by different letters is significantly different based on ANOVA using Tukey’s LSD test (*p* < 0.05).

Figure 2. Effects of acetamiprid on developmental duration of *C. septempunctata* within 2nd instar larval stage (mean ± SE). The same column followed by different letters is significantly different based on ANOVA using Tukey’s LSD test (*p* < 0.05).
Figure 3. Effects of acetamiprid on developmental duration of *C. septempunctata* within the 3rd instar larval stage (mean ± SE). The same column followed by different letters is significantly different based on ANOVA using Tukey’s LSD test (*p* < 0.05).

Figure 4. Effects of acetamiprid on developmental duration of *C. septempunctata* within 4th instar larval stage (mean ± SE). The same column followed by different letters is significantly different based on ANOVA using Tukey’s LSD test (*p* < 0.05).

As shown in Figure 1, when the first instar larvae were exposed to acetamiprid, the larval stage length of *C. septempunctata* in the first developmental period was the longest during treatment with sublethal concentration LC$_{20}$. Notably, this was significantly different from the larval stage length of the control group (ANOVA, *p* < 0.05). However, when the larvae grew, the larval stage length of the treatment groups shortened and decreased at the same stage with an increase of the sublethal concentration. The pupation stages of *C. septempunctata* treated with acetamiprid at LC$_{10}$ and LC$_{20}$ were significantly different from that of the control group.
In the second larval stage, the larvae were transferred to the drug film tube when the first instar larvae were to be fed in an insecticide-free environment. The development time of *C. septempunctata* larvae in the second instar development stage was the shortest under LC₅₀ treatment, which was significantly different from that of the control (ANOVA, *p* < 0.05, Figure 2). At the pupation stage, the developmental stage of *C. septempunctata* treated with acetamiprid at LC₅₀, LC₁₀₀, and LC₂₀₀ was significantly shorter than that of the control.

The larvae were not moved into the glass tube with acetamiprid until the larvae reached the third instar stage. As shown in Figure 3, there was no significant difference in the development duration between the control and treatment groups at the beginning of treatment when the larvae were removed from the drug film tube (ANOVA, *p* < 0.05). At the pupation stage, the pupation duration of the LC₂₀₀ group was significantly different from that of the control group.

The developmental period of the treatment groups was significantly longer than that of the control group when the fourth instar larvae were exposed to acetamiprid (ANOVA, *p* < 0.05). However, there was no significant difference in the pupation duration between the control and treatment groups (ANOVA, *p* < 0.05).

### 4. Discussion

The earlier life stages of larvae are often more sensitive to external chemical influences [25,32–36]. We determined the toxicity of *C. septempunctata* larvae at four instar stages. Our results showed that the toxicity of acetamiprid was the highest in the second instar larvae, followed by the first, third, and fourth instar stages. The acetamiprid-treated larvae moved slowly and had poor coordination. Previous studies have shown that the increased activity of detoxifying enzymes can lead to insect stage-dependent insecticide tolerance [37,38]. For example, enhanced oxidative detoxification and reduced permeability may cause differences in the susceptibility of *Spodoptera littoralis* [37]. We hypothesize that the first instar larvae feed less, which may have resulted in less exposure to acetamiprid.

Predation capacity is an important index for measuring the ability of predatory natural enemies to control pests, and it correlates strongly with the change in prey density [39]. When the first instar larvae of *C. septempunctata* were exposed to an acetamiprid concentration above LC₁₀₀, predation ability was weakened regardless of prey density. However, when prey density was the lowest, the effect of acetamiprid was not reflected in the second, third, and fourth instar larvae. Overall, the 24 h feed intake of larvae decreased with an increase in acetamiprid concentration, especially at LC₂₀₀. The highest voracity was observed during the last juvenile stage. The control group without insecticide treatment had the greatest predatory ability under the same aphid density, which means that the voracity of *C. septempunctata* larvae increased with prey density. Prey density affects larval development time and survival [40]. When insects are exposed to sublethal concentrations of insecticides, their biology and physiological functions are affected, and their feeding behavior changes [28,41]. Compared with pyrethroid and organophosphate insecticides, the neonicotinoids imidacloprid and thiamethoxam are less toxic but still could affect the aphid consumption of *C. septempunctata* [42].

The larvae of *C. septempunctata* showed a type II functional response after feeding on aphids treated with acetamiprid. The parameters of the Holling type II model obtained in our study indicated that sublethal exposure to acetamiprid significantly inhibited the predation ability of larvae. Neonicotinoid compounds (imidacloprid, thiamethoxam and thiacloprid) can seriously decrease the predation rate and foraging time of *C. septempunctata* [25,26,43]. When insecticides were used at LC₅₀, the predatory efficiency of both adult and larval *C. septempunctata* significantly decreased [42].

Developmental experiments indicated that acetamiprid exposure at LC₂₀₀ significantly shortened the 4th instar and pupation stages of *C. septempunctata* (ANOVA, *p* < 0.05). Additionally, the number of aphids consumed by the 1st, 2nd and 3rd instar larvae exposed to LC₂₀₀ was significantly higher than that consumed by the 4th instar of the
control group during the first 3 days (ANOVA, \( p < 0.05 \)). This may be because acetamiprid accelerated the predation of \textit{C. septempunctata} at the fourth instar stage, thus promoting the accumulation of pupation energy. Sublethal concentrations of clothianidin and thiamethoxam can significantly prolong the pupation period, whereas nitenpyram has little effect on the fourth instar and pupation stages \([44,45]\). Neonicotinoid insecticides act on nicotinic acetylcholine receptors (nAChRs) in the postsynaptic membrane of the insect nervous system and surrounding nerves. However, differences in toxicity effects can be caused by different binding sites. For example, in the American cockroach \textit{Periplaneta americana}, imidacloprid acts as an antagonist of nicotinic receptor 1 (nAChR1) instead of nAChR2 \([46–48]\). In contrast, acetamiprid binds to nAChR2 \([49,50]\). Acetamiprid is rapidly biotransformed into several compounds such as 6-chloronicotinic acid. In \textit{A. mellifera}, these compounds remain stable in the bodies except in the gut-free abdomen for at least 72 h, which may explain the short-term predation effect of acetamiprid \([51]\). This may also explain the short-term feeding effect of acetamiprid on \textit{C. septempunctata}.

5. Conclusions

The second instar larvae of \textit{C. septempunctata} were more sensitive to acetamiprid than the other instar larvae. Predation of the third instar larvae decreased significantly with increases in acetamiprid concentration. The neonicotinoid insecticide acetamiprid significantly affected the predation parameters of \textit{C. septempunctata} at LC_{10} and LC_{50}. Sublethal concentrations of acetamiprid could quickly reduce the predation activity of larvae and prolong the development duration of the instar during the treatment period. It is thus suggested that a sublethal concentration of acetamiprid may stimulate the growth of \textit{C. septempunctata}.

The neonicotinoid insecticide acetamiprid showed a high risk for \textit{C. septempunctata} under laboratory conditions, which may be different from the results in the field. Therefore, more studies are necessary, and should include multiple testing methods such as field trials and other sublethal concentrations.

Author Contributions: Y.Y., Z.Z., X.L., and H.W. conceived, designed, and performed the experiments and data analyses. J.Z. (Jie Zheng), J.Z. (Jianwei Zhao), and F.L. collected the data and performed the analysis. Y.C. and M.X. helped perform the analysis with constructive discussions. All authors contributed to manuscript preparation. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Special Foundation of Public Research Institutes of Fujian Province, China (2019RJ024–7), Innovation Team of Plant Protection and Fujian Academy of Agricultural Sciences (CXTD2021027) and “5511“ Collaborative Innovation Project of High-quality Agricultural Development and Surpassment in Fujian Province (XTCGC2021011 and XTCGJC2021017).

Conflicts of Interest: The authors declare no conflict of interest.

Reference

1. Doddamani, V.; Behere, G.; Firake, D.; Nongkynrih, B. Biology of \textit{Coccinella Septempunctata} on Mustard Aphid \textit{Lipaphis Erysimi}. \textit{Indian J. Hill Farming} 2017, 30, 41–44.
2. Yu, C.; Fu, M.; Lin, R.; Zhang, Y.; Yongquan, L.; Jiang, H.; Brock, T. Toxic effects of hexaflumuron on the development of \textit{Coccinella septempunctata}. \textit{Environ. Sci. Pollut. Res.} 2014, 21, 1418–1424.
3. Bahlai, C.A.; Colunga-Garcia, M.; Gage, S.H.; Landis, D.A. The role of exotic ladybeetles in the decline of native ladybeetle populations: Evidence from long-term monitoring. \textit{Biol. Invasions} 2015, 17, 1005–1024.
4. Cheng, Y.; Zhi, J.; Li, F.; Wang, H.; Zhou, Y.; Jin, J. Transcriptome sequencing of \textit{Coccinella septempunctata} adults (Coleoptera: Coccinellidae) feeding on artificial diet and \textit{Aphis craccivora}. \textit{PLoS ONE} 2020, 15, e0236249.
5. Cheng, Y.; Yu, Y.; Zhou, Y.H.; Li, F.L. An improved artificial diet for larvae of the seven-spotted ladybird beetle \textit{Coccinella septempunctata} L. (Coleoptera: Coccinellidae). \textit{Chemosphere} 2019, 216, 168–178.
7. Qi, X.; Zhang, L.; Han, Y.; Ren, X.; Huang, J.; Chen, H. De novo transcriptome sequencing and analysis of Coccinella septempunctata L. in non-diapause, diapause and diapause-terminated states to identify diapause-associated genes. BMC Genom. 2015, 16, 1086.

8. Yu, Y.; Cheng, Y.; Zhou, Y.; Li, F. Effects of various components of artificial diets on survival, development and reproduction of Coccinella septempunctata L. (Coleoptera: Coccinellidae). Biocontrol Sci. Technol. 2022, 32, 1122–1131.

9. Jiang, J.; Wang, Y.; Mu, W.; Zhang, Z. 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. 2020, 27, 4178–4189.

10. Casida, J.E. Neonicotinoid Metabolism: Compounds, Substituents, Pathways, Enzymes, Organisms, and Relevance. J. Agric. Food Chem. 2011, 59, 2923–2931.

11. Phogat, A.; Singh, J.; Kumar, V.; Malik, V. Toxicity of the acetamiprid insecticide for mammals: A review. Environ. Chem. Lett. 2022, 20, 1453–1478.

12. Jeschke, P.; Nauen, R.; Schindler, M.; Elbert, A. Overview of the status and global strategy for neonicotinoids. J. Agric. Food Chem. 2011, 59, 2897–2908.

13. Yang, Y.; Ma, S.; Liu, F.; Wang, Q.; Wang, X.; Hou, C.; Wu, Y.; Gao, J.; Zhang, L.; Liu, Y. Acute and chronic toxicity of acetamiprid, carbaryl, cypermethrin and deltamethrin to Helicoverpa armigera S. and sublethal effects on predator voracity and on functional response to the whitefly. J. Pest Sci. 2014, 87, 711–719.

14. Horgan, A. Acetamiprid: Novel neonicotinoid systemic insecticide. Asp. Appl. Biol. 2007, 83, 47.

15. Xu, X.; Guo, Y.; Wang, L.; He, K.; Guo, Y.; Wang, X.; Gunasekaran, S. Hapten-grafted programmed probe as a corecognition element for a competitive immunosensor to detect acetamiprid residue in agricultural products. J. Agric. Food Chem. 2018, 66, 7815–7821.

16. Morrissey, C.A.; Mineau, P.; Devries, J.H.; Sanchez-Bayo, F.; Liess, M.; Cavallaro, M.C.; Liber, K. Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: A review. Environ. Int. 2015, 74, 291–303.

17. Elbert, A.; Haas, M.; Springer, B.; Thielert, W.; Nauen, R. Applied aspects of neonicotinoid uses in crop protection. Pest Manag. Sci. 2010, 66, 1099–1105.

18. Cloyd, R.A.; Bethke, J.A. Impact of neonicotinoid insecticides on natural enemies in greenhouse and interiorscape environments. Pest Manag. Sci. 2011, 67, 3–9.

19. Tirello, P.; Pozzebon, A.; Duso, C. The effect of insecticides on the non-target predatory mite *Kampimodromus aberrans*: Laboratory studies. Chemosphere 2013, 93, 1139–1144.

20. Gentijo, P.C.; Moscardini, V.F.; Michaud, J.P.; Carvalho, G.A. Non-target effects of chlorantraniliprole and thiamethoxam on *Chrysoperla carnea* when employed as sunflower seed treatments. J. Pest Sci. 2014, 87, 711–719.

21. Cheng, S.; Lin, R.; Zhang, N.; Yuan, S.; Zhou, X.; Huang, J.; Ren, X.; Wang, S.; Jiang, H.; Yu, C. Toxicity of six insecticides to predatory mite *Amblyseius cucumeris* (Oudemans) (Acari: Phytoseiidae) in-and off-field. Ecotoxicol. Environ. Saf. 2018, 161, 715–720.

22. Jiang, J.; Liu, X.; Huang, X.; Yu, Z.; Zhang, W.; Fang, X.; Mu, W. Comparative ecotoxicity of neonicotinoid insecticides to three species of *Trichogramma* parasitoid wasps (Hymenoptera: Trichogrammatidae). Ecotoxicol. Environ. Saf. 2019, 183, 109587.

23. Jamil, R.Z.R.; Vandervoort, C.; Wise, J.C. Residual toxicity of insecticides to *Neoseiulus fallacis* (Acari: Phytoseiidae) in apples. J. Econ. Entomol. 2019, 112, 2262–2267.

24. Cabral, S.; Soares, A.O.; Garcia, P. Voracity of *Coccinella undecimpunctata*: Effects of insecticides when foraging in a prey/plant system. J. Pest Sci. 2011, 84, 373–379.

25. Yao, F.L.; Zheng, Y.; Zhao, J.W.; Desneux, N.; He, Y.-X.; Weng, Q.-Y. Lethal and sublethal effects of thiamethoxam on the whitefly predator *Serangium japonicum* (Coleoptera: Coccinellidae) through different exposure routes. Chemosphere 2015, 128, 49–55.

26. He, Y.; Zhao, J.; Zheng, Y.; Desneux, N.; Wu, K. 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*. Ecotoxicology 2012, 21, 1291–1300.

27. Yu, C.; Lin, R.; Fu, M.; Zhou, Y.; Zong, F.; Jiang, H.; Lv, N.; Piao, X.; Zhang, J.; Liu, Y. Impact of imidacloprid on life-cycle development of *Coccinella septempunctata* in laboratory microcosms. Ecotoxicol. Environ. Saf. 2014, 110, 168–173.

28. Desneux, N.; Decourtye, A.; Delpuech, J.M. The sublethal effects of pesticides on beneficial arthropods. Annu. Rev. Entomol. 2007, 52, 81–106.

29. Santos, M.; Santos, R.; Tomé, H.; Barbosa, W.; Martins, G.; Guedes, R.; Oliveira, E. Imidacloprid-mediated effects on survival and fertility of the Neotropical brown stink bug *Euschistus heros*. J. Pest Sci. 2016, 89, 231–240.

30. Holling, C.S. Some characteristics of simple types of predation and parasitism. Can. Entomol. 1959, 91, 385–398.

31. Wang, S.; Qi, Y.; Desneux, N.; Shi, X.; Biondi, A.; Gao, X. Sublethal and transgenerational effects of short-term and chronic exposures to the neonicotinoid nitenpyram on the cotton aphid *Aphis gossypii*. J. Pest Sci. 2017, 90, 389–396.

32. Galvan, T.L.; Koch, R.L.; Hutchison, W.D. Toxicity of indoxacarb and spinosad to the multicolored Asian lady beetle, *Harmonia axyridis* (Coleoptera: Coccinellidae), via three routes of exposure. Pest Manag. Sci. 2006, 62, 797–804.

33. Kianpour, R.; Fathipour, Y.; Kamali, K.; Omkar. Effects of mixed prey on the development and demographic attributes of a generalist predator, *Coccinella septempunctata* (Coleoptera: Coccinellidae). Biocontrol Sci. Technol. 2011, 21, 435–447.

34. Kumar, R.; Kranthi, S.; Nitharwal, M.; Jat, S.; Monga, D. Influence of pesticides and application methods on pest and predatory arthropods associated with cotton. Phytoparasitica 2012, 40, 417–424.
35. Kanawi, E.; Budd, R.; Tjeerdema, R.S. Environmental fate and ecotoxicology of fenpropatrin. Rev. Environ. Contam. Toxicol. 2013, 225, 77–93.
36. Douglas, M.R.; Tooker, J.F. Meta-analysis reveals that seed-applied neonicotinoids and pyrethroids have similar negative effects on abundance of arthropod natural enemies. PeerJ 2016, 4, e2776.
37. Christie, P.T.; Wright, D.J. Activity of abamectin against larval stages of Spodoptera littoralis Boisdruval and Heliothis armigera Hübner (Lepidoptera: Noctuidae) and possible mechanisms determining differential toxicity. Pestic. Sci. 1990, 29, 29–38.
38. Jansen, J.-P. Toxicity of two neonicotinoid insecticides via the food chain for larvae of the two spot ladybird Adalia bipunctata. IOBC-WPRS Bull. Pestic. Benef. Org. 2012, 82, 19–26.
39. Murdoch, W.W.; Briggs, C.J.; Nisbet, R.M. Consumer-Resource Dynamics; Princeton University Press: Princeton, New Jersey, USA, 2003.
40. Xia, J.Y.; Van, D.W.W.; Rabbinge, R. Temperature and Prey Density on Bionomics of Coccinella septempunctata (Coleoptera: Coccinellidae) Feeding on Aphis gossypii (Homoptera: Aphididae) on Cotton. Environ. Entomol. 1999, 28, 307–314.
41. Xiao, D.; Zhao, J.; Guo, X.; Chen, H.; Qu, M.; Zhai, W.; Desneux, N.; Biondi, A.; Zhang, F.; Wang, S. Sublethal effects of imidacloprid on the predatory seven-spot ladybird beetle Coccinella septempunctata. Ecotoxicology 2016, 25, 1782–1793.
42. Afza, R.; Riaz, M.A.; Afzal, M. Sublethal Effect of Six Insecticides on Predatory Activity and Survival of Coccinella Septempunctata (Coleoptera: Coccinellidae) Following Contact with Contaminated Prey and Residues. Gesunde Pflanz. 2020, 72, 77–86.
43. Martinou, A.F.; Seraphides, N.; Stavrinides, M.C. Lethal and behavioral effects of pesticides on the insect predator Macroplus pygmaeus. Chemosphere 2014, 96, 167–173.
44. Jiang, J.; Zhang, Z.; Yu, X.; Ma, D.; Yu, C.; Liu, F.; Mu, W. Influence of lethal and sublethal exposure to clothianidin on the seven-spotted lady beetle, Coccinella septempunctata L. (Coleoptera: Coccinellidae). Ecotoxicol. Environ. Saf. 2018, 161, 208–213.
45. Jiang, J.; Ma, D.; Zhang, Z.; Yu, C.; Liu, F.; Mu, W. Favorable compatibility of nitenpyram with the aphid predator, Coccinella septempunctata L. (Coleoptera: Coccinellidae). Environ. Ence Pollut. Res. 2018, 25, 27393–27401.
46. Courjaret, R.; Lapied, B. Complex intracellular messenger pathways regulate one type of neuronal alpha-bungarotoxin-resistant nicotinic acetylcholine receptors expressed in insect neurosecretory cells (dorsal unpaired median neurons). Mol. Pharmacol. 2001, 60, 80–91.
47. Tan, J.; Galligan, J.J.; Hollingworth, R.M. Agonist actions of neonicotinoids on nicotinic acetylcholine receptors expressed by cockroach neurons. NeuroToxicology 2007, 28, 829–842.
48. Thany, S.H.; Courjaret, R.; Lapied, B. Effect of calcium on nicotine-induced current expressed by an atypical alpha-bungarotoxin-insensitive nAChR2. Neurosci. Lett. 2008, 438, 317–321.
49. Bodereau-Dubois, B.; List, O.; Calas-List, D.; Marques, O.; Communal, P.-Y.; Thany, S.; Lapied, B. Transmembrane Potential Polarization, Calcium Influx, and Receptor Conformational State Modulate the Sensitivity of the Imidacloprid-Insensitive Neuronal Insect Nicotinic Acetylcholine Receptor to Neonicotinoid Insecticides. J. Pharmacol. Exp. Ther. 2012, 341, 326–339.
50. Calas-List, D.; List, O.; Quinchard, S.; Thany, S.H. Calcium pathways such as cAMP modulate clothianidin action through activation of alpha-bungarotoxin-sensitive and -insensitive nicotinic acetylcholine receptors. NeuroToxicology 2013, 37, 127–133.
51. Brunet, J.-L.; Badiou, A.; Belzunces, L. In vivo metabolic fate of [14C]-acetamiprid in six biological compartments of the honeybee, Apis mellifera L. Pest Manag. Sci. 2005, 61, 742–748. https://doi.org/10.1002/ps.1046.