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

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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 LC_{50} 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

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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 imidacloprid 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., Haikou, 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., Hangzhou, 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

LC_5, LC_{10}, and LC_{20} 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]:

$$Na = \frac{a'TN_0}{(1 + a'T_hN_0)} \quad (1)$$

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), N_0 is the prey density, and T_h is the handling time for a predator to catch each prey.

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

$$S = \frac{a'}{(1 + a'T_hN)} \quad (2)$$

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.

Table 1. The 48 h LC_{50} of acetamiprid for *C. septempunctata* within different larval stages.

Larval Stage	Regression Equation	SE [a]	χ^2 [b]	*df* [c]	P [d]	R^2 [e]	LC50 [f] (mg a.i. L^{-1}) [g]	95% Confidence Interval (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	0.898	0.968	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	0.351	0.916	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	0.567	0.932	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	0.934	0.984	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 LC_{10}, the predatory capacity of the 1st instar larvae began to decrease significantly.

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 LC_{20} in the different prey density groups. Predatory capacity was significantly weakened in the LC_5 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 LC_{20} reduced the predation ability of the 3rd instar larvae. When the aphid density was $\geq$100, pesticide treatment at LC_{10} 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 $\geq$ 100, LC_{10} 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, $p < 0.05$).

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	Prey Density (the Number of Aphids Per Tube)	Control	LC_5	LC_{10}	LC_{20}	*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
2nd	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
	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
3rd	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
	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
4th	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
	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 ($p < 0.05$).

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.

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 acetamprid at LC_{20} 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 3. Predatory functional response model and parameters of *C. septempunctata* in different larval stages.

	Treatment	Equation of Predator Functional Response	$R^{2\,a}$	The Rate of Successful Attack (a′)	Handling Time of Predatory (T_h/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
2nd Instar	Control	Na = 0.817N/(1 + 0.00057N)	0.997	0.817	0.0007
	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
3rd Instar	Control	Na = 0.938N/(1 + 0.00019N)	0.999	0.938	0.0002
	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
4th Instar	Control	Na = 0.992N/(1 + 0.00120N)	0.974	0.920	0.0013
	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.

Table 4. Searching efficiency of C. *septempunctata* after exposing to acetamiprid in different larval stages.

Developmental Time	Prey Density (The Number of Aphids Per Tube)	Treatments			
		Control	LC_5	LC_{10}	LC_{20}
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
2nd Instar	5	0.815	0.813	0.810	0.808
	10	0.805	0.751	0.747	0.744
	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
3rd Instar	30	0.934	0.931	0.928	0.924
	50	0.922	0.908	0.903	0.898
	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
4th Instar	50	0.868	0.821	0.779	0.741
	100	0.707	0.867	0.807	0.755
	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.

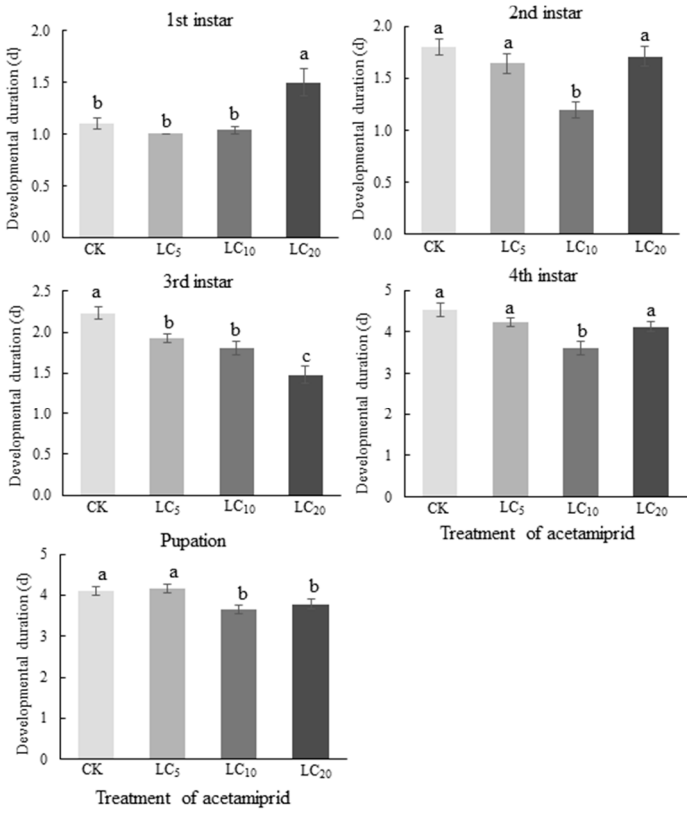

Figure 1. Effects of acetamiprid on *C. septempunctata* developmental duration within the 1st instar larval stage (mean ± SE). The same column followed by different letters is significantly different based on ANOVA using Tukey's LSD test ($p < 0.05$).

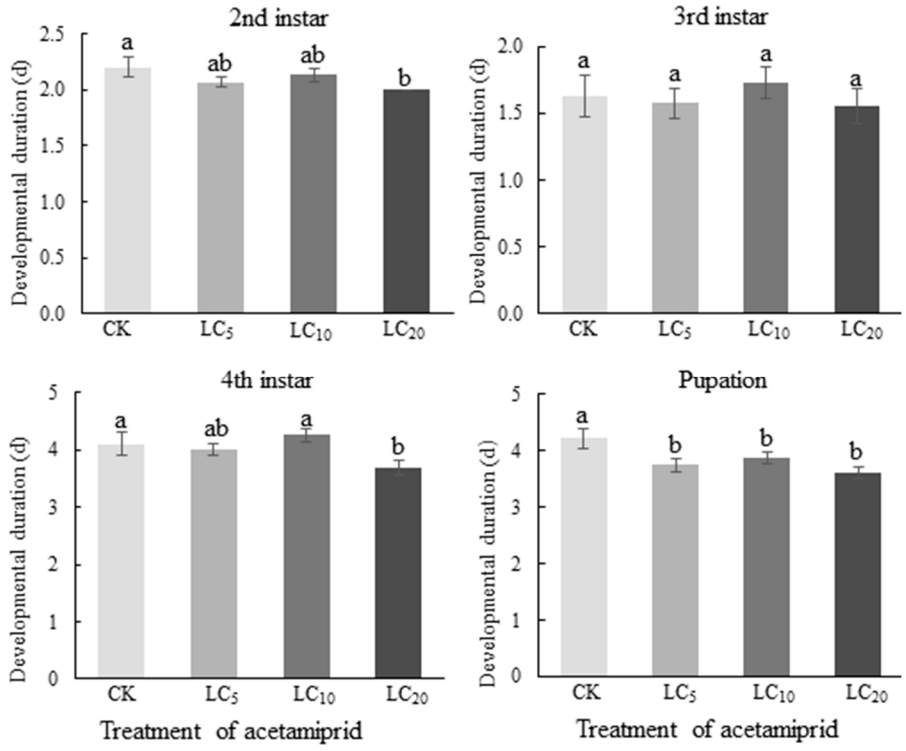

Figure 2. Effects of acetamiprid on developmental duration of *C. septempunctata* within 2nd instar larval stage (mean ± SE). The same column followed by different letters is significantly different based on ANOVA using Tukey's LSD test ($p < 0.05$).

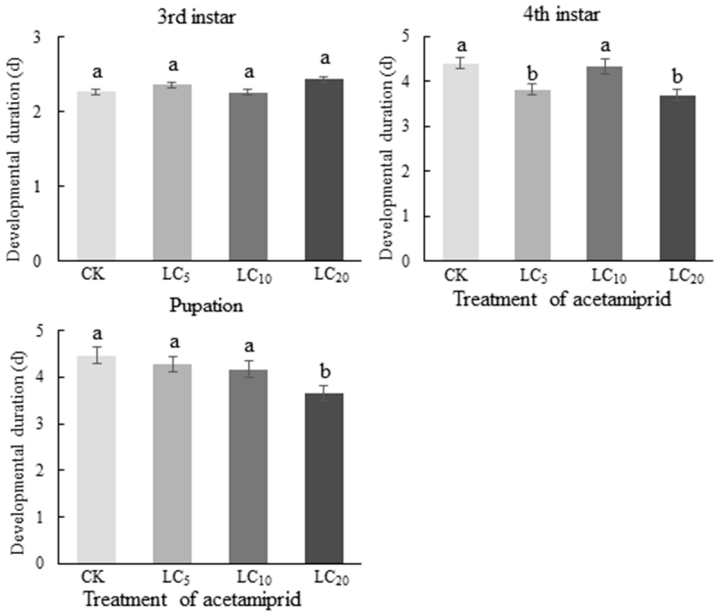

Figure 3. Effects of acetamiprid on developmental duration of *C. septempunctata* within the 3rd instar larval stage (mean ± SE). The same column followed by different letters is significantly different based on ANOVA using Tukey's LSD test ($p < 0.05$).

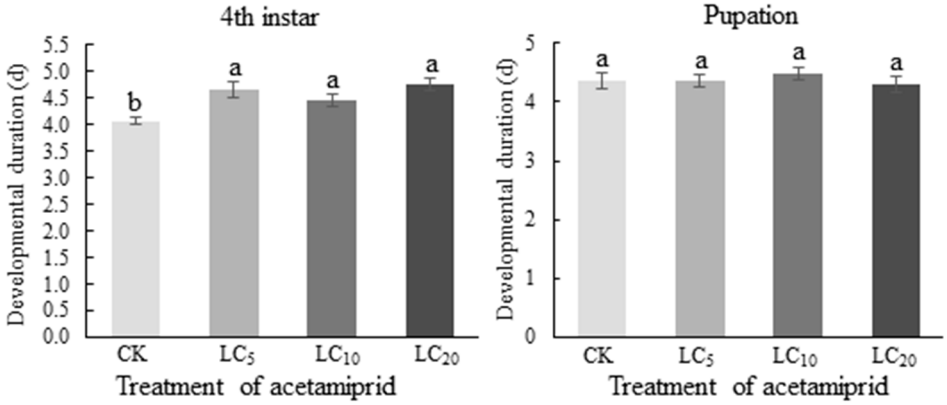

Figure 4. Effects of acetamiprid on developmental duration of *C. septempunctata* within 4th instar larval stage (mean ± SE). The same column followed by different letters is significantly different based on ANOVA using Tukey's LSD test ($p < 0.05$).

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 LC_{20} 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 LC_5, LC_{10}, and LC_{20} 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 LC_{20} 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 LC_{10}, 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 LC_{20}. 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 LC_{30}, the predatory efficiency of both adult and larval *C. septempunctata* significantly decreased [42].

Developmental experiments indicated that acetamiprid exposure at LC_{20} 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 LC_{20} 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 *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 *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 *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 *C. septempunctata*.

5. Conclusions

The second instar larvae of 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 C. *septempunctata* at LC_{10} and LC_{20}. 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 *C. septempunctata*.

The neonicotinoid insecticide acetamiprid showed a high risk for 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.

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