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Selectivity and sublethal effects of some frequently-used biopesticides on the predator *Cyrtorhinus lividipennis* Reuter (Hemiptera: Miridae)

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Abstract

The green miridbug, *Cyrtorhinus lividipennis*, is an important predator of the rice brown planthopper, *Nilaparvata lugens*. In this study, the selective toxicity of seven commercial biopesticides for *C. lividipennis* was examined under laboratory conditions; abamectin was the most selective to *C. lividipennis*, followed by matrine and azadirachtin. Veratridine, rotenone, *Bacillus thuringiensis* and *Beauveria bassiana* showed less selectivity for *C. lividipennis*. Subsequently, matrine, abamectin and azadirachtin were selected for sublethal assessments with respect to *C. lividipennis* due to their high toxicities to *N. lugens*. *C. lividipennis* treated with sublethal concentrations (LC_{10} and LC_{20}) of the three biopesticides could distinguish volatiles released from healthy and *N. lugens*-infested plants indicating that the biopesticides tested did not affect the foraging ability of surviving miridbugs. Azadirachtin decreased the consumption capability of *C. lividipennis* when the densities of *N. lugens* were 20, 30, 40 and 50 insects per vial. Sublethal concentration treatment did not impact the pre-oviposition period or egg hatchability of *C. lividipennis*. However, the fecundity of *C. lividipennis* exposed to azadirachtin and abamectin increased by 27–41% compared to the untreated individuals. In summary, abamectin or matrine together with *C. lividipennis* could be considered an effective, sustainable pest management strategy for rice.

Keywords: biopesticides, *Cyrtorhinus lividipennis*, selective toxicity, sublethal effects

1. Introduction

Pesticides are used extensively in agriculture because they are inexpensive, easy and effective means of managing pests, diseases and weeds (Stanley and Preetha 2016).

However, the consistent and injudicious application of chemical pesticides leads to the development of resistance in insect pests, the destruction of beneficial organisms, and increases of pesticide residue, which can endanger human health and disrupt the ecological balance in the living biome (Rizvi *et al.* 2009). Biopesticides derived from natural resources are attracting global attention because they are environmentally friendly and less toxic to humans (Qi *et al.* 2001). A highly successful biopesticide is *Bacillus thuringiensis*, which produces crystalline proteins that are toxic to certain insect species among the orders Lepidoptera, Diptera, and Coleoptera (Schnepf *et al.* 1998). More recently, biopesticides have been used to control rice pests. For example, extracts from neem seed kernels *Azadirachta indica* A. Juss (Sapindales: Meliaceae) and *Vitex negundo* L. (Lamiales: Verbenaceae) leaves suppressed both feeding

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and larval growth of the rice leaffolder *Cnaphalocrocis medinalis* (Guenée) (Lepidoptera: Pyralidae) (Nathan et al. 2005). The plant-derived alkaloid veratridine and the fungus *Beauveria bassiana* were used to efficiently manage the population of brown planthoppers *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae) in rice fields (Hu et al. 2010).

Entomophages, namely, parasitoids and predators, are natural enemies of insect pests and play important roles in pest control in agroecosystems. Biopesticides have been generally considered safe compared to synthetic chemical insecticides (Raguraman and Kannan 2014). However, adverse effects of biopesticides on natural enemies have been reported. For example, spinosad, a biopesticide derived from the bacterium *Saccharopolyspora spinosa*, exhibited high level of toxicity against adults of parasitoids *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) (Sattar et al. 2011), *Hyposoter didymator* (Thunberg) (Hymenoptera: Ichneumonidae) (Medina et al. 2008) and *Bracon nigricans* (Hymenoptera: Braconidae) (Biondi et al. 2013). In addition to direct toxicity, sublethal effects of biopesticides on natural enemies have also been reported. Sublethal effects are physiological or behavioral impacts on individuals surviving pesticide exposure (Desneux et al. 2007) and include changes in parasitism rate, longevity, egg viability, and consumption rate or behavior (Ruberson et al. 1998). For example, the biocontrol activity of *B. nigricans* females was significantly impacted by the neurotoxic insecticides of emamectin benzoate and abamectin when used in tomatoes to control *Tuta absoluta* (Lepidoptera: Gelechiidae) (Biondi et al. 2013). When treated with neem oil, changes in cocoon spinning and pronounced cytotoxic effects in the midgut of *Ceraeochrysa claveri* (Neuroptera: Chrysopidae), a common polyphagous predator in neotropical agroecosystems were observed (Scudeler and dos Santos 2013; Scudeler et al. 2016). However, the impact of biopesticides on beneficial arthropods distributed in rice fields remains unclear.

The green miridbug, *Cyrtorhinus lividipennis* Reuter (Hemiptera: Miridae) is widely distributed in rice fields in Southeast Asia and the Pacific (Heinrichs 1994). It feeds mainly on the eggs and young nymphs of rice planthoppers, which include *N. lugens*, *Sogatella furcifera* (Horvath), *Laodelphax striatella* (Fallén) (Homoptera: Delphacidae), and the green rice leafhopper *Nephotettix virescens* Distant (Homoptera: Euscelidae) (Lou and Cheng 1996). *C. lividipennis* plays a significant role in natural management of rice pests, and the conservation of this predator is extremely important. Unfortunately, some organophosphates, carbamate, phenylpyrazole and neonicotinoids insecticides have been reported to be toxic to *C. lividipennis* (Lakshmi et al. 2010; Preetha et al. 2010). Disrupted orientation behavior due to a pyrethroid (deltamethrin) (Zhang et al. 2015) and

reduced predation caused by an anthranilic diamide (chlorantraniliprole) (Wang et al. 2012) have also been reported for *C. lividipennis*.

In view of the importance of *C. lividipennis* as a biological control agent for rice pests and the scarcity of information regarding the impact of biopesticides on this predator, we examined selectivity and sublethal effects of commercial biopesticides on *C. lividipennis* in the laboratory. We first determined the acute toxicity of seven biopesticides for nymphs of *N. lugens* and female adults of *C. lividipennis* and then compared the insecticidal selectivity ratio of the biopesticides. Subsequently, we determined sublethal concentrations of three selected biopesticides, and investigated their sublethal on *C. lividipennis*. Three aspects of sublethal effects including orientation behavior, predation, and reproduction were examined.

2. Materials and methods

2.1. Rice, insects and insecticides

The hybrid rice line, II-You 118 susceptible to *N. lugens*, was utilized for raising insects and for the experiments described below. Rice seeds were germinated indoors and transplanted to plastic pots (20 cm diameter×15 cm height) as 30-day old seedlings. Potted plants containing three hills (3–4 plants per hill) were grown in the greenhouse at (28±2)°C with a minimum day length of 14 h. Rice plants were used in the experiments approximately 30–40 days after transplanting.

N. lugens was originally obtained as a laboratory strain maintained at China National Rice Research Institute (CNRRI), Fuyang, Zhejiang, China. Laboratory cultures of *C. lividipennis* were originally collected from rice fields in Yangzhou (32°23'N, 119°26'E). *N. lugens* and *C. lividipennis* were reared on II-You 118 as described by Lu et al. (2017). Rice plants and insects were maintained in a growth chamber at (28±2)°C with a 14 h:10 h (light:dark) photoperiod and 70–80% RH. Adults of *C. lividipennis* were used in experiments within 24 h after emergence.

Seven insecticides belonging to three classes (botanical, microbial, and agricultural antibiotics, see Table 1) were evaluated. These included matrine (1.3% AS) (Shangxi Dewei Biochemical Co., Ltd., Shangxi, China), veratridine (0.5% SL) (Hebei Fuji Biological Technology Co., Ltd., Hebei, China), azadirachtin (0.3% EC) (Chengdu Lvjin Biological Technology Co., Ltd., Chengdu, China), rotenone (6% ME) (Beijing Sanpu Baicao Green Plant Preparation Co., Ltd., Beijing, China), *B. thuringiensis* (8 000 IU µL⁻¹ SC) (Hebei Qingyuan Pesticide Co., Ltd., Hebei, China), *B. bassiana* (15 billion spores g⁻¹ WP) (Jiangxi Tianren Ecological Co., Ltd., Jiangxi, China), and abamectin (1.8% EW) (Zhejiang

Table 1 Median lethal concentration (LC₅₀) of biopesticides to *Nilaparvata lugens* and *Cyrtorhinus lividipennis* and risk assessment of tested biopesticides on *C. lividipennis*

Biopesticides	<i>N. lugens</i>				<i>C. lividipennis</i>				Selectivity ratio	Selectivity Category
	Regression equation	Related coefficient	LC ₅₀ (95% confidence interval, mg a.i. L ⁻¹)	Regression equation	Related coefficient	LC ₅₀ (95% confidence interval, mg a.i. L ⁻¹)	Regression equation	Related coefficient		
Botanical pesticides										
Matrine	y=6.4644+1.7085x	r=0.9952	0.14 (0.11–0.17)	y=5.1341+1.8812x	r=0.9999	0.85 (0.66–1.04)			6.11	Selective
Veratridine	y=0.3625+2.3396x	r=0.9859	95.98 (83.69–109.86)	y=1.8441+2.4596x	r=0.9899	19.19 (16.01–22.54)			0.20	Non-selective
Azadirachtin	y=1.7253+1.7751x	r=0.9880	69.95 (59.53–81.94)	y=-2.0183+2.9437x	r=0.9928	242.20 (210.25–281.67)			3.46	Selective
Rotenone	y=0.5994+2.1526x	r=0.9900	110.75 (95.57–127.78)	y=1.9781+1.9922x	r=0.9981	32.87 (26.98–39.59)			0.30	Non-selective
Microbial pesticides										
<i>Bacillus thuringiensis</i>	y=-2.2483+2.5449x	r=0.9996	728.29 (641.56–830.92)	y=0.5617+1.8687x	r=0.9997	237.20 (195.05–291.96)			0.33	Non-selective
<i>Beauveria bassiana</i>	y=0.4399+1.7497x	r=0.9847	403.90 (337.75–475.21)	y=1.9185+2.1126x	r=0.9923	28.75 (23.28–34.47)			0.07	Non-selective
Agricultural antibiotics										
Abamectin	y=6.2756+2.1249x	r=0.9921	0.25 (0.22–0.29)	y=1.7448+1.9137x	r=0.9954	50.24 (41.14–60.93)			200.16	Selective

Qianjiang Biochemical Co., Ltd., Zhejiang, China).

2.2. Establishment of concentration-mortality relationships

The toxicities of biopesticides to *N. lugens* and *C. lividipennis* were determined in the laboratory using the rice-stem dipping method described by Zhuang (2000). For each biopesticide, a preliminary test was conducted to determine the concentration necessary to cause 10–90% mortality in *N. lugens* and *C. lividipennis*. The biopesticides were diluted to the appropriate concentrations by adding tap water, and tap water served as the control in all experiments. Several rice stems were dipped into glass cups (7 cm diameter×12 cm high) containing 200 mL of each biopesticide concentration; rice stems were immersed for 30 s. When treated rice stems were dry, 15 third instar nymphs of *N. lugens* or female adults of *C. lividipennis* were introduced into the a vial (7 cm×12 cm) containing rice stems and maintained at (28±2)°C with a 14-h photoperiod at 70–80% RH. Twenty to 30 *N. lugens* nymphs were replenished daily on rice stems as a food source for *C. lividipennis*. Two days later, the numbers of dead brown planthoppers and predators were counted and recorded. Moribund insects lacking obvious movements were considered dead. For each test, six to seven biopesticide concentrations were evaluated. Approximately 60 *C. lividipennis* and 90 *N. lugens* individuals were examined for each biopesticide concentration. A concentration-mortality relationship was established by exposing *N. lugens* or *C. lividipennis* to serial concentrations of each biopesticide, and the LC₁₀, LC₂₀ and LC₅₀ were estimated by regression analysis.

2.3. Selective toxicity

The toxicity of biopesticides was evaluated by the selectivity ratio, which was calculated as described by Preetha *et al.* (2010) using the following formula:

$$\text{Selectivity ratio} = \text{LC}_{50} \text{ of } C. \textit{lividipennis} / \text{LC}_{50} \text{ of } N. \textit{lugens}$$

Values of 1 and <1 indicated that a biopesticide was more toxic to *C. lividipennis* than *N. lugens* (non-selective), and values >1 indicated that the biopesticide was less toxic to *C. lividipennis* than *N. lugens* (selective).

2.4. Sublethal effects

The LC₁₀ and LC₂₀, which represent sublethal concentrations, were estimated by regression analysis. Female adults of *C. lividipennis* were exposed to sublethal biopesticide concentrations (LC₁₀ and LC₂₀) for 48 h and surviving miridbugs were used to investigate sublethal effects including orientation behavior, predation and reproduction. *C. lividipennis* exposed to tap water was used as the control.

Effects of biopesticides on the orientation behavior of *C. lividipennis*

The olfactory responses of surviving *C. lividipennis* females exposed to sublethal biopesticide concentrations (LC₁₀ and LC₂₀) for 48 h were measured in a two-choice H-shaped olfactometer as described by Zhang (2015). The experiments were conducted between 08:30 and 17:30 in a climate-controlled room maintained at (28±2)°C, (70–80)% RH, and a 14-h

photoperiod. In these assays, volatile odors were contained in two glass arms (12 cm diameter×30 cm high), which were connected by a glass tube (6 cm diameter×15 cm long). The ends of the glass tube were covered with nylon mesh, and the central section contained a small opening (1 cm in diameter) for release of *C. lividipennis*.

Two sources of volatile odors were included in each test. One source consisted of rice seedlings infested with *N. lugens* at a rate of 100 gravid females per 10 plants for one day; the other source consisted of healthy rice plants.

To prepare *N. lugens*-infested plants as an odor source, rice seedlings were rinsed with running water and thinned to 10 plants per pot. Each rice seedling was individually inoculated with 10 gravid *N. lugens* females. After a 24-h exposure period, plants infested with 100 females were used as an odor source to represent *N. lugens*-infested plants in subsequent assays. Non-inoculated rice seedlings were also thinned to 10 plants per pot, and these served as a source of odors from healthy rice plants.

Surviving females ($n=20$) exposed to sublethal concentrations of bioinsecticides and starved for 12 h were then introduced into the glass tube of the olfactometer, which was covered with a black cloth to exclude light (Rapusas *et al.* 1996). After 3 h, the black cloth was gently removed, and the number of predators distributed in each half of the glass tube was calculated. Predators distributed within the 1 cm diameter release area were considered as 'no response' individuals. A group containing 20 surviving *C. lividipennis* female was considered as a replicate, and each treatment had five replications.

Effects of biopesticides on the predatory capacity of *C. lividipennis* Three rice plants, with their roots wrapped in absorbent cotton, were placed into vials (5 cm diameter×20 cm high), and 3rd instar *N. lugens* nymphs were transferred into the vials at densities of 5, 10, 20, 30, 40, and 50 per vial. One surviving *C. lividipennis* female, which had been treated with sublethal biopesticides (LC_{10} and LC_{20}) for 2 days and starved for 12 h, was introduced into each vial. One day later, the predator was removed, and the dead, wizened nymphs consumed by the predator were counted. The experiment was conducted in a climate-controlled room at $(28\pm 2)^{\circ}\text{C}$, with a 14-h photoperiod and 70–80% RH. One predator was considered as a replicate, and each treatment had 10 replications.

Effects of biopesticides on the reproduction of *C. lividipennis* Newly emerged female adults of *C. lividipennis* were exposed to sublethal biopesticide concentrations (LC_{10} and LC_{20}) as described above. After 48 h, a surviving female and an untreated male were paired and transferred to glass cups containing rice stems (15 cm) and 3rd instar *N. lugens* nymphs. The paired *C. lividipennis* were incubated

15 days at $(28\pm 2)^{\circ}\text{C}$ with a 14 h:10 h photoperiod (light:dark) for egg deposition. Rice stems and *N. lugens* nymphs were renewed daily, and an untreated female was used as a control. The nymphs of *C. lividipennis* that hatched from rice stems were recorded and removed daily until the experiment was terminated, and the number of unhatched eggs was counted using a microscope. The total number of eggs laid per female was calculated as the sum of nymphs and unhatched eggs. Egg hatchability was defined as the ratio of the number of nymphs to the total number of eggs. The pre-oviposition period was defined as the interval from adult emergence to the onset of oviposition. Therefore, in our study, the pre-oviposition period included the time from the female being paired to the onset of oviposition and the 2 days of biopesticide exposure. One paired predator was considered as a single replicate, and each treatment consisted of 20 replicates (20 mating pairs).

2.5. Statistical analysis

In the establishment of concentration-mortality relationships, the mortality values obtained in the control treatments were used to correct the mortality in each biopesticide treatment as described by Abbott (1987). A regression model for mortality was developed using the method described by Chen (1991) and was based on the logarithmic transformation of concentrations and probit transformation of mortalities (Finney 1971). The choice of surviving *C. lividipennis* for two odors was analyzed using a *t*-test. Data for predation quantity, the pre-oviposition period, number of eggs laid by per female, and egg hatchability were analyzed by analysis of variance (ANOVA). Multiple comparisons of the means were evaluated using Tukey's honestly significant difference test. All analyses were performed using Statistica (Tang *et al.* 2013).

3. Results

3.1. Selective toxicity

The LC_{50} values of seven biopesticides for *N. lugens* and *C. lividipennis* are shown in Table 1. Substantial differences in the toxicity of biopesticides to *N. lugens* and *C. lividipennis* were observed. Among the biopesticides tested, matrine was the most toxic to *N. lugens*, followed by abamectin and azadirachtin. Veratridine, rotenone, *B. bassiana* and *B. thuringiensis* were less toxic to *N. lugens*. Matrine was also the most toxic biopesticide for *C. lividipennis*, followed by veratridine, *B. bassiana*, rotenone and abamectin; *B. thuringiensis* and azadirachtin showed less toxicity for the predator. Based on the selectivity ratio, abamectin was

the most selective biopesticide for *C. lividipennis*, followed by matrine and azadirachtin, whereas veratridine, rotenone, *B. thuringiensis* and *B. bassiana* were found to be harmful to *C. lividipennis* (Table 1).

3.2. Sublethal effects

Determination of sublethal concentrations Since matrine, abamectin and azadirachtin were more toxic to *N. lugens*, we assessed their sublethal effects on *C. lividipennis*. Regression analysis indicated that the concentrations of matrine causing 10 and 20% mortality were 0.18 and 0.30 mg a.i. L⁻¹, respectively; thus, 0.20 and 0.30 mg a.i. L⁻¹ were chosen to represent the LC₁₀ and LC₂₀ for matrine (Appendix A). The estimated concentrations of abamectin causing 10 and 20% mortality were 10.75 and 18.25 mg a.i. L⁻¹, respectively, and 11 and 18 mg a.i. L⁻¹ were used as the LC₁₀ and LC₂₀. For azadirachtin, 90 and 125 mg a.i. L⁻¹ were chosen to represent the LC₁₀ and LC₂₀, respectively (Appendix A).

Effects of sublethal concentrations of biopesticides on the orientation behavior of *C. lividipennis* For matrine, the exposed and control predators did not significantly differ in their orientation behaviors (Table 2). The number of matrine-exposed *C. lividipennis* (LC₁₀ and LC₂₀) and the unexposed controls orienting to *N. lugens*-infested plants was greater than those to healthy rice plants (Table 2). These results indicated that both pesticide-exposed and control predators could distinguish volatiles released from *N. lugens*-infested and healthy plants (Table 2). Similarly, in the other two treatments, odors from *N. lugens*-infested plants were more attractive to the pesticide-exposed and control predators than those emanating from healthy rice plants (Table 2). Our results indicated that sublethal concentrations of biopesticides had no negative effects on the foraging ability of surviving predators.

Effects of sublethal concentrations of biopesticides on the predation of *C. lividipennis*

Sublethal concentrations of matrine had little effect on the predatory capacity of *C. lividipennis* when the densities of *N. lugens* were 5, 10, 20, 30, 40 and 50 per vial (Fig. 1-A; Table 3). Similarly, sublethal concentrations of abamectin did not cause significant changes in consumption by treated predators (Fig. 1-B; Table 3). However, azadirachtin decreased the consumption ability of *C. lividipennis* when the densities of *N. lugens* were greater than or equal to 20 per vial. For example, the numbers of *N. lugens* nymphs (at density of 20 per vial) consumed by *C. lividipennis* in the LC₁₀ and LC₂₀ azadirachtin treatments were 2.20±0.29 and 2.00±0.26 per vial, respectively, which was 33 and 39% lower than the control (3.30±0.21 per vial). Significant reductions in mean consumption by azadirachtin-treated *C. lividipennis* were also observed at densities of 30, 40 and 50 per vial (Fig. 1-C; Table 3).

Effect of sublethal concentrations of biopesticides on the reproduction of *C. lividipennis*

Exposure of *C. lividipennis* to the two sublethal concentrations of matrine had no influence on the pre-oviposition period when compared to the control group ($F=0.11$, $df=2$, 57, $P=0.8982$) (Fig. 2-A). Moreover, sublethal concentrations of abamectin and azadirachtin had no significant effect on the pre-oviposition period when compared to control groups (abamectin, $F=0.93$, $df=2$, 57, $P=0.3994$; azadirachtin, $F=0.64$, $df=2$, 57, $P=0.5296$) (Fig. 2-A).

Sublethal concentrations of matrine (LC₁₀ and LC₂₀) did not affect the fecundity of *C. lividipennis* ($F=0.60$, $df=2$, 57, $P=0.5533$). However, sublethal concentrations of abamectin significantly increased the fecundity of *C. lividipennis* (Fig. 2-B). The numbers of eggs laid by *C. lividipennis* in the LC₁₀ and LC₂₀ treatments were 91.8±7.0 and 93.2±4.7 eggs per female, respectively, which were 39 and 41% higher than the control (66.3±3.0 eggs per female) ($F=8.53$, $df=2$, 57, $P=0.0006$).

Table 2 Behavioral responses of surviving *Cytorhinus lividipennis* treated with sublethal insecticide concentrations of biopesticides to volatiles released from rice plants infested with *Nilaparvata lugens*

Biopesticide	Treatment ¹⁾	Amount of <i>C. lividipennis</i> preferring to different odors		Amount of no-response <i>C. lividipennis</i>	t-test
		<i>N. lugens</i> -infested plants	Healthy rice plants		
Matrine	Control	17.00±0.32	2.40±0.24	0.60±0.40	36.50**
	LC ₁₀	17.00±0.32	2.60±0.40	0.40±0.24	28.24**
	LC ₂₀	17.00±0.32	2.60±0.24	0.40±0.24	36.00**
Abamectin	Control	17.00±0.32	2.40±0.24	0.60±0.24	36.50**
	LC ₁₀	17.00±0.55	2.80±0.49	0.20±0.20	19.32**
	LC ₂₀	16.80±0.37	2.80±0.37	0.40±0.24	26.46**
Azadirachtin	Control	17.00±0.32	2.40±0.24	0.60±0.24	36.50**
	LC ₁₀	14.40±0.24	4.80±0.37	0.80±0.37	21.47**
	LC ₂₀	13.40±0.40	6.00±0.32	0.60±0.40	14.51**

¹⁾ Control indicates *C. lividipennis* exposed only to tap water; LC₁₀ and LC₂₀ for each biopesticide were listed in Appendix A.

Data are mean±SE. ** indicates significant differences between the insecticide treatment and corresponding control at $\alpha=0.01$ level, $df=8$, $t=3.355$.

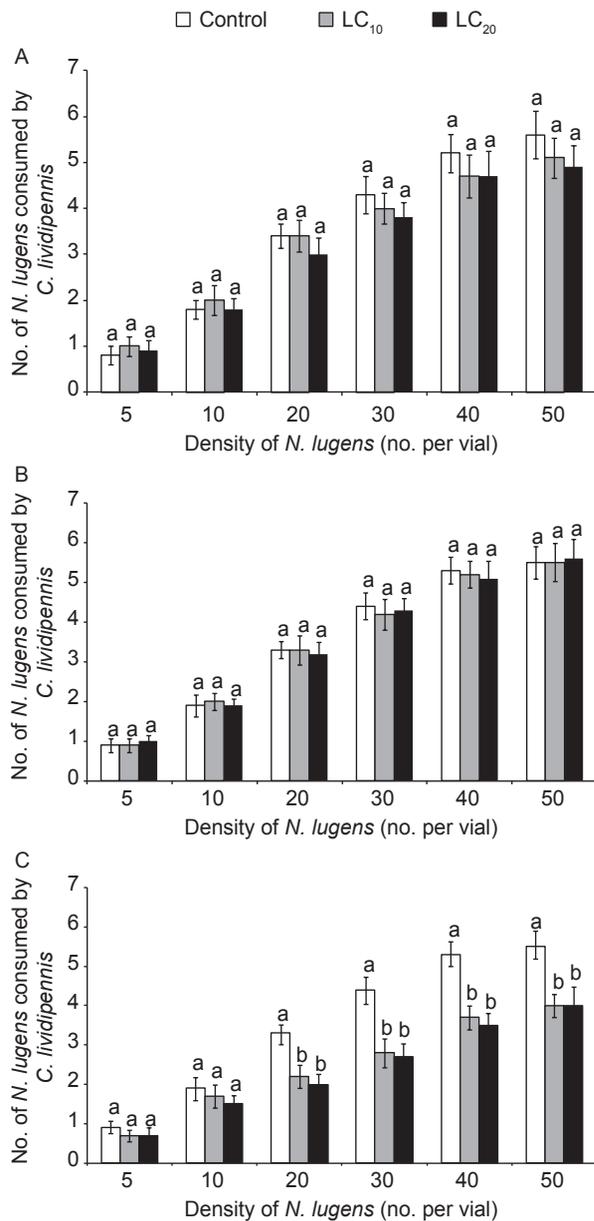


Fig. 1 Mean consumption of *Nilaparvata lugens* by *Cyrtorhinus lividipennis* in response to sublethal concentrations of matrine (A), abamectin (B), and azadirachtin (C). Data are mean±SE. Different letters indicate significant differences between the insecticide treatment and its untreated control within the same density of *N. lugens* ($P < 0.05$, Tukey's honestly significant difference test).

Sublethal concentrations of azadirachtin also significantly stimulated the fecundity in *C. lividipennis* increasing by 27–31% ($F=9.92$, $df=2$, 57 , $P=0.0002$) (Fig. 2-B).

Matrine, abamectin and azadirachtin had no significant sublethal effects on the egg hatchability of *C. lividipennis* (matrine, $F=0.10$, $df=2$, 57 , $P=0.9090$; abamectin, $F=0.95$, $df=2$, 57 , $P=0.3919$; and azadirachtin: $F=1.73$, $df=2$, 57 , $P=0.1895$) (Fig. 2-C).

Table 3 Statistics from the analysis for the predatory capacities of surviving *Cyrtorhinus lividipennis* treated with sublethal concentrations of biopesticides

Biopesticide	Density of <i>Nilaparvata lugens</i> (no. per vial)	Statistics
Matrine	5	$F=0.22$, $df=2$, 27 , $P=0.8071$
	10	$F=0.19$, $df=2$, 27 , $P=0.8301$
	20	$F=0.50$, $df=2$, 27 , $P=0.6120$
	30	$F=0.51$, $df=2$, 27 , $P=0.6077$
	40	$F=0.35$, $df=2$, 27 , $P=0.7060$
	50	$F=0.56$, $df=2$, 27 , $P=0.5753$
Abamectin	5	$F=0.12$, $df=2$, 27 , $P=0.8915$
	10	$F=0.07$, $df=2$, 27 , $P=0.9370$
	20	$F=0.04$, $df=2$, 27 , $P=0.9629$
	30	$F=0.08$, $df=2$, 27 , $P=0.9196$
	40	$F=0.07$, $df=2$, 27 , $P=0.9291$
	50	$F=0.02$, $df=2$, 27 , $P=0.9839$
Azadirachtin	5	$F=0.40$, $df=2$, 27 , $P=0.6771$
	10	$F=0.55$, $df=2$, 27 , $P=0.5811$
	20	$F=7.48$, $df=2$, 27 , $P=0.0026$
	30	$F=7.65$, $df=2$, 27 , $P=0.0023$
	40	$F=9.84$, $df=2$, 27 , $P=0.0006$
	50	$F=4.77$, $df=2$, 27 , $P=0.0169$

4. Discussion

The present study demonstrated that abamectin, matrine and azadirachtin showed a high selectivity ratio and favored survival of *C. lividipennis* (Table 1). Abamectin, an antibiotic produced by the soil bacterium, *Streptomyces avermitilis*, was used to control mites, leafminers, suckers, beetles and fire ants (Copping and Duke 2007) in trees, herbs, vegetables and some annual crops including rice (Bai and Ogbourne 2016). Previous studies indicated that abamectin was harmless to many parasitoids including *Ageniaspis citricola* Longvinovskaya (Hymenoptera: Encyrtidae) (Morais et al. 2016), *B. nigricans* (Biondi et al. 2013) and *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) (Lira et al. 2015). However, abamectin was toxic to the generalist predator *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) (Biondi et al. 2012). Abamectin exhibits some species-specific selectivity, and it was notably less toxic to *C. lividipennis* vs. *N. lugens* in our study. Such selectivity was probably due to differences in the rate of biopesticide uptake by these two species, with *C. lividipennis* assimilating pesticide primarily through the cuticle by contact rather than by contact and feeding, which was the case for *N. lugens*. Furthermore, abamectin has a short half-life (<24 h) on the plant surface (Bai and Ogbourne 2016); thus, the rapid adsorption of pesticides by the foliage would significantly increase the amount available for *N. lugens* uptake.

Matrine is an alkaloid that occurs abundantly in the roots of *Sophora* spp. (Fabaceae) (Mao and Henderson 2007). Du et al. (2004) showed that matrine perturbed

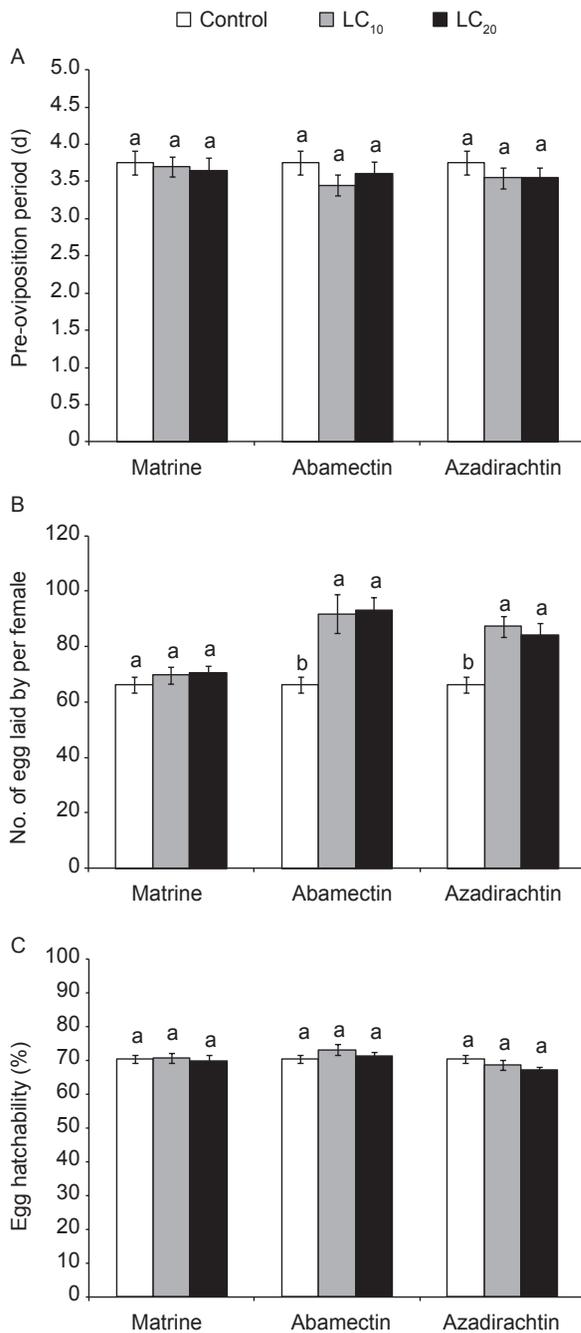


Fig. 2 Effect of sublethal concentrations of biopesticides on the reproduction of *Cyrtorhinus lividipennis*. A, pre-oviposition period. B, number of eggs laid per female. C, egg hatchability. Data are mean±SE. Bars with different letters represent means that differ significantly at $P=0.05$.

sodium channels in the insect nervous system. More recently, matrine has been used in isolation or in mixtures with other botanical extracts and synthetic pesticides for the control of termites, aphids, leafhoppers, caterpillars, mites, fungi, bacteria and nematodes in vegetables, fruits, flowers and teas (Zanardi et al. 2015). In addition to acute

toxicity, matrine promoted an antifeedant effect against the Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) (Mao and Henderson 2007), and the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) (Bakr et al. 2012). One explanation for the favorable selectivity ratio of matrine for *C. lividipennis* was a potential antifeedant effect that is specific for *N. lugens*; thus, *C. lividipennis* consumed the prey (*N. lugens*) rarely contaminated by matrine.

Azadirachtin-based neem products have emerged as prominent plant-based biopesticides. The non-target effects of azadirachtin on beneficial arthropods has received considerable attention. Recently, Raguraman and Kannan (2014) reviewed more than 230 publications and concluded that neem products have slightly to moderately deleterious effects on parasitoids, predators and honeybees. Azadirachtin was also shown to be harmless to the survival of predatory bug, *O. laevigatus* (Biondi et al. 2012). Azadirachtin-based products act by inhibiting the production of juvenile hormones and ecdysone; these compounds are responsible for insect growth and may cause high mortality rates when applied to immature insects (Ghazawy et al. 2010). Therefore, we speculate that azadirachtin showed a high selectivity ratio because it functions to disrupt molting, which was not a factor in our experiments using adult *C. lividipennis*. The effect of these biopesticides on nymphs of the predator needs further study. Low toxicities of the above mentioned biopesticides to the predator may also be related to acting mechanisms of the biopesticides and sensitivities of targets (acted by biopesticides) in insects. Further study seemed to be necessary to confirm this assumption.

Plants respond to herbivore feeding by producing unique blends of volatile, organic compounds that are important cues used by predators and parasitoids to locate preys or hosts (Dicke et al. 1990; Turlings et al. 1990). Numerous studies have demonstrated that predators and parasitoids can discriminate odors from healthy and herbivore-infested plants (Rijk et al. 2013; Vet and Dicke 1992). However, the orientation behavior of natural enemies can be disrupted by sublethal concentrations of insecticides, which may adversely affect the performance of the carnivore (Liu et al. 2010, 2012). Previous studies have shown that *C. lividipennis* is significantly attracted to the volatiles emitted by rice plants damaged by *N. lugens* (Rapusas et al. 1996). Previously, Zhang et al. (2015) investigated the sublethal effect of four synthetic insecticides (triazophos, deltamethrin, chlorantraniliprole, and pymetrozine) on the foraging ability of *C. lividipennis* and showed that only deltamethrin disturbed the foraging behavior of the miridbug. In the current study, the orientation behavior of *C. lividipennis* was not influenced by sublethal concentrations of matrine

or abamectin, azadirachtin (Table 2). Reports documenting the effects of sublethal insecticide concentrations on the host-searching behavior of beneficial insects are relatively scarce, and this topic warrants further study.

The effects of insecticides on the predatory capacities of predators varied with the insects examined and insecticides used. For example, the consumption of *Helicoverpa armigera* by *Mallada signatus* larvae (Schneider) (Neuroptera: Chrysopidae) increased significantly in response to 200 mg a.i. L⁻¹ azadirachtin (Qi et al. 2001). Sublethal concentrations of triazophos and deltamethrin reduced the mean consumption of *C. lividipennis*, but sublethal concentrations of chlorantraniliprole and pymetrozine exhibited no influence on the predatory capability of the predator (Zhang et al. 2015). In the current study, the predation of *C. lividipennis* was reduced by azadirachtin when prey densities were high; however, matrine and abamectin did not impact predation regardless of density (Table 3; Fig. 1). Azadirachtin may cause a secondary antifeedant effect in insects which is triggered by hormonal disturbances that suppress the gut peristalsis and/or the synthesis or release of digestive enzymes (Timmins and Reynolds 1992; Trumm and Dorn 2000). We speculate that the reduced predation of *C. lividipennis* exposed to azadirachtin may be associated with an antifeeding effect, although further experiments are needed to confirm this hypothesis.

Since reproductive traits represent an important parameter in the population development, we examined the effects of sublethal concentrations of biopesticides on the reproduction of *C. lividipennis*. Our results clearly show that the pre-oviposition period and egg hatchability of *C. lividipennis* did not change in response to matrine, abamectin or azadirachtin (Fig. 2-A and C). However, the mean number of eggs laid by *C. lividipennis* exposed to abamectin and azadirachtin increased significantly in comparison to untreated control predators (Fig. 2-B). Previous studies indicated that sublethal concentrations of abamectin severely reduced the fecundity of the predatory mite, *Phytoseius plumifer* (Acari: Phytoseiidae) (Hamedi et al. 2011), and the predaceous bug *Deraeocoris brevis* (Uhler) (Hemiptera: Miridae) (Kim et al. 2006). Azadirachtin impaired fecundity, production of adult progeny and the sex ratio of the predatory pirate bug, *Blaptostethus pallascens* (Heteroptera: Anthocoridae) (Celestino et al. 2014). On the other hand, Joshi et al. (1982) reported that neem seed kernel extracts increased the fecundity of the egg parasitoid, *Telenomus remus* (Hymenoptera: Platygasteridae). We recently reported that the fecundity of *C. lividipennis* could be induced by sublethal concentrations of triazophos, deltamethrin and imidacloprid (Lu et al. 2017). Further studies are needed to explore the mechanism and

ecological outcome of insecticide-induced reproduction in *C. lividipennis*.

5. Conclusion

Our study describes the selectivity and sublethal effects of biopesticides on *C. lividipennis* adults. Among the biopesticides tested, matrine, azadirachtin and abamectin were toxic to *N. lugens* but selective to *C. lividipennis*. Matrine had no obvious sublethal effects on orientation behavior, predation, or reproduction; whereas abamectin showed a positive effect on fecundity. Azadirachtin exhibited conflicting sublethal effects, e.g., decreased predatory capacity and increased fecundity. Azadirachtin may be compatible with the use of *C. lividipennis*, although further experiments are needed for reasonable use of this biopesticide integrated with *C. lividipennis*. In summary, we conclude that abamectin and matrine are safe to *C. lividipennis* and can be integrated with the predator for effective and sustainable management of rice pests, particularly in organic cultivation systems.

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Appendix associated with this paper can be available on <http://www.ChinaAgriSci.com/V2/En/appendix.htm>

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