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Potential Positive Effects of Pesticides Application on *Sesamia inferens* (Walker) (Lepidoptera: Insecta)

Guo-qing Yang, Shang-Gen Du, Li Li, Li-Ben Jiang and Jin-cai Wu

Horticulture and Plant Protection College, Yangzhou University, Yangzhou, China.

ABSTRACT: In China, the pink stem borer (PSB) *Sesamia inferens* (Walker) (Lepidoptera: Noctuidae) has become a rice pest in some rice-producing regions. The cause of this shift from secondary to major pest is unknown. The major purpose of this study was to examine the effect of five commonly used pesticides in rice fields on reproduction of PSB and on biochemical substances of rice plants. The results showed that the weight of pupae developed from 1st instar larvae treated with 2 mg/L triazophos and the number of eggs laid by emerged females from the treatment were significantly greater than those of the control, increasing by 26.2% and 47%, respectively. In addition, a nontarget insecticide, pymetrozine 100 mg/L, and a target insecticide, chlorantraniliprole 2 mg/L, stimulated reproduction of PSB. Biochemical measurement showed that foliar sprays of these pesticides resulted in significant reductions of contents of resistant substances, flavonoids and phenolic acids, in rice plants. For example, flavonoids and phenolic acids of rice plants treated with triazophos reduced by 48.5% and 22.4%, respectively, compared to the control. Therefore, we predicted that the application of some pesticides, eg triazophos and chlorantraniliprole, may be the cause of the increase in the population numbers of PSB in rice fields.

KEYWORDS: pesticide, *Sesamia inferens*, population growth, resistant properties

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CORRESPONDENCE: jincaiwu1952@sina.com

Introduction

The pink stem borer (PSB) *Sesamia inferens* (Walker) (Lepidoptera: Noctuidae) occurs in China, the Indian subcontinent, Pakistan, Japan, and Indonesia as a polyphagous pest.[1,2] It damages a variety of crops (rice, corn, and sugarcane) and has become one of the major rice pests in China since the 1990s.[3,4] However, PSB was once considered to be only a minor pest of rice in China and other countries.[3,5–7] In some paddies, eg the coastal of Jiangsu or along the Yangtse River of Anhui, the population of PSB has exceeded those of the traditional borer pests, for example, *Chilo suppressalis*, and has caused serious yield loss.[8,9] Generally, it is considered that the population dynamics of PSB is probably associated with some factors, such as changes in the agro-ecosystem, variety rotation, or climate change.[9] However, experimental evidence of population increase caused by these factors has not been attained to date. We hypothesized that some pesticide applications may be associated with the outbreak of PSB. For example, pesticide-induced stimulation of reproduction of pests is attributed to the effect of sublethal doses or concentration of pesticides.[10] Pesticides such as triazophos and jinggangmycin can stimulate reproduction of *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae).[11–13] But there are no reports about stimulation effects of chlorantraniliprole, pymetrozine, and chlorpyrifos. Also flavonoids and soluble phenolics in plants are the functional defence and can help plants to reduce the damage caused by insects or pathogens.[14]

In fact, the aggravated damage caused by PSB is partly attributed to chemical control. For example, some insecticides have often been used to control the main Lepidoptera pests of rice, eg *C. suppressalis* (Walker) (Lepidoptera: Pyralidae) and *Cnaphalocrocis medinalis* (Guenée) (Lepidoptera: Pyralidae), which resulted in an unexpected effect on PSB. It is reported that fipronil showed high control efficacy for *C. suppressalis*, but low efficacy for PSB, which has been an important indirect factor contributing to the increase in population density of PSB.[15,16] In addition, previous studies have indicated that pesticides at certain concentrations, especially at sublethal concentrations, could stimulate the reproduction of some insect pests of rice, such as the resurgence of *Nilaparvata lugens* (Hemiptera: Delphacidae).[17] The number of eggs laid by *Scirpophaga incertulas* (Walker) (Lepidoptera: Pyralidae) treated with imidacloprid increased significantly.[18] Thus, we selected five commonly used pesticides in rice fields (triazophos, chlorantraniliprole, chlorpyrifos, pymetrozine, and jinggangmycin) to examine stimulation effect on reproduction of PSB.

Triazophos is an organophosphate classic induced-resurgent insecticide that stimulated the reproduction of *N. lugens*.[11,19,20] Triazophos is often used to control lepidopterous pests at 180–300 g a.i. ha^{-1}, especially rice borers. Chlorantraniliprole is a novel alternative insecticide and was introduced into China in 2008 as a novel insecticide to control Lepidopterous pests at 15–25 g a.i. ha^{-1}. It has been reported that chlorantraniliprole suppressed reproduction of *N. lugens*.[21] Chlorpyrifos is an organophosphate insecticide that is used to control rice borers at 200–400 g a.i. ha^{-1}. Pymetrozine is a selective insecticide and used to control planthoppers at 200–400 g a.i. ha^{-1}. Jinggangmycin, an antibiotic compound developed in China, is a fungicide that is mainly used in controlling the rice sheath blight *Rhizoctonia solani* in China at 112–255 g a.i. ha^{-1}, which stimulated reproduction of *N. lugens*.[22] However, the stimulation effect of all the above pesticides on the reproduction of PSB is not clear. The objective of this study was to examine the stimulating effect of several pesticides on the reproduction of PSB and its possible mechanism from the view of changes in biochemical substances of rice plants caused by pesticides.

Materials and Methods

Insects and rice cultivar. The larvae of PSB, collected from the farm field of Yangzhou University, were maintained on *Zizania latifolia* (Griseb.) Turcz. ex Stapf in the laboratory until the next generation. Newly hatched neonates (<24 h) were used for the tests.

The rice cultivar Wuyujing 3 (japonica rice) was used. Rice seeds were sown in rice nursery after soaking and germination. Seedlings were transplanted in each of 30-cm diameter plastic pots, with four hills (three plants) per plot, when they were 30 days old (five-leaf stage).

Pesticides and applications. Pesticides applied in this study were acquired from different pesticide companies. These pesticides include triazophos (Changqin Pesticide Co. Ltd., Jiangsu, China), chlorantraniliprole (Xianfeng Pesticide Co. Ltd., Jiangsu, China), chlorpyrifos (Xianfeng Pesticide Co. Ltd., Jiangsu, China), pymetrozine (Anpon Electrochemical Co. Ltd., Jiangsu, China), and jinggangmycin (Jiangsu Shenhua Biochemical Co. Ltd., Jiangsu, China).

For the experiment on effects of pesticides on life history parameters of PSB population, five neonates or 3rd instar larvae were released onto each hill of potted rice plants at the maximum tillering stage. The plants were covered with a plastic bag after two hours to avoid larval drifting. Then, the plants were sprayed with different pesticides at two days after release using a Yangtse River 08 Model Sprayer (SeeSa Ltd. Co., Zhejiang, China) with a nozzle of 1 mm diameter. Pesticide concentrations were as follows: According to our former tests on the toxicity of three target insecticides on PSB, the sublethal concentrations of triazophos, chlorantraniliprole, and chlorpyrifos were determined as 2, 2, and 100 mg/L to 1st instar larvae, and 10, 40, and 300 mg/L to 3rd instar larvae, respectively; spray concentrations of pymetrozine and jinggangmycin were at 50, 100, and 200 mg/L and 100, 200, and 400 mg/L for both 1st and 3rd instar larvae, respectively. For the experiment on the effects of pesticides on biochemistry of rice plants, spray concentrations of five pesticides were as follows: 2, 50, and 100 mg/L for triazophos, 2, 10, and 40 mg/L for chlorantraniliprole, 50, 100, and 300 mg/L for chlorpyrifos, 50, 100, and 200 mg/L for pymetrozine, and 100, 200, and 400 mg/L for jinggangmycin, respectively. Spray quantity of pesticides was calculated based on the area of potted rice plants. Tap water was used as a control. Treatments and control were replicated five times. Treated and control rice plants were maintained under natural conditions and arranged randomly.

Population growth parameters of PSB. Larvae were treated with different pesticides during the 1st or 3rd instars, respectively. To exclude natural enemies, the potted rice plants were covered with a nylon cylindrical cage. During the population growth, the developmental duration of larvae of every treatment was recorded before the emergence of pupa. All pupas were collected and weighed individually, and then were maintained in plastic boxes covered with cheesecloth and matted with wet filter paper to maintain moisture. All emerged adults were collected for mating and oviposition on the rice plants. Then, all egg masses of every treatment and control were collected and brought to the laboratory for larval hatching, and total eggs per adult female were counted.

Measurement of contents of flavonoids, soluble phenolics, and silicon in rice plants. Rice plants at 3, 7, and 14 days after pesticide application were clipped to maintain at an ultra cold storage freezer in the laboratory for later measurement. Contents of flavonoids in rice stems were measured by using the method of Ge et al,[23] and total phenolic contents were measured colorimetrically with Folin Ciocalteu reagent.[24] The surface silicon contents of rice leaf sheath were tested with scanning electron microscopy (SEM) and energy spectrum technology.[25]

Statistical analysis. Data for the population growth parameters (growth duration, pupal weight, and number of eggs laid per female) of PSB and resistant substances (flavonoids, soluble phenolics, and silicon) of rice plants treated with pesticides were analyzed using one-way ANOVAs.[26] Means were compared using Fisher's protected least significant difference (LSD-test) at $P < 0.05$.

Results

Population growth parameters of PSB treated with pesticides. Three population growth parameters (development duration, pupal weight, and the number of eggs laid per female) of PSB feeding on rice plants treated with pesticides were investigated. When treated during the 1st or 3rd instar (Table 1), the duration of instars of PSB prolonged for some treatments, such as triazophos (2 mg/L), chlorantraniliprole (2 mg/L), jinggangmycin (100 and 200 mg/L), and pymetrozine (200 mg/L). There were no significant differences in the duration of pupas and adults compared to that of control. The application of pesticides during the 1st instar also influenced pupal weight at some concentrations (Fig. 1). The pupal weight of PSB treated with 2 mg/L triazophos increased significantly by 26.2% as compared to control for 1st instar. The treatment with jinggangmycin increased the pupal weight by 20.0% as compared to control at the concentration of 100 mg/L for 1st instar, but reduced it by 13.3% as compared to control at the higher dose (400 mg/L). The number of eggs laid per female obviously varied with different treatments (Fig. 2). Among these treatments during the 1st instar, triazophos resulted in the maximum fecundity, which increased the number of eggs laid by 1.47 fold than control. The number of eggs laid for pymetrozine treatment increased significantly at a concentration of 100 mg/L. Application of chlorantraniliprole at a sublethal concentration during the 3rd instar also enhanced the number of eggs laid significantly.

Content of flavonoids and soluble phenolics in rice plants treated with pesticides. Five pesticide foliar sprays decreased contents of flavonoids at 3, 7, and 14 days after treatments (Table 2). With the increasing concentration of triazophos and jinggangmycin, obviously means of the contents of flavonoids were lower than control by 48.5% and 42.1%, respectively.

Application of these pesticides also indicated a similar changing pattern in soluble phenolics content in rice plants (Table 3). Three days after treatment, except for jinggangmycin and chlorantraniliprole, contents of soluble phenolics reduced

Table 1. Developmental duration of *Sesamia inferens* after treatment with pesticides.

SPRAY TIME (LARVAL INSTAR)	PESTICIDES AND DOSE (mg/L)		LARVAL DURATION (d)	PUPA DURATION (d)	ADULT DURATION (d)
First	CK		29.70 ± 0.13b	9.71 ± 0.45a	5.81 ± 0.17a
	Triazophos	2	30.74 ± 0.13a	8.82 ± 1.07a	5.61 ± 0.35a
	Chlorantraniliprole	2	31.25 ± 0.25a	9.00 ± 0.43a	6.22 ± 0.19a
	Chlorpyrifos	100	30.88 ± 0.74b	10.14 ± 0.3a	6.17 ± 0.29a
	Pymetrozine	50	30.53 ± 1.78b	8.54 ± 0.69a	6.26 ± 0.60a
		100	29.66 ± 0.36b	9.54 ± 1.15a	5.89 ± 0.84a
		200	30.13 ± 0.14b	9.73 ± 0.25a	5.56 ± 0.10a
	Jinggangmycin	100	31.30 ± 0.30a	8.98 ± 0.75a	5.67 ± 0.58a
		200	30.73 ± 0.93a	9.19 ± 0.97a	5.44 ± 0.10a
		400	29.52 ± 0.43b	9.95 ± 0.69a	5.86 ± 0.48a
Third	CK		30.32 ± 0.88a	10.19 ± 1.55a	6.19 ± 0.17a
	Triazophos	40	30.47 ± 0.56a	10.37 ± 0.95a	6.02 ± 0.64a
	Chlorantraniliprole	10	31.42 ± 1.52a	10.75 ± 0.90a	6.00 ± 0.33a
	Chlorpyrifos	300	30.88 ± 0.74a	10.39 ± 0.60a	6.17 ± 0.29a
	Pymetrozine	50	29.92 ± 0.20b	11.00 ± 1.49a	6.20 ± 0.50a
		100	30.49 ± 0.62a	9.12 ± 0.98a	6.14 ± 0.48a
		200	31.14 ± 0.48b	9.53 ± 1.09a	5.89 ± 0.10a
	Jinggangmycin	100	30.66 ± 0.24a	10.57 ± 0.69a	6.09 ± 0.30a
		200	29.88 ± 0.83a	10.16 ± 0.69a	5.75 ± 0.87a
		400	30.60 ± 0.53a	9.86 ± 0.75a	5.83 ± 0.41a

Note: Means ± SE followed by different letters within a column of the same larval instar are significant difference at 0.05 levels, respectively.

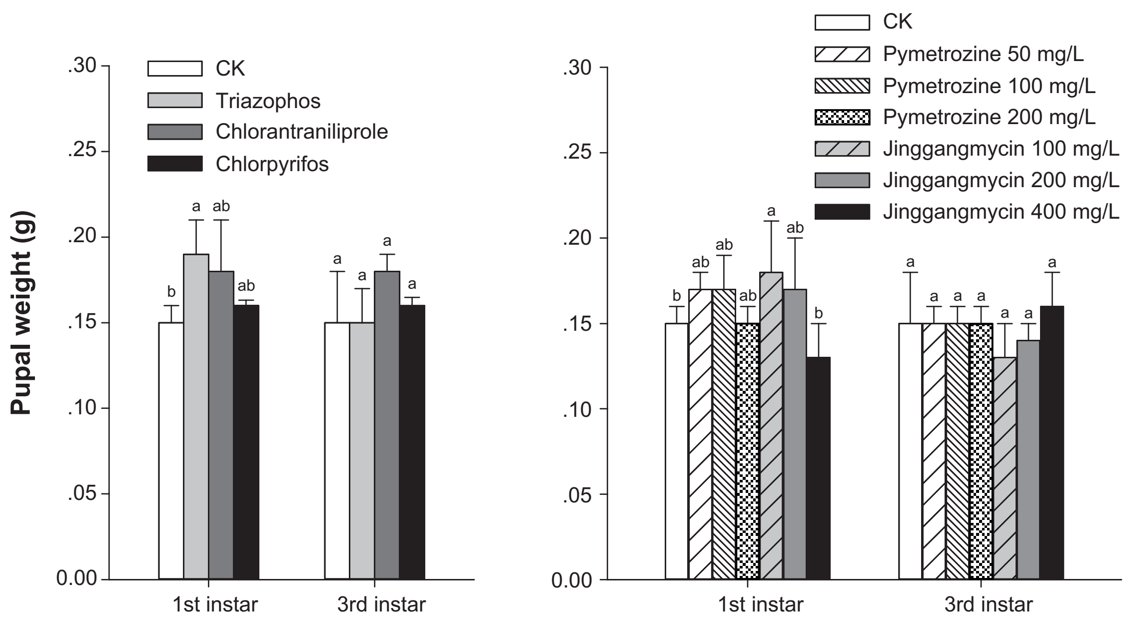

Figure 1. Pupal weights of *Sesamia inferens* after treatment with pesticides. Bars of the same larval instar with different letters indicate that there is significant difference at $P < 0.05$.

significantly. Furthermore, the effect was concentration dependent. At 7 and 14 days after jinggangmycin treatment except for 200 mg/L at 7th day, soluble phenolics contents were significantly lower than those of control.

Surface silicon contents of rice leaf sheath treated with pesticides. Pesticides influenced the surface silicon contents of rice leaf sheath at different concentrations to some extent (Fig. 3). Contrary to the decrease of flavonoids and soluble phenolics, the surface silicon contents at 7 days after chlorantraniliprole spray and at 14 days after jinggangmycin spray increased markedly compared to those of the control, respectively. However, no significant influence on the surface silicon contents of rice leaf sheath was found at 7 days or 14 days after other treatments.

Discussion

Occurrence of the natural population of PSB in rice paddies is complex. PSB has several overlapping generations and several instars per year. Larvae of several instar stadia

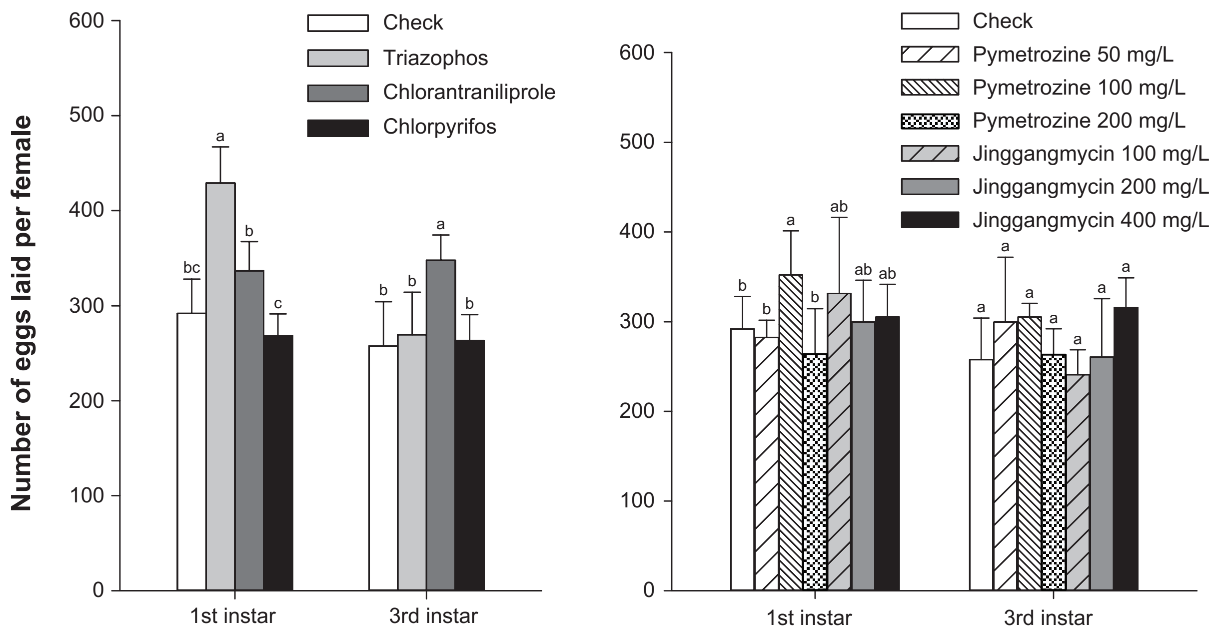

Figure 2. Number of eggs laid per female of *Sesamia inferens* after treatment with pesticides. Bars of the same larval instar with different letters indicate that there is significant difference at $P < 0.05$.

Table 2. Contents of flavonoids in rice plants treated with pesticides in China, 2013–2014.

PESTICIDES	CONCENTRATION (mg/L)	AMOUNT OF FLAVONOIDS (DRY WEIGHT, mg/g)		
		3d	7d	14d
Check	0	5.19 ± 0.38a	5.21 ± 0.96a	6.49 ± 0.80a
Pymetrozine	50	5.17 ± 0.48a	4.11 ± 0.16b	5.23 ± 0.51a
	100	5.04 ± 0.22a	3.96 ± 0.20b	5.36 ± 1.38a
	200	4.34 ± 0.25b	3.76 ± 0.26b	5.42 ± 0.66a
Jinggangmycin	100	3.74 ± 0.39b	3.46 ± 0.19b	4.66 ± 0.82b
	200	3.62 ± 0.39b	4.34 ± 0.48ab	3.34 ± 0.36c
	400	4.35 ± 0.68ab	3.67 ± 0.33b	3.29 ± 0.63c
Chlorpyrifos	50	4.99 ± 1.66a	3.83 ± 0.19b	6.01 ± 1.06ab
	100	5.56 ± 0.81a	4.63 ± 0.90ab	5.66 ± 1.10ab
	300	4.42 ± 0.55a	3.81 ± 0.14b	4.79 ± 0.41b
Chlorantraniliprole	2	5.02 ± 0.41a	4.49 ± 1.11a	4.77 ± 0.61b
	10	4.77 ± 0.37a	3.82 ± 0.28ab	4.91 ± 0.37b
	40	4.02 ± 0.29b	3.71 ± 0.68ab	5.14 ± 0.22b
Triazophos	2	4.86 ± 1.11ab	5.79 ± 0.16a	3.55 ± 0.68bc
	50	3.77 ± 0.11b	3.48 ± 0.67b	2.67 ± 0.52c
	100	4.21 ± 0.13ab	4.02 ± 0.36b	3.79 ± 0.48b

Note: Means ± SE followed by different letters within a column of the same time after treatment are significant difference at 0.05 levels, respectively.

can appear simultaneously. PBS has a complex generation cycle, which lasts about 40–50 days. To control PSB larvae, several pesticides (pymetrozine, jinggangmycin, and chlorantraniliprole) have been used to target planthoppers, rice sheath blight, and rice leaffolder, which may influence present investigation. Newly hatched neonates and transferred larvae from rice plant to plant can be directly exposed to these pesticides although PSB is a borer pest. In addition, some pesticides can be systemically transported to the position of larval feeding through rice plants. Therefore,

Table 3. Contents of phenolics in rice plants treated with pesticides in China, 2013–2014.

PESTICIDES	CONCENTRATION (mg/L)	AMOUNT OF PHENOLICS (DRY WEIGHT, mg/g)		
		3d	7d	14d
Check	0	5.40 ± 0.73a	6.96 ± 1.64a	6.05 ± 1.55a
Pymetrozine	50	4.52 ± 0.32b	4.98 ± 0.47b	4.72 ± 0.13a
	100	4.52 ± 0.15b	5.29 ± 0.73ab	5.50 ± 0.57a
	200	4.44 ± 0.35b	4.71 ± 0.61b	5.56 ± 1.14b
Jinggangmycin	100	5.59 ± 0.63a	5.19 ± 0.39b	5.77 ± 0.92a
	200	5.72 ± 0.20a	7.37 ± 0.86a	4.05 ± 0.16b
	400	5.38 ± 0.18a	4.57 ± 0.49c	4.04 ± 0.48b
Chlorpyrifos	50	5.90 ± 0.41a	4.96 ± 0.16	5.18 ± 0.61a
	100	4.36 ± 0.61b	6.36 ± 1.50a	5.54 ± 0.73a
	300	3.94 ± 0.28b	5.35 ± 0.35ab	5.08 ± 1.07a
Chlorantraniliprole	2	4.05 ± 0.16b	5.19 ± 0.27c	5.33 ± 0.09a
	10	4.21 ± 0.22b	5.29 ± 0.4bc	6.08 ± 0.79a
	40	4.58 ± 0.39b	7.56 ± 0.52a	4.90 ± 0.13a
Triazophos	2	4.68 ± 1.14ab	5.25 ± 0.80ab	4.85 ± 0.20ab
	50	3.94 ± 0.14b	5.61 ± 0.15ab	4.67 ± 0.79ab
	100	3.96 ± 0.17b	5.98 ± 1.83ab	4.86 ± 0.46ab

Note: Means ± SE followed by different letters within a column of the same time after treatment are significant difference at 0.05 levels, respectively.

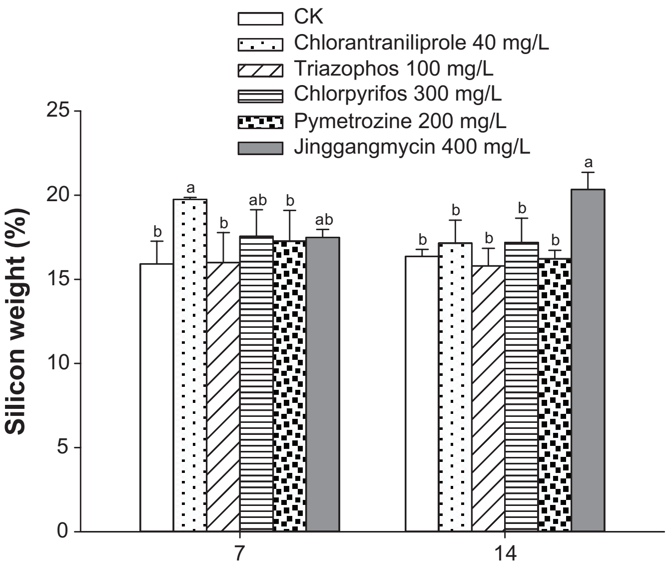

Figure 3. Contents of surface silicon of rice leaf sheath treated with pesticides. Bars of the same time after treatment with different letters indicate that there is significant difference at $P < 0.05$.

we conducted experiments of reproduction of adult females developed from larvae of two instar stadia (1st and the 3rd instars) treated with five pesticides. The present findings show the reproduction of adult females developed from 1st instars treated with triazophos and with 100 mg/L pymetrozine. In addition, triazophos treatment prolonged the duration of instars, and increased pupal weight. Previous studies indicated that the susceptibility of PSB to triazophos was higher than *Chilo suppressalis* in some rice-growing regions,[5] and chlorantraniliprole showed a better control efficacy to PSB.[27] However, PSB is frequently exposed to sublethal concentrations of triazophos and chlorantraniliprole in rice fields because of boring biological characteristics, larva instar overlap, and genetic diversity.[28] Thus, the present results can, in part, explain causes of increase of natural population of PSB.

Generally, causes of the resurgence of insect pests following pesticide applications mainly include direct and indirect factors. On one hand, it has been reported that ecological mechanisms for resurgence of some insect pests could be explained as the reduction in natural enemies[29] and stimulation of reproduction by pesticides.[19,20,22] The present experiment showed that PSB resurgence may be attributed to stimulation of reproduction by some pesticides. Moreover, previous studies showed that alterations in physiology and biochemistry, eg oxalic acid and soluble sugar, of rice plant after pesticide application are beneficial to the feeding, survival, and reproduction of *N. lugens*.[18,30] The present experiment demonstrated that contents of flavonoids and soluble phenolics in rice plants decreased following pesticide application, such as triazophos and jinggangmycin. Whereas, contents of silicon, acting as an indicator of physical resistance to insect pests, did not vary with pesticide application, except for significant increase at 7 days after chlorantraniliprole treatment and at 14 days after jinggangmycin treatment. Thus, it is considered that reduction of resistant substances in rice plants treated with pesticides is beneficial to larva feeding and development, and it stimulated reproduction of PSB adults via alteration of rice nutrimental substances. Molecular mechanism of triazophos-, chlorantraniliprole-, and pymetrozine-induced stimulation of reproduction of PSB will be further investigated.

It has been reported that chlorantraniliprole suppressed reproduction of *Nilaparvata lugens*,[21] but stimulated reproduction of PSB. Therefore, chlorantraniliprole should not be used to control rice leaffolder during PSB occurrence because it has the possibility of resulting in PSB resurgence. Thus evaluation of effects of pesticide application on various pest species is very important for harmonic control of pests in rice ecosystems.

Conclusion

The present study showed that reduction of resistant substances (flavonoids, soluble phenolics, and silicon) in rice plants treated with some pesticides (especially triazophos) is beneficial to larva feeding and development, and it stimulated reproduction of PSB adults via alteration of rice nutrimental substances. But not each pesticide has these effects in our study, and this requires further testing. We also found that the application of some pesticides, eg triazophos and chlorantraniliprole, may lead to increase in the population numbers of the PSB *Sesamia inferens* (Walker) in rice fields, which is associated with the reduction of resistant substances in rice plants treated with pesticides.

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Author Contributions

Conceived and designed the experiments: G-QY. Analyzed the data: S-GD. Wrote the first draft of the manuscript: G-QY. Contributed to the writing of the manuscript: S-GD, LL, L-BJ. Agree with manuscript results and conclusions: G-QY, S-GD, LL, L-BJ, J-CW. Jointly developed the structure and arguments for the paper: G-QY, S-GD, LL, L-BJ, J-CW. Made critical revisions: J-CW. All authors reviewed and approved of the final manuscript.

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