Toxic Effects of Nitenpyram on the Brown Planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae)[1]

Jie Zhang, Liangxiong Hu, Shanfeng Ling, Jie Liu, Haidong Chen, and Runjie Zhang[2]

State Key Laboratory for Biocontrol and Institute of Entomology, Sun Yat-sen University, Guangzhou 510275, P.R. China

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Abstract Laboratory assays explored the potential of nitenpyram, a novel neonicotinoid insecticide developed by Takeda Chemical Industry Co., Ltd. against the brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae). All nymphal instars were sensitive to nitenpyram with the first and second instars being the most susceptible (median lethal concentrations [LC50] of 0.14 mg/L at 72 h after treatment). Fifth-instar nymphs were the least susceptible. Mortality of first - second instars treated with 2 mg/L peaked at 48 h after treatment. The five concentrations of nitenpyram tested (2, 1, 0.5, 0.25, 0.125 mg/L) decreased the weight of brown planthopper nymphs following treatment with the insecticide. These laboratory results indicate that nitenpyram may prove to be an effective alternative for the control of brown planthopper in rice culture.

Key Words brown planthopper, nitenpyram, toxicity, laboratory assays, median lethal concentration

The brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae), is one of the most serious pests of rice, causing substantial yield loss in most rice-producing countries and inducing symptoms commonly referred to as 'hopper burn' (Shepard et al. 1991, Heong et al. 1992, Heinrichs 1994, Cheng et al. 2003, Qiu et al. 2004, Backus et al. 2005, Wang and Wang 2007). In China, outbreaks of this planthopper have occurred frequently in recent years. This monophagous pest causes severe damage to rice plants through direct sucking, ovipositing, and virus disease transmission. Because of its highly adaptive capacity to changing cultural practices and high reproductive potential, integrated control to this insect pest is necessary to manage populations below economic levels (Ding and Su 2002).

Insecticides have been extensively used for control of this pest (Zhao 2000, Endo and Tsurumachi 2001, Yoo et al. 2002) resulting in the development of resistance in brown planthopper populations in different countries and areas (Nagata 1982, Kilin et al. 1981, Hirai 1993). Certain insecticides also have been implicated in the stimulation of brown planthopper reproduction (Heinrichs et al. 1982, Reissig et al. 1982). Progressive rice farmers in China commonly apply insecticides 3 - 4 times each growing season. Such insecticide usage, although aimed at only 1 or 2 target pests, usually has an adverse effect on other insects and the ecosystem, including natural enemies. This

[1]Received 20 August 2009; accepted for publication 22 December 2009.
[2]Address inquiries (email: lsszrj@mail.sysu.edu.cn.)

could result in resurgence (Heinrichs et al. 1982) and development of insecticide resistance (Ahn et al. 1993, Yoo et al. 2002).

To avoid these problems, it is necessary to minimize the use of chemical insecticides, rotate classes or types of insecticides, or use new types of insecticides for brown planthopper control. Nitenpyram is a neonicotinoid insecticide developed in Japan during the 1990s (Obana et al. 2003). It binds to nicotinic acetylcholine receptors, interfering with normal nerve transmission (Nagata et al. 1999, Chatellier 2001) and has low mammalian toxicity and high toxicity to insect pest species, including fleas, muscids, and fruit flies (Kashiwada 1996, Tomizawa et al. 1996). It is also considered to be an ideal insecticide for the control of sucking insect pests such as aphids, whiteflies, etc. (Horowitz and Ishaaya 2004).

To date, little knowledge exists regarding the response of brown planthopper to nitenpyram. Thus, the objective of this study was to investigate the toxic effects of nitenpyram on the nymphal stages of brown planthopper using laboratory immersion assays.

Materials and Methods

An insecticide-susceptible strain of brown planthopper was maintained on rice seedlings at 26 ± 1°C with a 16:8 h (L:D) photophase for more than 2 yrs at the Institute of Entomology, Sun Yat-Sen University, Guangzhou, China, without any exposure to insecticide. Nitenpyram toxicity to brown planthoppers was assayed using the rice-stem dipping method described by Zhuang et al. (1999) and Wang et al. (2008a). Technical grade (98%) nitenpyram, provided by the Plant Protection Research Institute of Guangdong Academy of Agricultural Sciences, was diluted in distilled water to 5 concentrations (2.0, 1.0, 0.5, 0.25, 0.125 mg/L). Rice plants at tillering to booting stage were washed thoroughly. Rice stems (about 10 cm long) with roots were cut and air dried to remove excess water. Three rice stems were grouped and dipped into different insecticide solutions for 30 sec. After the rice stems were air dried, moistened cotton was used to wrap the rice roots. The treated rice stems were then placed into a 500-ml plastic cup. Twenty first - second nymphal instars, third - fourth nymphal instars, and fifth nymphal instars were introduced into each plastic cup using a vacuum device. Each concentration was replicated 3 times; a distilled water treatment was used as a control. Mortality of the nymphs was recorded every 24 h after treatment. At 72 h after treatment, surviving larvae were weighed using a Mettler Toledo AB-E balance (Mettler Toledo Instruments (Shanghai) Co. Ltd.). Treated insects were maintained at a temperature of 26 ± 1°C with a 16: 8 h (L: D) photophase. The nymphs were considered dead if they failed to move after being gently prodded with a fine brush.

Mortality and weight data were subjected to analysis of variance (ANOVA of arcsine, logarithmic and square root transformed percentages). Significant differences were determined by using Tukey's multiple range test ($P < 0.05$) with SPSS®, Version 11.5. Mortality was corrected using Abbott's formula, if necessary. Lethal concentrations (both LC50 and LC90) were calculated using probit analysis; values were expressed as means ± SE (SE) of 3 replicates.

Results

Nymphal instars of brown planthopper showed different levels of susceptibility to nitenpyram in these laboratory assays (Table 1). Among all nymphal instars, the

Table 1. Susceptibility of different brown planthopper instars to nitenpyram.

Time	Instar	Number	LC_{50} mg/L (95% CL)	LC_{90} mg/L (95% CL)	Slope ± SE
24	1 - 2	60	0.93 (0.71 - 1.31)	4.48 (2.68 - 11.38)	1.87 ± 0.22
	3 - 4	60	2.27 (1.76 - 3.46)	8.11 (4.85 - 11.10)	2.32 ± 0.37
	5	60	3.18 (2.16 - 2.57)	16.51 (7.64 - 17.87)	1.79 ± 0.32
48	1 - 2	60	0.20 (0.16 - 0.24)	0.68 (0.54 - 0.94)	2.45 ± 0.29
	3 - 4	60	0.63 (0.50 - 0.80)	3.96 (2.56 - 7.85)	1.60 ± 0.20
	5	60	0.72 (0.57 - 0.95)	5.23 (3.16 - 9.88)	1.49 ± 0.20
72	1 - 2	60	0.14 (0.11 - 0.17)	0.41 (0.34 - 0.53)	2.80 ± 0.36
	3 - 4	60	0.24 (0.19 - 0.30)	1.35 (1.02 - 2.02)	1.72 ± 0.20
	5	60	0.28 (0.21 - 0.35)	1.83 (1.27 - 3.22)	1.57 ± 0.20

Time is the hours after treatment. Number is the number of larvae tested. CL is the 95% confidence limits. Slope ± SE is the slope ± standard error.

first - second instars were the most susceptible with LC50 values of 0.93 (χ^2 = 9.51; df = 13; $P > 0.05$), 0.20 (χ^2 = 11.64; df = 13; $P > 0.05$), and 0.14 (χ^2 = 10.97; df = 13; $P > 0.05$) mg/L at 24, 48, and 72 h after treatment, respectively. The fifth instars were the least susceptible to nitenpyram with the LC50 values of 3.18 (χ^2 = 4.26; df = 13; $P > 0.05$), 0.72 (χ^2 = 6.25; df = 13; $P > 0.05$), 0.28 (χ^2 = 2.83; df = 13; $P > 0.05$) mg/L at 24, 48, and 72 h after treatment, respectively. The LC50s of the third - fourth instars were 2.44, 3.15, and 1.71 greater than those of the first - second instars.

Cumulative mortality of brown planthopper nymphs treated with the 5 concentrations of nitenpyram are shown in Fig. 1. Greater numbers of nymphs were killed at a faster rate when treated with the higher concentrations of nitenpyram (2, 1 mg/L) than at the median (0.5 mg/L) and lower (0.25, 0.125 mg/L) concentrations, and control mortality (treated with distilled water) remained low. No significant differences in mortality of the first - second instars treated with 2 and 1 mg/L were observed at 24, 48 and 72 h after treatment. However, significant differences ($P < 0.05$) were observed among the other concentrations of nitenpyram and the control. For third - fourth and fifth instars, mortality levels were significantly different ($P < 0.05$) among all concentrations of nitenpyram and the control at this same posttreatment interval. Nymphs treated with 2 and 1 mg/L showed significantly higher mortality than that observed with other concentrations of nitenpyram or the control.

Almost all first - second instars treated with nitenpyram died, thus, preventing weighing survivors. However, at 72 h after treatment, the weights of the third - fourth and fifth instars were significantly reduced to 4.85 and 6.05 mg at 2 mg/L, respectively, when compared with the controls ($P < 0.05$). Furthermore, weights of third - fourth and fifth instars showed a gradual decrease with increasing concentration of the chemical (Table 2).

Discussion

The neonicotinoids are the most important new synthetic insecticides developed in the past 3 decades. These are increasingly used for systemic control of plant-sucking

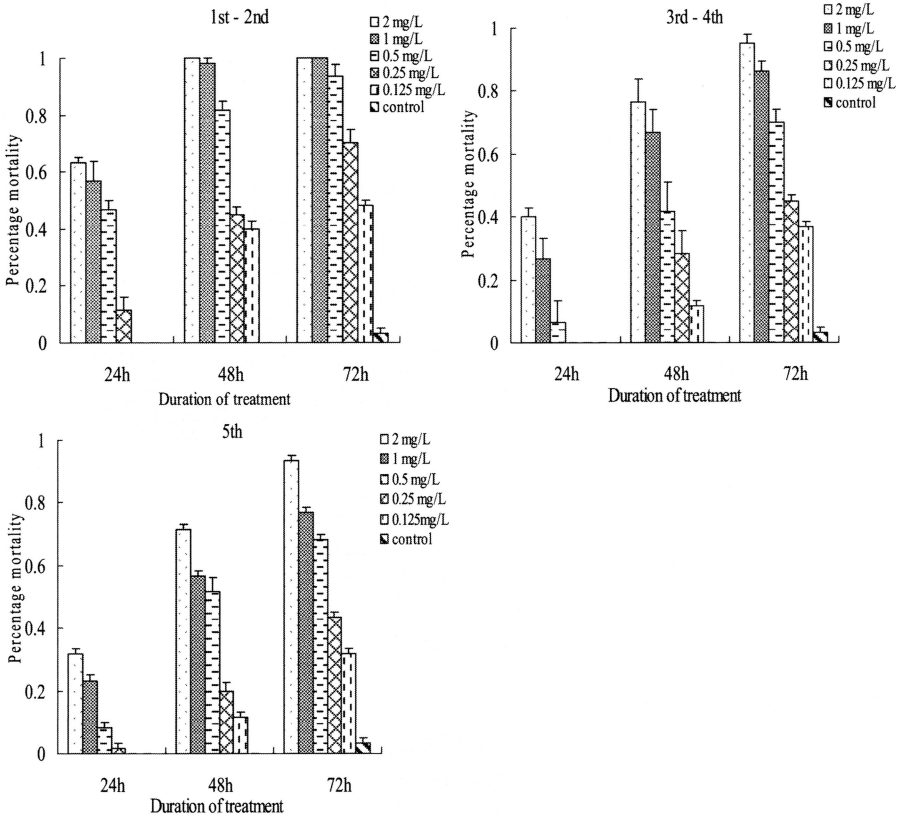

Fig. 1. Corrected mortality of brown planthopper instars following treatment with nitenpyram.

insects, replacing the organophosphate and methylcarbamate compounds, which have decreased effectiveness because of development of resistance or increased regulatory restrictions. Neonicotinoids are also important in animal health care (Ralf and Ian 2005, Yamamoto and Casida 1999, Motohiro and John 2003).

The broad spectrum of nitenpyram efficacy, together with systemic and translocation activity, pronounced residual activity, and a unique mode of action, make it rapidly expanding insecticidal class (Minamida et al. 1993, Alfred et al. 2008). It is considered to mimic the mode of action of nicotine, acting as an agonist of nicotinic acetylcholine receptors in postsynaptic nerve membranes (Bai et al. 1991, Chao et al. 1997). Its systemic properties and long residual activity make these insecticides ideal against sucking pests (Horowitz and Ishaaya 2004).

Our results show that nitenpyram affected various instars of brown planthopper (first - second, third - fourth, and fifth) at different concentrations (0.125, 0.25, 0.5, 1.0 and 2.0 mg/L). The data suggest that nitenpyram is a highly toxic insecticide against this insect. LC50 values showed gradual decreases with time after treatment and insect stage (e.g., first - second instars are more susceptible than third - fourth instars

Table 2. Nymphal weights of brown planthoppers following exposure to nitenpyram 72 h earlier (weight (mean±S.E.) of ten nymphs).

Nitenpyram mg/L	first-second instar	third-fourth instar	fifth instar
2	-	4.85 ± 1.16a	6.05 ± 1.75a
1	-	7.92 ± 2.05ab	8.36 ± 2.05ab
0.5	-	11.02 ± 3.45bc	12.10 ± 2.85bc
0.25	-	13.04 ± 3.94c	15.42 ± 3.55c
0.125	-	13.35 ± 3.33c	15.54 ± 3.35c
control	0.50 ± 0.04	17.34 ± 5.08c	20.58 ± 4.25c

Means within columns not followed by the same letter are significantly different (Tukey's test, $P < 0.05$).

and fifth instars). LC50 values with first - second instars were 0.20 mg/L at 48 h after treatment and 0.14 mg/L at 72 h after treatment, whereas LC50s with third - fourth instars were 0.63 mg/L at 48 h and 0.24 mg/L at 72 h after treatment, and the LC50s for the fifth instars were 0.72 mg/L at 48 h and 0.28 mg/L at 72 h after treatment. The LC50 with third - fourth instars after 72 h treatment is similar to the result of Wang et al. (2008b). Differences could be from the use of brown planthopper populations from distinctly different geographic areas.

Higher rates of mortality were obtained with higher concentrations. In general, the increase of mortality was related to both time and nitenpyram concentrations. Nitenpyram caused 100% mortality of the first - second instars at 2 mg/L at 48 h after treatment. There were significant differences in mortality among various concentrations for third - fourth instars and fifth instars after 72 h. Mortality of various instars differ from the control treatment and were significantly higher than those observed with higher concentrations. Mortality was associated mainly with failure to molt. Besides the toxic effect, the present study revealed numerous sublethal effects of nitenpyram which would hinder survival and molting activity of the insect.

In our study, the nitenpyram also affected the weight of the third - fourth and fifth instars. When exposed to the highest concentration (2 mg/L), the weights of third - fourth and fifth instars decreased significantly by 78.0% and 70.6% compared with the control. At the lowest concentration (0.125 mg/L), the weights of third - fourth and fifth instars also were decreased compared with the control, but these were not statistically significant.

In conclusion, nitenpyram had significant effects on brown planthopper nymphs, causing increased mortality and decreased weights of survivors as concentration was increased. Nitenpyram appears to be a potential candidate for further development for the management of brown planthopper in rice production.

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