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

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Abstract Laboratory assays explored the potential of paichongding, a novel neonicotinoid insecticide, against the brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae). Cumulative mortality of *N. lugens* following exposure was concentration and time dependent. *Nilaparvata lugens* nymphs were more sensitive to paichongding compared with the adults, and the first and second instars were the most susceptible. Median lethal concentration (LC_{50}) values to instars I-II at 24, 48, and 72 h after treatment were 7.00, 0.46, and 0.13 mg/L, respectively. Brachypterous adults were more susceptible than macropterous adults, and the macropterous females were the least susceptible with LC_{50} values at 24, 48, and 72 h after treatment of 97.05, 58.70, and 1.26 mg/L, respectively. The median lethal time (LT_{50}) significantly decreased with increasing concentration of paichongding. The LT_{50} for instars I-II decreased from 62.38 h at 0.2 mg/L to 25.13 h at 3.2 mg/L, whereas the LT_{50} values for macropterous females were 92.67 h at 0.2 mg/L and 52.65 h at 3.2 mg/L. These laboratory results suggested that paichongding has potential in controlling populations of *N. lugens*. Our preliminary study laid foundations for development and future use of paichongding in rice culture.

Key words *Nilaparvata lugens*, paichongding, toxicity, median lethal concentration, median lethal time

Rice, *Oryza sativa* L., is the staple food for almost half the world's population (Ooi 1991). More than 60% of the Chinese people are living with rice, and rice production is directly related to food security (Lou and Cheng 2011). The brown planthopper, *Nilaparvata lugens* (Stål), is a major insect pest of rice in Asia (Dyck and Thomas 1979). Feeding damage causes hopper burn characterized by dry leaves and wilted shoots (Bae and Pathak 1970). In China, outbreaks of *N. lugens* have occurred frequently in recent years (Gao et al. 2006). Macropterous adults are able to migrate long distances, thus creating difficulties in controlling this pest (Syobu et al. 2002, Huang et al. 2003).

Nilaparvata lugens is a typical r-strategist and, therefore, chemical control is the first choice for management (Endo and Tsurumachi 2001, Yoo et al. 2002). Worldwide, rice now accounts for more insecticide usage than any other crop, with 80%

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used in Asia (Woodburn 1990). However, an ideal insecticide which could kill the target pest with little or no side effects on other organisms is rare (Way 1977).

The escalating use of insecticides to control herbivorous insects has been implicated in resurgence of primary pests and outbreaks of secondary herbivores (Ripper 1956, Raupp et al. 2001, Frampton and Dorne 2007, Liang et al. 2007). *Nilaparvata lugens* is a classical insecticide-induced resurgent pest whose degree of damage is positively correlated to insecticide use (Chelliah and Heinrichs 1980, Reissig et al. 1982a, Heinrichs and Mochida 1984, Kenmore et al. 1984, Gao et al. 1988). In general, outbreaks of *N. lugens* following applications of insecticides are generally thought to arise due to: (1) elimination of natural enemies or decrease of their foraging abilities (Ripper 1956, Croft and Brown 1975, Gu and Waage 1990); (2) changes in plant quality increase feeding rate and fecundity of *N. lugens* causing outbreaks (Chelliah and Heinrichs 1980, Heinrichs and Mochida 1984, Wu et al. 2001, 2003, Yin et al. 2008), and; (3) stimulation of fecundity as a result of exposure to sublethal doses of pesticides (Chelliah and Heinrichs 1980, Heinrichs et al. 1982, Reissig et al. 1982b).

Dicyclic nitromethylene neonicotinoid with cis-configuration, 1-[(6-Chloropyridin-3-yl) methyl]-7-methyl-8-nitro-5-propoxy-1, 2, 3, 5, 6, 7-hexahydroimidazo [1, 2-α] pyridine, named paichongding, has exhibited higher insecticidal activities than imidacloprid (Li et al. 2009). It is especially active against sucking insects such as planthoppers and aphids, and it is considered to be an ideal candidate for development for the control of *N. lugens* (Li et al. 2009, Xu et al. 2009, Wu et al. 2009). However, little knowledge exists regarding the response of *N. lugens* to paichongding. In this study, our goal was establish a dose-mortality response of *N. lugens* and paichongding relative to various life stages of the insect.

Materials and Methods

An insecticide-susceptible strain of *N. lugens* has been maintained with rice seedlings at 28 ± 0.5°C, RH (70 ± 5) % and a photoperiod of 14:10 h (L:D) for more than 3 yrs at the Institute of Entomology, Sun Yat-Sen University, Guangzhou, China without any exposure to insecticide. *Nilaparvata lugens* nymphs and adults (3 d old) obtained from this colony were used as test insects in this study. Paichongding (10%) was purchased from Jiang Su Kesheng Group Co., Ltd. (Jiangsu, China).

A bioassay was conducted using the rice-stem dipping method (Zhuang et al. 1999, Wang et al. 2008). Paichongding was diluted in distilled water to 5 concentrations of 3.2, 1.6, 0.8, 0.4, 0.2 mg/L. Rice plants (about 60 d) at tillering phase were collected. Rice stems (about 10 cm in length) with roots were cut and washed thoroughly, and then air dried to remove residual water. Three rice stems were grouped and dipped into the respective paichongding solution for 30 sec, then allowed to air dry. After that, moistened sponges were used to wrap the rice roots. The treated rice stems were placed into a 500-ml plastic cup. Sixty adults or nymphs of specific group were introduced into each plastic cup using a vacuum device. A distilled water treatment served as a control. Both control and insecticide treatments were replicated 5 times. Mortality was observed at 12-h intervals and corrected according to Abbott's formula (Abbott 1925). The treated insects were maintained at 28 ± 0.5°C, RH 70 ± 5% and a photoperiod of 14:10 h (L:D). Individuals were considered dead if they showed no response after being gently prodded with a fine brush.

All data were analyzed by SPSS®, version 17.0, and the median lethal concentration and time estimates were determined by probit analysis. Significant differences were determined by using Tukey's multiple range test ($P < 0.05$).

Results and Discussion

The cumulative mortality of *N. lugens* in different concentrations of paichongding was concentration and time dependent (Fig. 1). Generally, the cumulative mortality of *N. lugens* from instars I-II to macropterous females increased as the concentration of paichongding increased. Significant differences ($P < 0.05$) were observed among different concentrations of paichongding treatment. For nymphs of different stages and adults of different forms, mortality levels were significantly different ($P < 0.05$) among most of concentrations at the same posttreatment interval. Insects treated with 3.2 mg/L showed significantly higher mortality than that observed with other concentrations of paichongding ($P < 0.05$). At 96 h after treatment, the observed mortality of instars I-II was 100, 98.89, 95.56, 87.78 and 74.44% at 3.2, 1.6, 0.8, 0.4 and 0.2 mg/L, whereas mortality of macropterous females was 87.78, 81.11, 78.89, 67.78 and 60.00%, for the respective concentrations.

The median lethal concentrations (LC_{50}) varied with insect stage (Table 1). At 72 h after treatment, the LC_{50} value was 0.13 mg/L ($\chi^2 = 8.63$; df = 13; $P > 0.05$) for instars I-II, 0.31 mg/L ($\chi^2 = 11.87$; df = 13; $P > 0.05$) for instars III-IV, 0.52 mg/L ($\chi^2 = 6.67$; df = 13; $P > 0.05$) for instar V, 0.90 mg/L ($\chi^2 = 9.07$; df = 13; $P > 0.05$) for brachypterous females, 0.86 mg/L ($\chi^2 = 7.97$; df = 13; $P > 0.05$) for brachypterous males, 1.26 mg/L ($\chi^2 = 7.96$; df = 13; $P > 0.05$) for macropterous females, and 1.21 mg/L ($\chi^2 = 6.43$; df = 13; $P > 0.05$) for macropterous males.

The median lethal times (LT_{50}) for nymphs and adults were dramatically decreased with increasing concentration of paichongding (Table 2). The LT_{50} values for adults were significantly longer than those for nymphs, macropterous adults especially macropterous females showed the greatest LT_{50} values. The LT_{50} for instars I-II decreased from 62.38 h ($\chi^2 = 14.60$; df = 13; $P > 0.05$) to 25.13 h ($\chi^2 = 31.78$; df = 13; $P > 0.05$) with the increasing concentration from 0.2 - 3.2 mg/L, whereas the LT_{50} for macropterous females were decreased from 92.67 h ($\chi^2 = 9.88$; df = 13; $P > 0.05$) to 52.65 h ($\chi^2 = 28.98$; df = 13; $P > 0.05$) over the same concentrations. The LT_{50} values for *N. lugens* instars III-IV and V at 0.2 mg/L were 73.62 h ($\chi^2 = 25.24$; df = 13; $P > 0.05$) and 74.40 h ($\chi^2 = 26.00$; df = 13; $P > 0.05$) and were reduced to 29.81 h ($\chi^2 = 27.03$; df = 13; $P > 0.05$) and 41.21 h ($\chi^2 = 27.77$; df = 13; $P > 0.05$) at 3.2 mg/L. The LT_{50} values for macropterous males, brachypterous females and males were also significantly reduced with increasing concentrations of paichongding.

Neonicotinoid insecticides possess high activity, broad spectrum and low toxicity for mammals and aquatic animals. They are widely used in controlling plant-sucking insects. Imidacloprid has been used to control *N. lugens* for more than 10 yrs in China. Its continued use has resulted in a gradual decrease of its efficacy, with the pest resistance levels significantly increased (Hirai 1993, Wang et al. 2009). Systemic properties and long residual activity of paichongding make it an ideal insecticide for potential development and use against sucking insect pests.

Our results revealed that instars I-II are easier to control with paichonding than instars III-IV, V and the adults with an observed toxicity by stage being instars I-II > instars III-IV-V > adults. These results are similar to those of Zhang et al. (2010) in their study of nitenpyram activity against *N. lugens*. Furthermore, macropterous adults

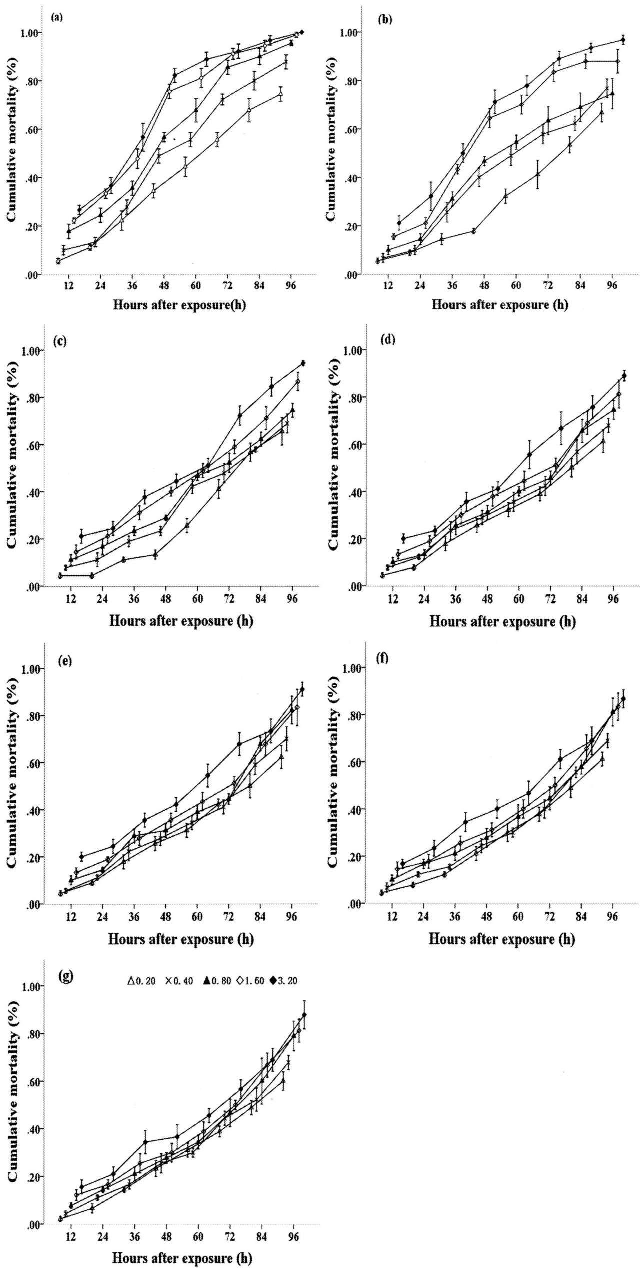

Fig. 1. Trends in mean mortalities (± SE) of *N. lugens* nymphs and adults after exposure to different concentrations of paichongding: instars I-II (a), instars III-IV (b) and instars V (c), brachypterous females (d) and brachypterous males (e), macropterous males (f) and macropterous females (g).

Table 1. Susceptibility of *N. lugens* nymphs and adults to paichongding.

Time	*N. lugens*	No	Slope ± SE	LC_{50} mg/L (95% CL)	χ^2	df	P
24 h	I-II	60	0.80 ± 0.11	7.00 (5.67 - 7.58)	4.07	13	> 0.05
	III-IV	60	0.78 ± 0.09	15.06 (12.18 - 17.29)	6.09	13	> 0.05
	V	60	0.74 ± 0.13	21.58 (18.56 - 23.98)	7.01	13	> 0.05
	BF	60	0.65 ± 0.06	67.16 (62.30 - 69.76)	6.28	13	> 0.05
	BM	60	0.55 ± 0.08	60.43 (58.68 - 61.89)	5.87	13	> 0.05
	MM	60	0.83 ± 0.10	75.35 (68.63 - 76.57)	8.58	13	> 0.05
	MF	60	0.93 ± 0.09	97.05 (87.53 - 98.93)	7.08	13	> 0.05
48 h	I-II	60	1.12 ± 0.12	0.46 (0.38 - 0.57)	9.51	13	> 0.05
	III-IV	60	1.17 ± 0.11	0.90 (0.71 - 1.16)	8.24	13	> 0.05
	V	60	0.79 ± 0.12	4.05 (3.41 - 4.27)	8.79	13	> 0.05
	BF	60	0.67 ± 0.04	13.04 (10.48 - 14.79)	6.33	13	> 0.05
	BM	60	0.58 ± 0.05	12.91 (9.24 - 13.98)	8.86	13	> 0.05
	MM	60	0.84 ± 0.07	15.93 (14.45 - 16.72)	5.43	13	> 0.05
	MF	60	0.90 ± 0.05	58.70 (56.66 - 60.14)	6.66	13	> 0.05

Table 1. Continued

Time	*N. lugens*	No	Slope ± SE	LC_{50} mg/L (95% CL)	χ^2	df	P
72 h	I-II	60	1.17 ± 0.18	0.13 (0.09 - 0.20)	8.63	13	> 0.05
	III-IV	60	1.21 ± 0.16	0.31 (0.25 - 0.41)	11.87	13	> 0.05
	V	60	0.63 ± 0.06	0.52 (0.48 - 0.61)	6.67	13	> 0.05
	BF	60	0.55 ± 0.04	0.90 (0.73 - 1.08)	9.07	13	> 0.05
	BM	60	0.92 ± 0.07	0.86 (0.68 - 1.01)	7.97	13	> 0.05
	MM	60	0.88 ± 0.04	1.21 (0.88 - 1.27)	6.43	13	> 0.05
	MF	60	0.85 ± 0.04	1.26 (0.94 - 1.48)	7.96	13	> 0.05

Time is the hours after treatment. No is the number of insects tested. CL is the 95% confidence limits. Slope ± SE is the slope ± SE. I-II (instars I-II), III-IV (instars III-IV) and V (instars V), brachypterous females (BF) and brachypterous males (BM), macropterous males (MM) and macropterous females (MF)

Table 2. LT_{50} values of *N. lugens* nymphs and adults to paichongding.

Concn	*N. lugens*	No	Slope ± SE	LT50 h (95% CL)	χ^2	df	P
0.2 mg/L	I-II	60	2.76 ± 0.23	62.38 (59.26 - 64.52)	14.60	22	>0.05
	III-IV	60	2.51 ± 0.15	73.62 (72.90 - 74.17)	25.24	22	> 0.05
	V	60	2.98 ± 0.23	74.40 (73.34 - 75.11)	26.00	22	> 0.05
	BF	60	2.36 ± 0.14	86.50 (81.79 - 88.05)	12.57	22	>0.05
	BM	60	2.37 ± 0.24	84.82 (77.46 - 87.66)	13.00	22	>0.05
	MM	60	2.46 ± 0.18	90.09 (87.98 - 95.40)	16.54	22	>0.05
	MF	60	2.68 ± 0.27	92.67 (89.86 - 96.58)	9.88	22	>0.05
0.4 mg/L	I-II	60	2.98 ± 0.19	47.79 (45.06 - 48.73)	15.93	22	> 0.05
	III-IV	60	2.64 ± 0.23	60.16 (57.09 - 61.18)	16.73	22	> 0.05
	V	60	2.35 ± 0.18	63.59 (59.19 - 64.68)	19.99	22	> 0.05
	BF	60	2.14 ± 0.12	76.84 (69.23 - 79.18)	17.45	22	>0.05
	BM	60	2.40 ± 0.18	75.22 (68.66 - 76.61)	18.73	22	>0.05
	MM	60	2.30 ± 0.15	81.68 (77.70 - 84.72)	23.47	22	>0.05
	MF	60	2.46 ± 0.15	83.29 (79.06 - 85.97)	16.15	22	>0.05

Table 2. Continued

Concn	*N. lugens*	No	Slope ± SE	LT50 h (95% CL)	χ^2	df	P
0.8 mg/L	I-II	60	2.91 ± 0.18	36.62 (33.08 - 38.17)	18.94	22	> 0.05
	III-IV	60	2.39 ± 0.12	53.39 (49.53 - 56.93)	17.20	22	> 0.05
	V	60	2.16 ± 0.21	60.58 (58.99 - 63.11)	20.77	22	> 0.05
	BF	60	2.23 ± 0.14	66.79 (64.07 - 67.59)	24.78	22	>0.05
	BM	60	2.32 ± 0.11	63.13 (59.31 - 64.81)	34.71	22	>0.05
	MM	60	2.20 ± 0.18	69.68 (65.91 - 72.64)	28.49	22	>0.05
	MF	60	2.39 ± 0.15	72.52 (70.63 - 73.56)	26.77	22	>0.05
1.6 mg/L	I-II	60	3.02 ± 0.29	28.72 (26.30 - 31.00)	29.13	22	> 0.05
	III-IV	60	2.80 ± 0.18	36.61 (33.27 - 38.94)	22.39	22	> 0.05
	V	60	2.23 ± 0.23	51.43 (47.47 - 52.10)	28.60	22	> 0.05
	BF	60	2.12 ± 0.17	59.38 (56.59 - 61.47)	28.42	22	> 0.05
	BM	60	2.16 ± 0.20	58.23 (55.74 - 60.07)	33.93	22	> 0.05
	MM	60	2.10 ± 0.12	61.62 (58.55 - 64.70)	22.67	22	> 0.05
	MF	60	2.20 ± 0.17	62.81 (60.52 - 64.37)	26.37	22	> 0.05

Table 2. Continued

Concn	*N. lugens*	No	Slope ± SE	LT50 h (95% CL)	χ^2	df	P
3.2 mg/L	I-II	60	3.08 ± 0.22	25.13 (22.80 - 28.29)	31.78	22	> 0.05
	III-IV	60	2.88 ± 0.21	29.81 (26.86 - 32.67)	27.03	22	> 0.05
	V	60	2.34 ± 0.09	41.21 (34.87 - 44.98)	27.77	22	> 0.05
	BF	60	2.15 ± 0.13	44.86 (39.64 - 46.80)	36.36	22	> 0.05
	BM	60	2.15 ± 0.15	44.46 (38.32 - 45.27)	35.73	22	> 0.05
	MM	60	2.06 ± 0.19	50.42 (43.78 –52.37)	32.38	22	> 0.05
	MF	60	2.12 ± 0.21	52.65 (49.29 - 54.87)	28.98	22	> 0.05

Concn is the concentration of paichongding. Time is the hours after treatment. No is the number of insects tested. CL is the 95% confidence limits. Slope ± SE is the slope ± SE. I-II (instars I-II), III-IV (instars III-IV) and V (instars V), brachypterous females (BF) and brachypterous males (BM), macropterous males (MM) and macropterous females (MF) appear to be less sensitive to paichonding than the brachypterous adults, and females are less susceptible than males of the same wing type.

Indeed, susceptibility of macropterous females to insecticides should be used as a standard for insecticide development and assessment. *Nilaparvata lugens* is a long-distance migratory rice pest in temperate eastern Asia (Cheng et al. 1979). Insect migration is an important reason for recurrent outbreaks due to the benefit of varying environments (Ma 1964, 1982). The reproduction rate of *N. lugens* populations significantly increases after long-distance migratory flight (Shen and Cheng 1998). Previous studies demonstrated that the flight capacity of adult females and males was enhanced markedly by insecticide treatment, especially that of adult females (Zhao et al. 2011). Outbreaks of *N. lugens* were associated with the number of immigrant insects in combination with several ecological factors. It was suggested that macropterous female migration is one kind of coping mechanism of *N. lugens* response to insecticide stress.

In conclusion, our data demonstrate that paichongding has significant toxicity effects on *N. lugens*, especially on *N. lugens* nymphs. It appears to be a potential candidate for further development for the management of *N. lugens* in rice production.

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