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

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Abstract Laboratory assays were conducted to assess the potential of pymetrozine, a novel azomethine pyridine insecticide, against the brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae). All nymphal instars were treated with the pymetrozine concentrations of 400, 200, 100, 50, 25 mg/l, and then maintained in incubators at 26 ± 1°C on a photoperiod of 16:8 h (L: D). At 24, 48, 72, and 120 h after treatment, mortality was recorded. Greater numbers of nymphs were killed at a faster rate with the higher concentrations (mortality of 70.7% with 400 mg/l and 63.8% with 200 mg/l at 24 h, first -second) than at the median concentrations (mortality of 51.7% with 100 mg/l and 50% with 50 mg/l at 24 h, first -second) and the lower concentration (mortality of 25.9% with 25 mg/l at 24 h, first -second), whereas mortality in the control remained low (3.3% with distilled water at 24 h, first -second). The first and second instars were the most susceptible with median lethal concentrations [LC_{50}] of 76.3, 35.5, 26.6 and 21.96 mg/L at 24, 48, 72 and 120 h after treatment, respectively. Fifth-instars were the least susceptible with the LC_{50} values of 5,887.53, 758.41, 236.15 and 67.23 mg/L at 24, 48, 72 and 120 h after treatment, respectively. The LC_{50}s of the third - fourth instars were 1737.89, 601.6, 96.21 and 50.14 mg/L at 24, 48, 72 and 120 h after treatment, respectively, and were significantly greater than those of the first - second instars. These results indicate that pymetrozine would be an effective alternative for the control of brown planthopper.

Key Words brown planthopper, pymetrozine, toxicity, laboratory assays, lethal concentrations

Brown planthopper, *Nilaparvata lugens*(Stål) (Homoptera: Delphacidae), is one of the most economically important insects of rice in most countries. It damages rice directly by removing nutrients and indirectly by transmitting rice pathogens, e.g., ragged stunt virus and grassy stunt virus (Sogawa 1982, Ghaffar et al. 2011). Integrated pest management (IPM) tactics, including pest-resistant cultivars, fertility management, naturally-occurring biological control, prudent use of insecticides when needed, have long been recommended for *N. lugens* management (Bottrell and Schoenly 2012).

Brown planthopper populations in different areas have reportedly developed resistance to almost all insecticides used in rice production, including but not restricted to imidacloprid and buprofezin. Imidacloprid with its unique chemical and biological properties, like broad-spectrum insecticidal activity, low application rates, and safety

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to natural enemies (Gorman et al. 2010), has become the first choice for brown planthopper control for growers all over the world. However, laboratory assays demonstrated the potential of brown planthopper to develop high levels of resistance to imidacloprid and, subsequently, resistance in field populations was reported in China from 2005 - 2007, only 10 yrs after the widespread use of imidacloprid (Wen et al. 2009).

Alternative chemistries must be developed for use against brown planthopper to better manage the development of resistance to imidacloprid. Pymetrozine is an azomethine pyridine with a spectrum of activity against sucking pests such as aphids, whiteflies and planthoppers. Pymetrozine irreversibly halts aphid feeding by blocking stylet penetration into the plant tissue, thus, causing death by starvation. It is systemic, highly mobile within plants, and has little, if any, direct impact on natural enemies (Sechser et al. 2002, Boina et al. 2011, Joseph et al. 2011). Furthermore, pymetrozine prevents transmission of cauliflower mosaic caulimovirus and reduces *potato virus Y* transmission on tobacco plants (Bedford et al. 1998, Margaritopoulos et al. 2010) with its impact on feeding mechanism. Development of pymetrozine resistance will likely be specific and will not impact development of resistance to imidacloprid, a neonicotinoid (Gorman et al. 2010). There is little information on the efficacy of pymetrozine against brown planthoppers; so, the objective of this study was to determine the effects of pymetrozine on the nymphal stages of brown planthopper using laboratory immersion assays.

Materials and Methods

Insects used for these assays were from a culture of an insecticide-susceptible strain of brown planthopper that had been maintained on rice seedlings at 26 ± 1°C with a 16:8 h (L:D) photophase for more than 3 yrs at the Institute of Entomology, Sun Yat-Sen University, Guangzhou, China, with no exposure to insecticides. A previously described rice stem dipping assay method was used (Zhuang et al. 1999, Zhang et al. 2010). Briefly, pymetrozine, under the brand name of Dingfeng™ (Syngenta AG. Switzerland) with a purity of 50% was diluted in distilled water to concentrations of 400, 200, 100, 50, and 25 mg/l. Rice plants at tillering stage were washed thoroughly, and rice stems (about 10 cm long) with roots were cut and air dried. Three rice stems were grouped and dipped into an appropriate insecticide solution for 30 sec. After the rice stems were air dried, moistened cotton was used to wrap the rice roots, and the stems were then placed into individual 500-ml plastic cups.

Brown planthopper nymphs were grouped into 3 groups: first - second nymphal instars, third - fourth nymphal instars, and fifth nymphal instars. Thirty nymphs of each group were placed into an appropriate plastic cup containing treated rice stems using a vacuum device. Each treatment concentration was replicated 3 times, and a distilled water treatment was used as a control. Treated insects were maintained in incubators maintained at 26 ± 1°C on a photoperiod of 16:8 h (L: D). Mortality of the nymphs was recorded at 24, 48, 72, and 120 h after treatment. Nymphs were considered dead if they failed to move after being gently touched with a fine brush.

Mortality 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 13.0. Mortality was

corrected using Abbott's formula, if necessary. Lethal concentrations (LC_{30}, LC_{50} and LC_{90}) were calculated using probit analysis.

Results

Among the nymphal age groups assayed, the first - second instars were the most susceptible with LC_{50} values of 76.26 ($\chi 2 = 3.05$; df = 3; $P > 0.05$), 35.5 ($\chi 2 = 3.43$; df = 3; $P > 0.05$), 26.6 ($\chi 2 = 2.55$; df = 3;$P > 0.05$), and 21.96 ($\chi 2 = 6.53$; df = 3; $P > 0.05$) mg/l at 24, 48, 72 and 120 h after treatment, respectively (Table 1). The fifth instars were the least susceptible to pymetrozine with the LC_{50} values of 5,887.53 ($\chi 2 = 0.48$; df = 3; $P > 0.05$), 758.41 ($\chi 2 = 2.86$; df = 3; $P > 0.05$), 236.15 ($\chi 2 = 1.19$; df = 3; $P > 0.05$) and 67.23 ($\chi 2 = 8.46$; df = 3; $P > 0.05$) mg/l at 24, 48, 72 and 120 h after treatment, respectively. The LC_{50}s of the third - fourth instars were 1737.89, 601.6, 96.21and 50.14 mg/l at 24, 48, 72 and 120 h after treatment, respectively, and were significantly greater than those of the first - second instars.

Greater numbers of nymphs were killed at a faster rate with the higher concentrations (mortality of 70.7% with 400 mg/l and 63.8% with 20 0mg/l at 24 h, first -second) than at the median concentratons (mortality of 51.7% with 100 mg/l and 50% with 50 mg/l at 24 h, first -second) and lower concentration (25.9% with 25mg/l at 24h, first -second) (Fig. 1), whereas mortality in the control (3.3% at 24 h, first -second) remained low. No significant differences in mortality of the first - second instars treated with 400 and 200 mg/L were observed at 24 and 48 h after treatment, but 100% mortality was observed by 72 h after treatment. However, significant differences ($P < 0.05$) in mortality were observed among the other 3 concentrations of pymetrozine and the control. For third - fourth and fifth instars, mortality levels were significantly different ($P < 0.05$) among all 3 concentrations of pymetrozine and the control. At 72 h after treatment, mortality in the third – fourth instars and the fifth instars treated with 400 and 200 mg/l was significantly higher than that observed in the other concentrations and the control.

Discussion

The requirements for an insecticide intended for modern pest control are an acceptable spectrum of activity, a new mode of action and safety to the environment. Pymetrozine is such a novel type of chemistry that selectively halts the feeding of hemipteran and homopteran insects (i.e., aphids, whiteflies, planthoppers, etc.), shows biological activity against brown planthopper and the aphid *Lipaphis erysini* (Kaltenbach) (Gu et al. 2000, Rezaei et al. 2007, Lashkari et al. 2007), is a contact poison that can be absorbed and transported in the insects, possesses residual activity, and is active against juvenile and adult stages. Laboratory and field studies show that pymetrozine is moderately toxic to *Cyrtorhinus lividipennis* (Reuter) (Hemiptera: Miridae) but safe to spiders (Acari) that are naturally-occurring predators of brown planthopper.

Initial feeding choice experiments, electrical penetration graph (EPG) data, and feeding recovery data reveal that pymetrozine has no deterrent or antifeeding effects on brown planthopper and did not block the stylet probing and xylem ingestion. However, phloem ingestion was markedly decreased following exposure, and the recovery of the treated insects from the inhibitive effects was slow (He et al. 2010, Lang et al. 2007).

Table 1. Susceptibility of different nymphal instars of *N. lugens* to pymetrozine

Time*(h)	stages	LC_{30} (mg/L) (95%CL**)	LC_{50} (mg/L) (95%CL)	LC_{90}(mg/L) (95%CL)	Slope±SE***
24	1 - 2	19.8 (13.57 - 28.89)	76.26 (52.26 - 111.29)	2109.6 (1445.52 - 3,078.77)	0.89 ± 0.08
	3 - 4	390.08 (237.78 - 639.96)	1737.89 (1059.22 - 2851.39)	68742 (41,874 - 112,851)	0.80 ± 0.11
	5	1042 (590.39 - 1839.05)	5887.53 (3,335.32 - 10,393)	418000 (236,608 - 738,454)	0.69 ± 0.13
48	1 - 2	16.54 (12.98 - 21.08)	35.5 (27.86 - 45.24)	232.79 (182.69 - 296.63)	1.57 ± 0.05
	3 - 4	162.4 (107.17 - 246.1)	601.6 (396.98 - 911.7)	15109 (9,967.45 - 22,903)	0.91 ± 0.09
	5	173.02 (117.96 - 253.77)	758.41 (517.07 - 1112.39)	28823 (19,647 - 42,285)	0.81 ± 0.08
72	1 - 2	15.27 (12.5 - 18.64)	26.60 (21.78 - 32.48)	104.32 (85.42 - 127.4)	2.16 ± 0.04
	3 - 4	38.85 (29.63 - 50.96)	96.21 (73.36 - 126.18)	896.48 (683.55 - 1175.74)	1.32 ± 0.06
	5	59.48 (42.75 - 82.77)	236.15 (169.71 - 328.58)	7032.71 (5,053.72 - 9,786.65)	0.87 ± 0.07
Time*(h)	stages	LC_{30} (mg/L) (95%CL**)	LC_{50} (mg/L) (95%CL)	LC_{90}(mg/L) (95%CL)	Slope±SE***
120	1 - 2	12.86 (10.48 - 15.78)	21.96 (17.90 - 26.94)	82.03 (66.87 - 100.63)	2.24 ± 0.05
	3 - 4	26.18 (21.2 - 32.34)	50.14 (40.59 - 61.93)	248.13 (200.89 - 306.48)	1.84 ± 0.05
	5	28.12 (22.7 - 34.82)	67.23 (54.28 - 83.27)	574.83 (464.09 - 712.01)	1.37 ± 0.05

*Time is the hours after treatment. **CL is the 95% confidence limits. ***Slope± SE is the slope ± standard error.

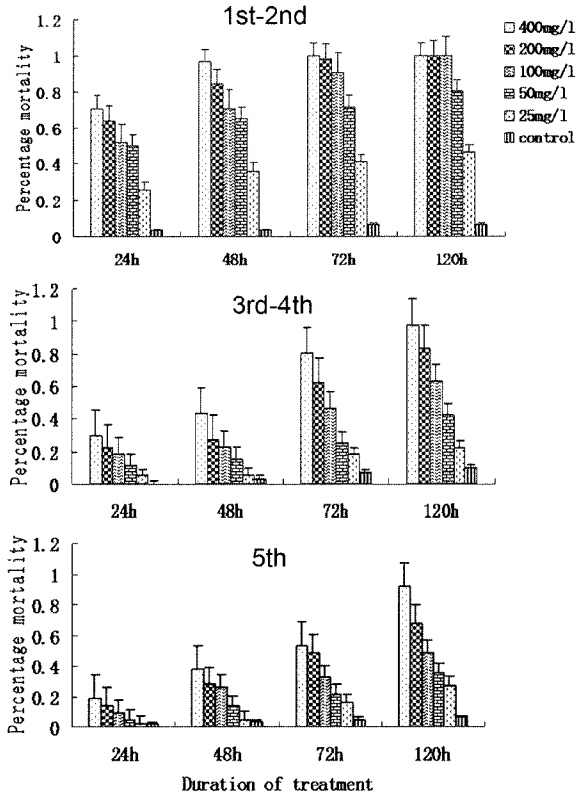

Fig. 1. Corrected mortality of brown planthopper instars following treatment with pymetrozine.

Our results show that pymetrozine affected various nymphal instars of brown planthopper at concentrations ranging from 25 - 400 mg/L. These data suggest that pymetrozine was not highly toxic to the insect, e.g., LC_{50} values showed gradual decreases with time after treatment and with older insect stages. The LC_{50} with first-second, third-fourth, and fifth instars of 21.96, 50.14 and 67.23 mg/l, respectively, 120 h after treatment is similar to the results of Xu et al. (2010).

In conclusion, pymetrozine had significant effects on brown planthopper nymphs by apparent inhibition of feeding resulting starvation. Cumulative mortality was related to both time after exposure and concentration. The response to time is likely tied to the mechanism of action of this insecticide. Pymetrozine should be investigated further for potential development against the brown planthopper.

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