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Reduced Toxicities of Insecticides Against Brown Planthopper (*Nilaparvata lugens* Stål) Collected from Rice Fields in Bangladesh

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ABSTRACT

The brown planthopper, *Nilaparvata lugens* (Stål), caused severe yield losses in rice production in many Asian countries. Chemical control is the key component of the integrated pest management tool to control rice brown planthopper (BPH). However, reduced toxicity of insecticides against this pest has been reported. We investigated the BPH mortality rate and the evolution of insecticide resistance in BPH collected from Bangladesh to commonly used insecticides by the rice-stem dipping method. Both the BPH laboratory strain and field populations were used in this study. The mortality rate of the Gazipur field population ranges from 15% to 80%, after exposure to imidacloprid in different concentrations. The highest mortality was found in response to dinotefuran followed by chlorpyrifos and etofenprox. The mortality rate increased after the time of exposure in relation to the increased concentrations of the insecticides. Additionally, the selected BPH strain shows a higher level of resistance to imidacloprid (resistance ratio = 179.45-fold), while other field populations show low to moderate levels of resistance. Increased detoxification enzyme activity, cytochrome P450 monooxygenase, was found in imidacloprid-resistant BPH. This study provides important information regarding the reduced toxicities of frequently used insecticides to control BPH.

Keywords: Brown planthopper, chemical control, mortality, resistance ratio, mechanism.

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INTRODUCTION

Rice is a major cereal crop around the globe after wheat. However, the production of rice faces constraints by several factors such as floods, drought, weeds, pests, and diseases (Balasubramanian, Sie, Hijmans, & Otsuka, 2007; Haque et al., 2021). Diseases like bacterial blight, blast, and destructive pests like brown planthopper (BPH), stem borer, and green leafhopper hampering rice production in many Asian countries (Seck, Diagne, Mohanty, & Wopereis, 2012). Among them the BPH (*Nilaparvata lugens* Stål) is considered a key rice pest that caused significant yield losses in major rice-growing areas, such as China, Vietnam, Thailand, Japan, Korea, India, Bangladesh, and the Philippines (Hereward et al, 2020; Nguyen et al, 2021). The sap-sucking nature of the pest caused the complete drying of the rice plants, widely known as 'hopper-burn' (Datta et al, 2021a). Chemical control is the primary pest management strategy to manage this pest. The key chemical insecticides used to control BPH are neonicotinoids, pyrethroids, carbamates, organophosphates, and insect growth regulators (Fujii et al, 2020; Datta & Banik, 2021). However, the toxicity against BPH has been reduced and the pest also evolved low to high levels of resistance to commonly used insecticides (Datta et al, 2021b). According to Arthropod Pesticide Resistance Database BPH evolved resistance to 33 active ingredients of insecticides (APRD, 2021). Neonicotinoids are the principal group of insecticides for BPH control followed by pyrethroids, carbamates, and organophosphates (Matsuda, Ihara, & Sattelle, 2020). Islam et al (2009) mentioned the first 'hopper-burn' in rice fields of Bangladesh in 1976 and an outbreak of BPH was reported in 1983. Although, chemical insecticide is widely used to control rice pests including BPH in Bangladesh, very limited studies have been carried out to monitor the toxicities of insecticides commonly used in this region. Therefore, this study aimed to investigate the mortality rates of BPH in response to common insecticides, the toxicities of different insecticides, and the metabolic mechanism that evolved in BPH.

MATERIALS AND METHODS

Insect

A total of four BPH strains were used in this study. Two field populations were collected from the rice fields of Gazipur and Dinajpur, and two laboratory strains (one susceptible and one resistant selected strain) of BPH were reared for several generations. The susceptible strain (Sus) was reared in the laboratory without exposure to any insecticides, in contrast, the resistance selected strain was reared with exposure to imidacloprid for eight generations. All of the collected insects were reared on susceptible rice variety, BR3, in a controlled environment (16/8 hour light/dark photoperiod).

Insecticides

Details of insecticides tested against brown planthopper were shown in Table 1. The commercial-grade insecticide was directly dissolved in water in a series of five to seven concentrations (mg/L). Water solution without any chemicals was considered as the control.

Table 1. Insecticides and their classifications used to monitor mortality rates.

Insecticide group	Insecticides	Purity	Classification[1]	Source
Neonicotinoids	Imidacloprid	20% SC	4A	Auto Crop Care Ltd., Bangladesh
	Dinotefuran	20% SC		ACI Formalities Ltd., Bangladesh
Organophosphate	Chlorpyrifos	22% SC	1B	Auto Crop Care Ltd., Bangladesh
Pyrethroids	Etofenprox	30% EC	3A	New Agro industries Ltd., Bangladesh

[1]Classification has been done by Insecticide resistance action committee (IRAC)

Bioassay

The biological assay of the rice-stem dipping method was performed with the fourth-instar nymphs of BPH. Rice plants at the tillering stage were used in monitoring mortality rates and detecting levels of resistance. Rice plants were washed thoroughly and then kept to dry. These rice stems are then dipped into an insecticide solution for up to 30 seconds and let dry. For each replicate, twenty-five nymphs of BPH were transferred onto a plastic bottle containing the treated rice plant by a homemade aspiration device. There were at least three replications for each concentration. Mortality rates of one field population (Gazipur) were assessed after 48, 72, and 96 hours (hr) of insecticide treatments. The nymphs were considered as dead if they were unable to move after gentle pushing with a soft brush.

Enzymatic assay

Biochemical assays were done to measure enzyme activities in the imidacloprid-resistant BPH strain. The activities of cytochrome P450 monooxygenase (P450) were determined according to the previously described method with the minor modification (Asperen, 1962). The protein concentration of all the enzyme solutions was determined by the Bradford method (Bradford, 1976).

Statistical analysis

Abbott's formula was used to correct the percent mortality rate of treated BPH (Abbott, 1925). The program DPS software (Data Processing System, version v15.10) was used for probit analysis to determine LC_{50} (median lethal concentration) value. The resistance ratio (RR) was calculated by dividing the LC_{50} value of a field population by the corresponding LC_{50} value of susceptible BPH strain. The resistance ratio was classified as: RR>100-fold as a high resistance level, 10<RR<100-fold as a moderate resistance level, 5<RR<10-fold as a low resistance level, and RR<5-fold as susceptibility.

RESULTS

Mortality rates of BPH to different insecticides

Mortality rates of the BPH field population (Gazipur) to imidacloprid, dinotefuran, chlorpyrifos, and etofenprox, in 48, 72, and 96 hr of insecticide application have been

presented in Fig. 1. The mortality rates of BPH when exposing to the insecticides increased with the time of treatment. The highest mortality rate was found in BPH exposed to dinotefuran after 96 hr of insecticide application followed by imidacloprid, chlorpyrifos, and etofenprox. In all four insecticides exposure, the mortality rate was significantly higher in the highest insecticide concentration and after 96 hr of insecticide application.

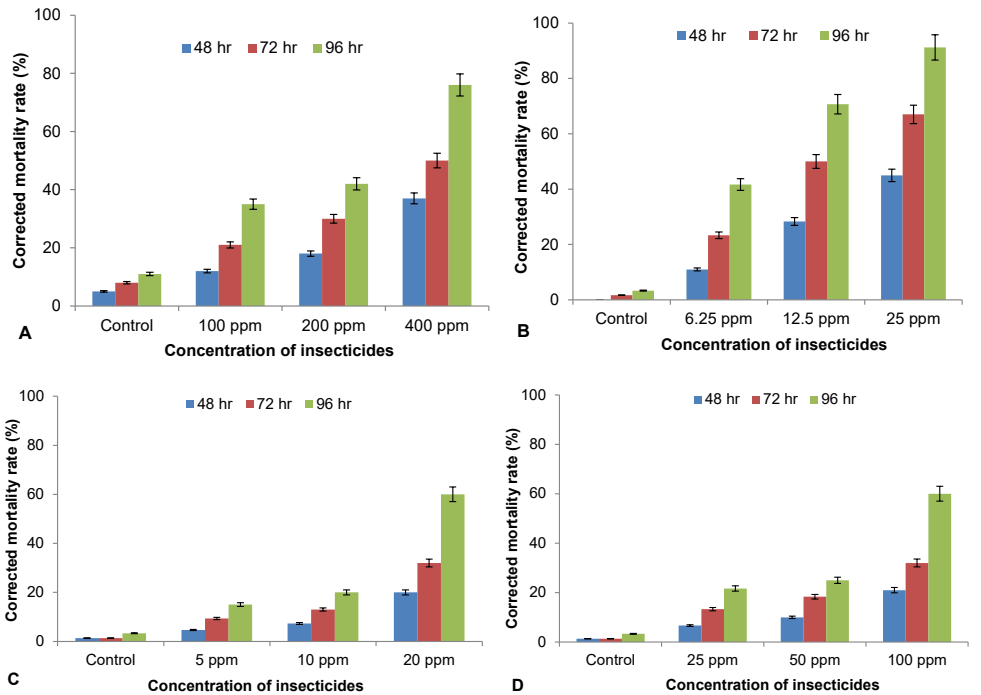

Fig 1. Mortality rates of Gazipur BPH field population after the application of insecticides, (A) imidacloprid, (B) dinotefuran, (C) chlorpyrifos, (D) etofenprox, at different doses.

Relative toxicity of different insecticides against BPH

The LC_{50} values of laboratory susceptible BPH strain to imidacloprid, dinotefuran, chlorpyrifos, and etofenprox were 1.54, 0.98, 1.25, and 9.37, respectively. The LC_{50} values of laboratory susceptible BPH strain were used as a baseline to calculate the resistance ratio of BPH (Table 2). The BPH strain, selected with imidacloprid for eight generations, showed a higher level of resistance to imidacloprid (RR = 179.45-fold), followed by dinotefuran (RR = 22.93-fold) and etofenprox (RR = 15.06-fold). The Gazipur field population showed low to moderate levels of resistance to different insecticides, with the lowest resistance to chlorpyrifos (6.31-fold), and a moderate resistance to imidacloprid (63.42-fold). Additionally, the Dinajpur BPH field population showed low levels of RR to etofenprox (8.53-fold), chlorpyrifos (9.30-fold), and dinotefuran (9.70-fold), while a moderate level of resistance to imidacloprid (87.12-fold) (Table 2).

Table 2. Toxicity of different insecticides to lab strain and field collected BPH population.

Strains	Insecticides	Slope±SE	LC_{50}	RR
Lab[1]	Imidacloprid	1.29±0.17	1.54	1
	Dinotefuran	2.21±0.20	0.98	1
	Chlorpyrifos	1.77±0.19	1.25	1
	Etofenprox	1.89±0.15	9.37	1
Selection[2]	Imidacloprid	2.42±0.30	276.36	179.45
	Dinotefuran	2.44±0.21	22.47	22.93
	Etofenprox	2.08±0.19	141.09	15.06
Field - Gazipur	Imidacloprid	2.12±0.25	97.67	63.42
	Dinotefuran	1.79±0.24	13.34	13.61
	Chlorpyrifos	1.02±0.20	7.89	6.31
	Etofenprox	1.78±0.30	82.67	8.82
Field - Dinajpur	Imidacloprid	2.39±0.32	134.17	87.12
	Dinotefuran	1.40±0.24	9.51	9.70
	Chlorpyrifos	1.52±0.30	11.62	9.3
	Etofenprox	1.43±0.20	79.89	8.53

[1]reared for more than 15 generations in controlled environment without any contact to insecticides

[2]BPH strain selected with imidacloprid for eight generations

Enzymatic activity of resistant BPH

Metabolic enzyme activity of P450 in imidacloprid-resistant BPH strain has been measured and compared with laboratory susceptible strain (Fig. 2). More than double fold increase (2.14-fold increase) in P450 activity in BPH resistant strain has been found compared to susceptible strain.

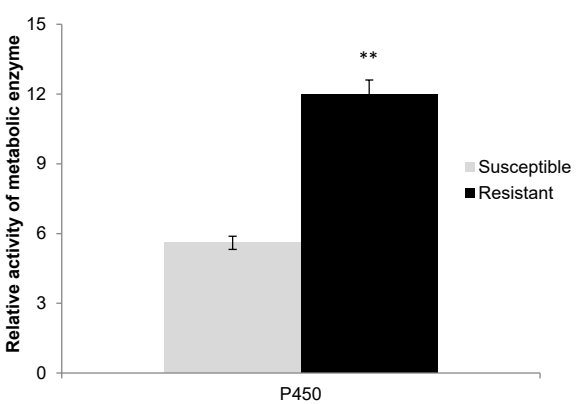

Fig. 2. Relative activity of P450 enzyme in imidacloprid-resistant BPH compared to laboratory susceptible BPH strain. Significant difference was indicated by asterisks above the error bar determined by student t-test ($P < 0.05$).

DISCUSSION

Currently, chemical control is considered as the key measure to manage major crop pests including BPH, small brown planthopper, stem borer, rice bugs, etc (Sparks & Lorsbach, 2017; Datta et al, 2021a). However, continuous and heavy use of chemical insecticides caused reduced toxicities of commonly used insecticides against BPH. Neonicotinoids became the more efficient and popular insecticides to control brown planthopper across the rice-producing countries in Asia (Ihara & Matsuda, 2018). Particularly, in Bangladesh more farmers are using neonicotinoids, insect growth regulators, and combinations of different insecticides to control BPH despite their reduced toxicity (Datta et al, 2021a). However, an increasing trend of resistance to commonly used insecticides was recently reported, which is worrying for other rice-producing countries (Khan, Kaleem-Ullah, Siddiqui, & Ali, 2020; Chakrabarty et al, 2022). In this study, we determined the mortality rates of BPH after exposure to insecticides (48, 72, and 96 hr of exposure), and also toxicities of insecticides and the metabolic enzyme activities have been measured. Our previous study showed low to extremely high levels of resistance has been evolved in BPH collected from three locations in Bangladesh. Therefore, in this study, we have collected BPH field populations that are different from previous locations. The mortality rates of BPH increased with time after exposure to insecticides, which is also related to the concentration of insecticides (Fig. 1). Banks, Banks, Joyner, & Stark (2008) presented a model with time-varying mortality rates with the levels of insecticide exposure. The mortality rates increased with the time after exposure but the rates may decline or stable after a certain period of time. There might be different reasons for the dissimilar mortality rates of BPH. One reason might be due to the mode of action and the other is the susceptibility of the pest to insecticides.

Extremely high levels of resistance to imidacloprid, and moderate resistance to dinotefuran had evolved in BPH collected from Bangladesh in 2020 (Datta et al, 2021a). The development of insecticide resistance to neonicotinoids in BPH has also been reported across Asian countries (Ding et al, 2013; Sanada-Morimura et al, 2019). In our investigation, we also found that BPH developed low to moderate levels of resistance to chlorpyrifos, etofenprox, dinotefuran, and imidacloprid (Table 2). The BPH has the potential to develop a higher level of resistance when constantly exposed to certain insecticides, for instance, we found a high level of resistance in BPH to imidacloprid after selection for several generations. Resistance in BPH to chlorpyrifos increased after selection for a particular period of time and the resistance ratio was significantly higher in resistant BPH strain (Lu et al, 2017).

Many studies identified metabolic resistance mechanism, increased detoxification enzyme, as the main mechanism that contributes to resistance development in BPH. Increased enzyme activities of P450, GST, and EST were found responsible for resistance development in BPH (Datta & Banik 2021). We found increased activities of P450 in imidacloprid resistant-BPH. This indicates that enhancement of P450 activity may contribute to imidacloprid resistance development in BPH. This result agrees with the previously reported data where P450 was suggested as the

key detoxification enzyme (Datta et al, 2021a). However, we did not investigate the molecular mechanisms and the candidate genes that encoded enzymatic activities involved in resistance development in BPH. Further studies are required to understand the molecular mechanism involved in resistant BPH strains.

Our data indicated that mortality rates increased as the exposure time and concentration of insecticides increased. There are also possibilities of rapid resistance development in BPH. To assess the mechanisms, we measured the metabolic enzyme activities and found enhanced P450 activity in imidacloprid-resistant BPH. This study outlines the importance of regular monitoring that is required to understand the trend of reduced toxicities. The finding will assist in future studies to understand the molecular mechanism of resistance development.

DECLARATIONS

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Conflict of Interest The authors declare that they have no conflict of interest.

Ethics Approval The authors did not perform any study with animals or humans' participants to prepare this manuscript.

REFERENCES

Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18, 265-267.

APRD. (2021). *Arthropod Pesticide Resistance Database*. Available https://www.pesticideresistance.org/search.php (Accessed 24 November, 2021).

Asperen, K. (1962). A study of housefly esterases by means of a sensitive colorimetric method. *Journal of Insect Physiology,* 8(4), 401-416.

Balasubramanian, V., Sie, M., Hijmans, R.J., & Otsuka, K. (2007). Increasing rice production in sub-Saharan Africa: challenges and opportunities. *Journal of Advances in Agronomy*, 94, 55-133.

Banks, H.T., Banks, J.E., Joyner, S.L., & Stark, J.D. (2008). Dynamic models for insect mortality due to exposure to insecticides. *Mathematical and Computer Modeling,* 48(1-2), 316-332.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry,* 72(1), 248-254. doi:https://doi.org/10.1016/0003-2697(76)90527-3

Chakrabarty, S., Chakraborty, P., Islam, T., Islam, A.K.M., Datta, J., Bhattacharjee, T., Minghui, J., & Xiao, Y. (2022). *Bacillus thuringiensis proteins: Structure, mechanism and biological control of insect pests*. In *Bacilli in Agrobiotechnology* (pp. 581-608). Springer, Cham.

Datta, J. & Banik, S.C. (2021). Insecticide resistance in the brown planthopper, *Nilaparvata lugens* (Stål): mechanisms and status in Asian countries. *Journal of the Entomological Research Society,* 23(3), 225-238. doi:0.51963/jers.v23i3.2016

Datta, J., Monsur, M.B., Chakraborty, P., Chakrabarty, S., Fahad, S., Hossain, A., Mondal, F.M., Ahmed, S., Ali, M.P., & Sabagh, A. (2021b). *Obstacle in controlling major rice pests in Asia: insecticide resistance and the mechanisms to confer insecticide resistance*. In S. Fahad, O. Sönmez, S. Saud, D. Wang, C. Wu, M. Adnan, M. Arif, & Amanullah (Eds.), *Engineering Tolerance in Crop Plants Against Abiotic Stress* (1st ed., pp. 336): CRC Press.

Datta, J., Wei, Q., Yang, Q., Wan, P.J., He, J.C., Wang, W.X., Lai, F.X., Ali, M.P., & Fu, Q. (2021a). Current resistance status of the brown planthopper *Nilaparvata lugens* (Stål) to commonly used insecticides in China and Bangladesh. *Crop Protection*, 150, 105789. doi:10.1016/j.cropro.2021.105789

Ding, Z., Wen, Y., Yang, B., Zhang, Y., Liu, S., Liu, Z., & Han, Z. (2013). Biochemical mechanisms of imidacloprid resistance in *Nilaparvata lugens*: over-expression of cytochrome P450 *CYP6AY1*. *Insect Biochemistry and Molecular Biology,* 43(11), 1021-1027. doi:10.1016/j.ibmb.2013.08.005

Fujii, T., Sanada-Morimura, S., Oe, T., Ide, M., Thanh, D.V., Chien, H.V., Tuong, P.V., Loc, P.M., Cuong, L.Q., Liu, Z.W., Zhu, Z.R., Li, J.H., Wu, G., Huang, S.H., Estoy, G.F., Sonoda, J.S., & Matsumura, M. (2020). Long-term field insecticide susceptibility data and laboratory experiments reveal evidence for cross resistance to other neonicotinoids in the imidacloprid-resistant brown planthopper *Nilaparvata lugens*. *Journal of Pest Management Science,* 76(2), 480-486.

Haque, M.M., Datta, J., Ahmed, T., Ehsanullah, M., Karim, M.N., Akter, M.S., Iqbal, M.A., Baazeem, A., Hadifa, A., Ahmed, S., & Sabagh, A.E. (2021). Organic amendments boost soil fertility and rice productivity and reduce methane emissions from paddy fields under sub-tropical conditions. *Sustainability,* 13(6). doi:10.3390/su13063103

Hereward, J.P., Cai, X., Matias, A.M.A., Walter, G.H., Xu, C., & Wang, Y. (2020). Migration dynamics of an important rice pest: The brown planthopper (*Nilaparvata lugens*) across Asia-Insights from population genomics. *Journal of Evolutionary applications,* 13(9), 2449-2459.

Ihara, M. & Matsuda, K. (2018). Neonicotinoids: molecular mechanisms of action, insights into resistance and impact on pollinators. *Current Opinion in Insect Science,* 30, 86-92.

Islam, Z., Catling, D., Hasan, M., Haque, S.S., Begum, M.A., & Haq, M. (2009). Influence of the green revolution on the insect pests of rice: with particular reference to Bangladesh. *Journal of Outlooks on Pest Management,* 20(1), 37-43.

Khan, M.M., Kaleem-Ullah, R.M., Siddiqui, J.A., & Ali, S. (2020). Insecticide resistance and detoxification enzymes activity in *Nilaparvata lugens* Stål against neonicotinoids. *Journal of Agricultural Science, 12*(5). doi:10.5539/jas.v12n5p24

Lu, K., Wang, Y., Chen, X., Zhang, Z., Li, Y., Li, W., & Zhou, Q., (2017). Characterization and functional analysis of a carboxylesterase gene associated with chlorpyrifos resistance in *Nilaparvata lugens* (Stål). *Journal of Comparative Biochemistry Physiology Part C: Toxicology Pharmacology*, 203, 12-20.

Matsuda, K., Ihara, M., & Sattelle, D. (2020). Neonicotinoid insecticides: molecular targets, resistance, and toxicity. *Annual Review of Pharmacology & Toxicology,* 60, 241-255.

Nguyen, C.D., Zheng, S.H., Sanada-Morimura, S., Matsumura, M., Yasui, H., & Fujita, D. (2021). Substitution mapping and characterization of brown planthopper resistance genes from indica rice variety, 'PTB33' (*Oryza sativa* L.). *Breeding Science*, 21034.

Sanada-Morimura, S., Fujii, T., Chien, H.V., Cuong, L.Q., Estoy, G.F., Jr., & Matsumura, M. (2019). Selection for imidacloprid resistance and mode of inheritance in the brown planthopper, *Nilaparvata lugens*. *Pest Management Science,* 75(8), 2271-2277. doi:10.1002/ps.5364

Seck, P.A., Diagne, A., Mohanty, S., & Wopereis, M. (2012). Crops that feed the world 7: Rice. *Journal of Food security,* 4(1), 7-24.

Sparks, T.C. & Lorsbach, B.A. (2017). Perspectives on the agrochemical industry and agrochemical discovery. *Pest Management Science,* 73(4), 672-677. doi:10.1002/ps.4457