



## CROSS-RESISTANCE TO IMIDACLOPRID IN BROWN PLANTHOPPER *NILAPARVATA LUGENS* (STÅL)

B SRAVANTHI<sup>1</sup>, Y SRIDHAR<sup>2\*</sup>, R SUNITHA DEVI<sup>1</sup>, D SANJEEVA RAO<sup>2</sup>,  
D DHYAN CHOWDARY<sup>2,3</sup> AND S M RAHMAN<sup>1</sup>

<sup>1</sup>Professor Jayashankar Telangana State Agricultural University,  
Rajendranagar, Hyderabad 500030, Telangana, India

<sup>2</sup>ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad 500030, Telangana, India

<sup>3</sup>Acharya N G Ranga Agricultural University, Lam, Guntur 522034, Andhra Pradesh, India

\*Email: yerramsridhar303@gmail.com (corresponding author); ORCID ID 0009-0002-3976-0894

### ABSTRACT

Cross-resistance pattern of imidacloprid resistance in brown planthopper, *Nilaparvata lugens* (Stål) to three insecticides viz., dinotefuran, pymetrozine and triflumezopyrim was evaluated with laboratory developed imidacloprid resistant (IMI-R) and susceptible populations (IMI-S). IMI-R population had a 6-fold resistance compared to IMI-S population. The  $LC_{50}$  values for dinotefuran in IMI-R and IMI-S populations were 466.6 and 129.2 mg/L with a resistance ratio of 3.6-fold. Whereas,  $LC_{50}$  values for triflumezopyrim and pymetrozine in IMI-R and IMI-S populations were 0.27 and 0.17 mg/L; 49.07 and 31.5 mg/L with resistant ratios of 1.58 and 1.55, respectively. These findings clearly demonstrated cross-resistance between imidacloprid and dinotefuran but no obvious cross-resistance to triflumezopyrim and pymetrozine.

**Key words:** Rice, insecticides, neonicotinoids, imidacloprid, dinotefuran, triflumezopyrim, pymetrozine, bioassay,  $LC_{50}$ , probit analysis

Brown planthopper *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae), is one of the most destructive monophagous insect pests of rice in Asia (Sogawa and Cheng., 1979). This insect sucks nutrient from the phloem causing the characteristic “hopper burn” symptom. In addition, it also acts as a vector of grassy stunt, ragged stunt and wilted stunt virus diseases. Neonicotinoids are effective class of insecticides for the *N. lugens* management and imidacloprid, the first one in this class was launched in the year 1991. Imidacloprid is a systemic insecticide with contact and stomach action and widely used for the management of sucking insects including the *N. lugens* in rice. Among the neonicotinoids, imidacloprid, thiamethoxam, dinotefuran, nitenpyram, acetamiprid, thiacloprid and imidaclothiz are the most widely used insecticides (Wu et al., 2022). However, each newly developed chemical initially provided efficient control of *N. lugens*, then became lesser effective due to development of resistance in treated populations. In most of the Asian countries including India, *N. lugens* was reported to have developed resistance to various insecticides including imidacloprid (Basanth et al., 2013). Further, development of resistance to multiple insecticide classes was a major problem in the management of the *N. lugens*. Several studies demonstrated cross-resistance of

imidacloprid resistance to other neonicotinoids (Wang et al., 2009; Zhang et al., 2016). Patterns of cross-resistance to neonicotinoids have also been reported in other insect pests such as Colorado potato beetle, *Leptinotarsa decemlineata* (Say) and tobacco whitefly, *Bemisia tabaci* Gennadius (Fujii et al., 2020). The present study investigates the cross-resistance patterns of imidacloprid resistance in *N. lugens* to dinotefuran, pymetrozine and triflumezopyrim.

### MATERIAL AND METHODS

Imidacloprid susceptible (IMI-S) population of *N. lugens* was being maintained in glasshouse for about 10 generations at ICAR-Indian Institute of Rice Research, Hyderabad without exposure to any insecticides. Imidacloprid resistant (IMI-R) population was selected by exposure to sub lethal doses of imidacloprid at every generation. These populations were maintained separately on 45-day old potted rice plants of TN-1 in insect proof cages at  $27 \pm 1^\circ\text{C}$  temperature 70-80% relative humidity and 16:8 h light: dark photoperiod. The test insecticides were neonicotinoid compounds, imidacloprid 17.8SL (Bayer Crop Science) and dinotefuran 20SG (Indofil Industries Limited); pyridine azomethine compound,

pymetrozine 50WG (Syngenta India Private Limited) and mesoionic compound triflumezopyrim 10SC (Corteva Agriscience). Rice seedling dip method (IRAC, 2012) with suitable modifications was adopted for bioassays. The experiment was conducted in the glasshouse on 45-day old rice plants of cultivar TN-1. One or two tillers were separated from the rice plant and cut above 20 cm from the base. The cut portion was checked and cleaned by removing the dead leaves and washed with water. Using a holder, the cut stem was placed in the centre of a plastic cup. This arrangement was placed inside of another plastic cup containing water in to which the bottom of the cut stem with a few roots was kept immersed (to prevent stress in plant due to feeding by the insects). These cut stems were confined by a mylar film cage. Rice seedlings were dipped into 6-9 concentrations of imidacloprid solution for 30 seconds and shade dried at room temperature. Initially, wide range of concentrations were attempted as part of 'range-finding' test. Final concentrations were chosen after initial exploratory trials in such a way that three concentrations resulted in mortalities below 50% and three concentrations result in mortalities above 50%. Tap water without any insecticide served as a control. Fifteen third instar nymphs were transferred to the rice seedling and the cup was covered with a mylar sheet. There were three replicates for each insecticide concentration. Mortality was recorded 72 hr of after exposure. The nymphs, unable to move even after a gentle prod with a fine bristle were considered dead. To assess the resistance to imidacloprid and cross-resistance to dinotefuran, pymetrozine and triflumezopyrim bioassays were conducted with IMI-R and IMI-S populations. Mortality data were subjected to probit analysis (PoloPlus 2.0 LeoOra). Further, resistance ratio (RR) was given as the ratio between

the  $LC_{50}$  of resistant population and  $LC_{50}$  of susceptible population.

## RESULTS AND DISCUSSION

Resistance to insecticides in target insects was a major factor responsible for the failure of insecticides. Widespread and intensive use of imidacloprid could be a major driving factor accelerating resistance development in *N. lugens*. Being a systemic insecticide, imidacloprid has prolonged residual activity, which was likely to generate persistent selection pressure leading to resistance development (Liu et al., 2003). In addition, cross-resistance to other insecticides exacerbates the problem. In this study,  $LC_{50}$  values of IMI-R and IMI-S populations were 1594.7 mg/ L and 263.6 mg/ L, respectively showing a 6-fold resistance. (Table 1). The  $LC_{50}$  values for dinotefuran in IMI-R and IMI-S populations were 466.6 mg/L and 129.2 mg/ L, respectively, showing a 3.6-fold cross-resistance. Earlier, Wang et al. (2009) also reported a low level of cross-resistance to dinotefuran in IMI-R *N. lugens* populations. Cytochrome P450 monooxygenase-mediated detoxification, specifically overexpression of CYP6ER1 gene was reported for the resistance to neonicotinoids including imidacloprid, thiamethoxam and dinotefuran in *N. lugens*. In addition, a significant correlation between  $LC_{50}$  values of imidacloprid and dinotefuran were found indicating a potential cross-resistance pattern (Sun et al., 2018; Khoa et al., 2018). However, a CYP6ER1 knockout strain constructed using CRISPER/Cas9 strategy showed a higher sensitivity to imidacloprid and thiacloprid as compared to acetamiprid, nitenpyram, clothianidin and dinotefuran as CYP6ER1 activity was found to be insecticide structure dependent (Zhang et al., 2023).

Table 1.  $LC_{50}$  of imidacloprid resistant (IMI-R) and susceptible (IMI-S) populations and cross-resistance in *N. lugens*

Insecticide	Population	$LC_{50}$ (mg a.i./ L)	95% fiducial limits	Slope	$\chi^2$	Resistance ratio
Imidacloprid	IMI-R	1594.75	814.2-4755.8	0.084± 0.024	2.39	6.0
	IMI-S	263.60	110.3-961.7	0.109± 0.031	1.47	
Dinotefuran	IMI-R	466.60	203.6-978.1	0.253± 0.057	6.45	3.6
	IMI-S	129.20	63.9-215.2	0.306± 0.064	6.47	
Triflumezopyrim	IMI-R	0.27	0.159-0.505	0.671± 0.109	0.89	1.58
	IMI-S	0.17	0.101-0.303	0.770± 0.119	0.91	
Pymetrozine	IMI-R	49.07	8.54-107.87	0.251± 0.062	3.27	1.55
	IMI-S	31.50	19.01-47.34	0.845± 0.126	7.70	

$LC_{50}$ : median lethal concentration, IMI-S: Imidacloprid susceptible, IMI-R- Imidacloprid resistant,  $\chi^2$ : chi square value

With respect to pymetrozine,  $LC_{50}$  values in IMI-R and IMI-S populations were 49.07 and 31.50 mg/L, respectively showing 1.55-fold cross-resistance. Earlier Yang et al. (2016) reported a 1.26-fold cross-resistance to pymetrozine in IMI-R population and no cross-resistance between imidacloprid and pymetrozine. Similarly, Khoa et al. (2018) found no cross-resistance between imidacloprid and pymetrozine. Pymetrozine has a unique mode of action wherein it disrupts the function of chordotonal mechanoreceptors (Ausburn et al., 2005) affecting sense of gravity, hearing, and coordination in insects. As a result, feeding and reproductive behaviour of the target insect are adversely affected. On the other hand, resistance to pymetrozine due to metabolic detoxification by overexpression of NICYP6CS1 gene also was demonstrated (Wang et al., 2021). Therefore, different modes of action and detoxification mechanisms for imidacloprid and pymetrozine could be reasons for lack of cross-resistance in *N. lugens*. No cross-resistance was observed to triflumezopyrim (1.58-fold) and  $LC_{50}$  values in IMI-R and IMI-S populations were 0.27 and 0.17 mg/L, respectively. However, Liao et al. (2021) reported a low level of resistance to triflumezopyrim (up to 7.3-fold) in *N. lugens*. Whereas, in small brown planthopper, *Laodelphax striatellus* (Fallén) Wen et al. (2021) reported 26.29-fold resistance to triflumezopyrim and resistant strain had no cross-resistance to imidacloprid, dinotefuran and pymetrozine. Different modes of action of triflumezopyrim by inhibition of orthosteric binding site of nAChRs (Holyoke Jr et al., 2015) and imidacloprid by binding of nAChRs agonistically, could be the factor for the absence of cross-resistance. This study clearly demonstrated a low level of cross-resistance between imidacloprid and dinotefuran but no cross-resistance to triflumezopyrim and pymetrozine. This information is of great importance in choosing insecticide options for the management of *N. lugens*.

#### ACKNOWLEDGEMENTS

The authors thank the College of Agriculture, Rajendranagar, Professor Jayashankar Telangana State Agricultural University, Hyderabad, Telangana and ICAR- Indian Institute of Rice Research, Hyderabad for providing the necessary facilities for conducting the research investigation.

#### FINANCIAL SUPPORT

The authors are thankful to Professor Jayashankar Telangana State Agricultural University,

Hyderabad-500030, Telangana, India and ICAR- Indian Institute of Rice Research, Hyderabad for providing necessary financial support.

#### AUTHOR CONTRIBUTION STATEMENT

Sravanthi has conducted the original experiment and wrote the original manuscript, Sridhar Y conceptualised the work and corrected the manuscript and others have supported during the work.

#### CONFLICT OF INTEREST

No conflict of interest.

#### REFERENCES

- Ausborn J, Wolf H, Mader W, Kayser H. 2005. The insecticide pymetrozine selectively affects chordotonal mechanoreceptors. *Journal of Experimental Biology* 208(23): 4451-4466.
- Basanth Y S, Veerappanavar S V T, Gowda S D K. 2013. Susceptibility of different populations of *Nilaparvata lugens* from major rice growing areas of Karnataka, India to different groups of insecticides. *Rice Science* 20: 371-378.
- Fujii T, Morimura S S, Oe T, Ide M, Van T D, Van C H, Van T P, Loc P M, Cuong L Q, Liu Z W, Zhu Z R. 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*. *Pest Management Science* 76(2): 480-486.
- Holyoke Jr C W, Zhang W, Pahutski Jr T F, Lahm G P, Tong M H T, Cordova D, Schroeder M E, Benner E A, Rauh J J, Dietrich R F, Leighty R M. 2015. Triflumezopyrim: Discovery and optimization of a mesoionic insecticide for rice. In discovery and synthesis of crop protection products. American Chemical Society 365-378.
- IRAC. 2012. [http://www.irac-online.org/content/uploads/Method\\_005\\_v4.1.pdf](http://www.irac-online.org/content/uploads/Method_005_v4.1.pdf).
- Khoa D B, Thang B X, Liem N V, Holst N, Kristensen M. 2018. Variation in susceptibility of eight insecticides in the brown planthopper, *Nilaparvata lugens* in three regions of Vietnam from 2015-2017. *PLoS one* 13(10): 1-14.
- Liao X, Xu P F, Gong P P, Wan H, Li J H. 2021. Current susceptibilities of brown planthopper *Nilaparvata lugens* to triflumezopyrim and other frequently used insecticides in China. *Insect Science* 28(1): 115-126.
- Liu Z W, Han Z J, Wang Y C, Zhang L C, Zhang H W, Liu C J. 2003. Selection for imidacloprid resistance in *Nilaparvata lugens*: cross-resistance patterns and possible mechanisms. *Pest Management Science* 59: 1355-1359.
- Sogawa K and Cheng C H. 1979. Economic thresholds, nature of damage, and losses caused by the brown planthopper. In *Brown Planthopper: Threat to Rice Production in Asia* Chapter 7; Pathak, M.D., Ed.; The International Rice Research Institute: Los Baños, Philippines. 125-142.
- Sun X, Gong Y, Ali S, Hou M. 2018. Mechanisms of resistance to thiamethoxam and dinotefuran compared to imidacloprid in the brown planthopper: Roles of cytochrome P450 monooxygenase and a P450 gene CYP6ER1. *Pesticide Biochemistry and Physiology* 150: 17-26.

- Wang L X, Tao S, Zhang Y, Jia Y L, Wu S F, Gao C F. 2021. Mechanism of metabolic resistance to pymetrozine in *Nilaparvata lugens*: over-expression of cytochrome P450 CYP6CS1 confers pymetrozine resistance. *Pest Manage Science* 77: 4128-4137.
- Wang Y H, Wu S G, Zhu Y C, Chen J, Liu F Y, Zhao X P, Wang Q, Li Z, Bo X P, Shen J L. 2009. Dynamics of imidacloprid resistance and cross-resistance in the brown planthopper, *Nilaparvata lugens*. *Entomologia Experimentalis et Applicata* 131: 20-29.
- Wen S, Xue Y, Du R, Liu C, Wang X, Wang Y, Liu C, Wang S, Wang J, Xia X. 2021. Toxicity and sublethal effects of triflumezopyrim on the development and detoxification enzymatic activities in the small brown planthopper (SBPH), *Laodelphax striatellus* (Fallen). *Crop Protection* 150: 105813.
- Wu S, He M, Xia F, Zhao X, Liao X, Li R, Li M. 2022. The cross-resistance pattern and the metabolic resistance mechanism of acetamiprid in the brown planthopper, *Nilaparvata lugens* (Stål). *International Journal of Molecular Sciences* 23(16): 1-16.
- Yang Y, Huang L, Wang Y, Zhang Y, Fang S, Liu Z. 2016. No cross-resistance between imidacloprid and pymetrozine in the brown planthopper: status and mechanisms. *Pesticide Biochemistry and Physiology* 130: 79-83.
- Zhang X, Liao X, Mao K, Zhang K, Wan H, Li J. 2016. Insecticide resistance monitoring and correlation analysis of insecticides in field populations of the brown planthopper *Nilaparvata lugens* (Stål) in China 2012-2014. *Pesticide Biochemistry and Physiology* 132: 13-20.
- Zhang Y C, Yu Z T, Gao Y, Ye W N, Peng Y X, Zhang S, Gao C F. 2023. Dinotefuran resistance in *Nilaparvata lugens*: resistance monitoring, inheritance, resistance mechanism and fitness costs. *Journal of Pest Science* 1-15.

(Manuscript Received: September, 2023; Revised: April, 2024;

Accepted: April, 2024; Online Published: May, 2024)

Online First in [www.entosocindia.org](http://www.entosocindia.org) and [indianentomology.org](http://indianentomology.org) Ref. No. e24674