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EFFECT OF SELECTION PRESSURE OF NOVEL INSECTICIDES ON BROWN PLANTHOPPER, *NILAPARVATA LUGENS* (STÅL.) INFESTING RICE

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ABSTRACT : The selection pressure placed by insecticides on the insect-pests of agricultural crops is the primary cause of burgeoning resistance in many pest species. The process of selection pressure alters the relative frequency of one or more genes within a population. As a result, the insect-pests have attained resistance against the major insecticidal modes of action currently on the market. Therefore, the baseline susceptibility and impact of selection pressure of two new insecticides having novel mode of action was assessed against brown planthopper (BPH), *Nilaparvata lugens* infesting rice during 2017-2018. Toxicity of insecticides, namely dinotefuran and pymetrozine against susceptible *N. lugens* population, maintained for over 20 generations were tested using stem-dip bioassay technique. In the studies on selection pressure, LC_{50} value of dinotefuron in base generation was 0.00021 per cent, which escalated to 0.00026 per cent in F_4 and 0.00034 per cent in F_8 generation. Similarly, the LC_{50} value of pymetrozine elevated from 0.0001 per cent in base generation to 0.0002 per cent and 0.0003 per cent in F_4 and F_8 generation, respectively. Resistance developed in *N. lugens* towards dinotefuran was 1.23 and 1.61-fold in F_4 and F_8 generation and towards pymetrozine was 1.6 and 2.8-fold in F_4 and F_8 generation, respectively. The overall data reveals the high baseline toxicity of two insecticides and development of low levels of resistance under selection pressure in comparison to conventional insecticides, thus unraveling the promising potential of their use in rice crop against brown planthoppers.

Key words : LC_{50}, *N. lugens*, selection pressure, stem-dip bioassay technique, toxicity.

INTRODUCTION

Rice, *Oryza sativa* L., is the staple food of more than half of the global population and over 90% of the world's rice is grown and utilized in Asia only (Rai, 2004). India, with an area of 43.79 million hectares and an annual production of 16.5 million tonnes, is one of the world's leading rice producers (FAO, 2017) and Punjab, with 3.05 million hectares area and an annual production of 18.8 million tonnes is one of the major rice producing states in India (Anonymous, 2018). The injury inflicted on rice crop by rice planthoppers often leads to a yield loss of up to 60% under epidemic conditions (Srivastava *et al*, 2009; Kumar *et al*, 2012).

The brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae), is a serious pest of rice crop in Asia (Pathak and Khan, 1994; Dupo and Barrion, 2009). This monophagous delphacid casts an adverse effect on rice crop through sucking of plant sap often causing "hopper burn", transmission of virus diseases and consequently the huge yield losses. The annual estimated losses caused by brown planthopper in Asia are more than $300 million (Min *et al*, 2014). Insecticides have been the prime line of defence against the planthoppers attacking rice on account of their effectiveness, ease of application and immediate results. However, their widespread, indiscriminate and injudicious use has induced the development of high levels of resistance to many of the major classes of insecticides including organophosphates, carbamates, pyrethroids, neonicotinoids, insect growth regulators and phenylpyrazoles (Wang *et al*, 2008a; Zhang *et al*, 2016). Insecticide resistance has been reported from China, Taiwan, Thailand, Japan and Korea while it is under suspicion in several rice growing habitats of India (Krishnaiah *et al*, 2006; Matsumura *et al*, 2009; Lakshmi *et al*, 2010).

BPH has developed resistance to 29 insecticides worldwide (Sparks and Nauen, 2015). Therefore, resistance monitoring is essential to understand the ongoing scenario of susceptibility of the field population of BPH to various insecticides. Early detection of resistance/susceptibility changes may encourage the adoption of alternative control measures, which are necessary for the successful management of this pest (Wang *et al*, 2008a). Further, it is generally accepted that,

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the resistance of field-collected populations of BPH are subject to changes in detoxifying enzymes (Wang *et al*, 2008b; Bass *et al*, 2011; Ding *et al*, 2013; Pang *et al*, 2014; Bao *et al*, 2016; Garrood *et al*, 2016). Many conventional insecticides though have been evaluated against *N. lugens*, yet, most of them have become ineffective and obsolete within a short span of time due to loss in efficacy, cross-resistance, residual toxicity problem and development of resistance in BPH against them. It is highly imperative to probe new insecticides that have novel mode of action from the diverse insecticide pool for the successful management of BPH. Pymetrozine being the sole representative of the pyridine azomethines, a new class of insecticides, causes an immediate and irreversible cessation of feeding, resulting in blockage of stylet penetration after exposure to the compound (Kayser *et al*, 1994). Similarly, dinotefuran, a new furanicotinyl insecticide, belonging to the 3rd generation of neonicotinoid group causes death by cessation of feeding within several hours of contact andingestion. An investigation was undertaken to generate the baseline susceptibility data of toxicity towards new chemistry molecules by application of selection pressure for the management of BPH in Punjab.

MATERIALS AND METHODS

Experiments were conducted at the Entomological Research Farm and Insect Physiology laboratory at the Punjab Agricultural University, Ludhiana during 2017-18.

Rearing and maintenance of *N. lugens* populations

The susceptible population of *N. lugens* was obtained from the rearing stock maintained in an insecticides-free environment for over 20 generations, at the Screen house of the Department of Entomology, P.A.U., Ludhiana.

Test insecticides

The following insecticides were used in the bioassay experiments :

Active substance	Trade name
Dinotefuran	Chess 50 WG
Pymetrozine	Osheen 20 SG

The test concentrations of these insecticides were prepared from the commercial formulations by adding required quantities of water.

Determination of toxicity in brown planthopper towards new insecticides

The experiment on development of insecticide resistance in brown planthopper following exposure to new insecticides, pymetrozine and dinotefuran insecticides was conducted on susceptible BPH population, maintained at Entomological Research Farm, PAU, Ludhiana. The adults of planthoppers were made to feed on rice plants treated with LC_{50} of the test insecticides. Planthoppers surviving from the treated populations were collected with the help of aspirator and released on month old potted rice plants covered with nylon mesh to obtain a batch of next generation while maintaining discrete generations. Adult hoppers that emerged from these plants were considered as F_1 and this was done up to three generations.The 4th generation adults were used for computing the new $LC_{50.}$ The same procedure was followed upto seven generations and the 8th generation adults were again used for computing new LC_{50}.

Determination of the insecticide resistance in *N. lugens*

The 'stem-dip' bioassay method (Zhuang *et al*, 1999) was used to determine LC_{50} values against the 4th instar nymphs of the susceptible BPH populations. Rice stems of test variety PR 121 sown in pots were pulled out along with the roots and thoroughly washed with tap water. The basal 10 cm long stems were cut, air dried and the excess water removed. Three such rice stems were pooled and soaked into appropriate insecticide test solution for 30 seconds with gentle agitation. After air drying for about an hour, these were placed in plastic cups containing 2-3 cm soil and covered with Mylar film cages with muslin cloth at the top. Twenty 4th instar nymphs were introduced into each cage. Mortality was recorded after 24 and 48 hours. The planthoppers were counted as dead, if they did not move in a coordinated way when prodded with a fine brush.

There were four replicates for each insecticide concentration along with the control that was dipped in water only. Results were expressed as percentage mortality with correction for untreated (control) mortality using Abbott's formula (Abbott, 1925). The mortality data was subjected to probit analysis (Finney, 1971) using the software package POLO-PC (LeOra Software, 1987) and the log concentration-mortality regression was estimated according to the calculations given by Finney (1971). The resistancein BPH population was calculated as:

$$\text{Resistance ratio} = \frac{LC_{50} \text{ of field collected population}}{LC_{50} \text{ of susceptible population}}$$

RESULTS AND DISCUSSION

Susceptibility of *N. lugens*

The mortality of 4th instar nymphs of the susceptible population when exposed to various concentrations of insecticides ranged from 0 to 100%.

Table 1 : Effect of selection pressure of pymetrozine on development of resistance in *N. lugens*.

Generations	Log concentration probit regression parameters				
	LC_{50} (%)	Fiducial limits at 95% CL	Heterogenity ÷^{2} (d.f.)	Slope	Resistance ratio
Base generation	0.0001	0.00004 to 0.0003	0.49(4)	0.794±0.134	1.00
4th generation	0.0002	0.00006 to 0.0006	0.24(4)	0.660±0.114	1.6
8th generation	0.0003	0.00010 to 0.0012	0.31(4)	0.635±0.113	2.8

Table 2 : Effect of selection pressure of dinotefuran on development of resistance in *N. lugens*.

Generations	Log concentration probit regression parameters				
	LC_{50} (%)	Fiducial limits at 95% CL	Heterogenity ÷^{2} (d.f.)	Slope	Resistance ratio
Base generation	0.00021	0.0001 to 0.0002	0.92 (3)	0.968±0.187	1.00
4th generation	0.00026	0.0002 to 0.0003	0.23 (3)	1.248±0.250	1.23
8th generation	0.00034	0.0002 to 0.0004	0.11 (3)	1.538±0.331	1.61

Pymetrozine

The LC_{50} value of pymetrozine for the base generation was found to be 0.0001 per cent, which increased to 0.0002 per cent in 4th generation and 0.0003 per cent in 8th generation. The selection pressure exerted by pymetrozine exhibited the development of 1.6 and 2.8 fold resistance in the 4th and 8th generation, respectively (Table 1). The current findings of high toxicity of pymetrozine to BPH are consistent with those of Liu *et al* (2013), who also recorded an initial toxicity of 0.0001% in the BPH population and reported that pymetrozine is an effective insecticidal substitute for the management of BPH. Bhanu (2015) also reported higher toxicity of pymetrozine in contrast to neonicotinoids and buprofezin.

Similarly, Mohan (2016) also observed low level of resistance development (1.3 to 1.8 folds) in BPH population against pymetrozine from Nalgonda district of Telangana. However, Yang *et al* (2014) reported 2.13 to 25.72 fold increase in resistance ratio over the five years of pymetrozine usage. Earlier, Peng *et al* (2013) reported an increase in resistance level from low (1.92 to 5.1 folds) to moderate level (15.7 to 25.4 folds) over the two years of pymetrozine usage.

Recently, Xiao *et al* (2018) conducted insecticide resistance studies in seven districts of China's Guangdong province and concluded that the use of thiamethoxam, imidacloprid and buprofezin should be suspended, and pymetrozine (resistance ratio 3.7 to 11-fold) should be used on rice so as to slow down the development of insecticide resistance in BPH populations. The unique mode of action of pymetrozine makes it outstand from conventional insecticides in defence line for controlling this delphacid pest. This sole pyridine azomethine compound do not cause direct toxicityto insects but acts by causing an immediate and irreversible cessation of feeding, leading to blockage of stylet penetration after exposure to the compound rather than causing a deterrent action (Kayser *et al*, 1994). Moreover, there is no risk of cross-resistance with conventional insecticides due to its novel mode of action.

Dinotefuron

After 48 hours of treatment, the LC_{50} value of base generation was calculated to be 0.00021 per cent, which inclined to 0.00026 per cent in the 4th generation showing 1.3-fold resistance. The LC_{50} value in the 8th generation was recorded as 0.00034 per cent, showing 1.6-fold resistance as compared to base generation (Table 2). The slopes of log concentration probit line were 0.968, 1.248 and 1.538 for base, 4th and 8th generation, respectively. BPH population was homogenous as indicated by the chi-square test.

The present findings of high baseline toxicity of dinotefuron (LC_{50} = 0.0002 per cent) against *N. lugens* are consistent with results of Zhang *et al* (2016), who reported the baseline susceptibility of 0.00014% towards BPH populations of China. Previously, Ghosh *et al* (2014) reported higher toxicity of dinotefuron to BPH compared to neonicotinoids and conventional organophosphate insecticides. The findings that dinotefuron developed very low level of resistance (1.23 and 1.61 fold after 4th and 8th generation, respectively) are in agreement with results of Basanth *et al* (2013), who observed a very low level of resistance (0.82 to 2.22 fold) development in BPH population of Karnataka. Earlier, Wen *et al* (2009) also reported toxicity of 0.0002% and a very low level of resistance (3.1 fold) in BPH populations of China. Mu *et* *al* (2016) recorded varying levels of resistance ratios developed in the susceptible BPH populations ranging from 0.96 to 16.6-folds, whereas, in the field populations, moderate levels of resistance were observed in BPH populations to dinotefuran. Similarly, Mohan (2016) also observed development of 1.4, 1.6 and 3.6-fold resistance in the susceptible BPH population from Nalgonda district of Telangana state at 24 hrs, 48 hrs and 72 hrs, respectively. The mode of action of dinotefuran differs from those of organophosphate, carbamate, and pyrethroid compounds as it neither inhibits cholinesterase nor interferes with sodium channels but halts the feeding of insects leading to death within few hours of contact and ingestion. It is postulated that dinotefuran acts as an agonist of insect nicotinic acetylcholine receptors by affecting the binding in a mode that differs from other neonicotinoid compounds due to the presence of tetrahydro-3-furylmethyl group instead of chlorinated pyridine rings.

CONCLUSION

On application of selection pressure, LC_{50} value of dinotefuron and pymetrozine increased at a slow pace from base generation to F_8 generation. The high baseline toxicity of two insecticides, development of low levels of resistance under selection pressure in comparison to conventional insecticides and their novel mode of action makes them promising for the control of brown planthoppers in rice. Thus, the two insecticides should be considered for field trials for further evaluation of their results so as to keep these molecules in the pipeline for management of brown planthoppers in Punjab.

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