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Status of insecticide resistance in rice brown planthopper (*Nilaparvata lugens*) in Punjab

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ABSTRACT

The present study was undertaken in 2017–18 at the entomological Research Farm, PAU, Ludhiana to determine the status of insecticide resistance and the role of enzymes in imparting resistance in *Nilaparvata lugens*. Toxicity of insecticides, viz. imidacloprid, chlorpyriphos, quinalphos, buprofezin and lambda cyhalothrin against the susceptible and field populations of *N. lugens* were evaluated. Based on LC_{50} values calculated, the susceptible population showed the highest sensitivity to buprofezin with lowest LC_{50} value of 0.0007% while the lowest sensitivity was observed for chlorpyriphos (0.0010%). Buprofezin proved most effective against planthopper population collected from Ludhiana and Patiala with LC_{50} values of 0.0032% and 0.0036% and toxicity ratios of 4.40 and 2.75, respectively, whereas quinalphos proved effective against Kapurthala population with LC_{50} value of 0.0027% and toxicity ratio of 2.85. Imidacloprid was the least effective insecticide at all test locations (LC_{50} values ranged from 0.0077% to 0.0141%). *N. lugens* population showed moderate level of resistance development to imidacloprid at all the test locations, viz. 19.09-folds in Ludhiana, 14.14-folds in Patiala and 11-folds resistance in Kapurthala. The resistance ratio for buprofezin was the lowest (4.57-folds) in case of Ludhiana population, whereas in case of Patiala and Kapurthala populations, resistance to quinalphos was the lowest (4.39 and 3.00-folds, respectively). The activity of esterases was significantly higher in the field populations collected from Ludhiana whereas the activities of acetylcholinesterase in resistant populations of Ludhiana and Patiala were significantly higher than in the susceptible population, indicating their probable role in imparting resistance.

Keywords: Acetylcholinesterase, Esterases, Insecticide resistance, LC_{50}, *Nilaparvata lugens*, Toxicity

Rice (*Oryza sativa* L.) is a dietary staple of more than half of world's population, and more than 90% of world's rice is grown and consumed in Asia alone (Bandumula 2017). India, with 43.79 million hectares area and 16.5 million tonnes annual production, is one of the leading producers of rice in the world (FAO 2017); Punjab with 3.05 million hectares area and 18.86 million tonnes annual grain production, is one of major rice producing states of India (Anonymous 2018). The brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae), is a serious pest of rice crop in Asia (Dupo and Barrion 2009). This monophagous pest causes severe damage to rice plants through sucking of plant sap often causing hopper burn, virus disease transmission and consequently huge losses in rice yields. The estimated losses caused by this insect pest in Asia are more than $300 million annually (Min *et al.* 2014). Insecticides have been the first line of defence against the rice planthoppers but their widespread, indiscriminate and injudicious use has led to development of high levels of resistance to many of the major classes of insecticides including organophosphates, carbamates, pyrethroids, neonicotinoids, insect growth regulators, and phenylpyrazoles (Zhang *et al.* 2016). Insecticide resistance in rice planthoppers has been reported from China, Taiwan, Thailand, Japan and Korea (Matsumura *et al.* 2009). In India, it has been reported from the southern states, Karnataka and Andhra Pradesh (Basanth *et al.* 2013, Lakshmi *et al.* 2010b). BPH has developed resistance to 29 insecticides in the world (Sparks and Nauen 2015). Early detection of changes in resistance/susceptibility can promote adoption of alternative control measures, which are essential for the successful management of this pest (Wang *et al.* 2008). Further, the changes in detoxifying enzymes also contribute to the resistance of field-collected populations of BPH (Garrood *et al.* 2016). The present study was therefore, undertaken to assess the current status of insecticide resistance in *N. lugens* to conventional and commonly used insecticides in the Punjab and to study the role of enzymes involved in imparting insecticide resistance.

MATERIALS AND METHODS

Experiments were conducted at the Entomological

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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, PAU, Ludhiana. The field populations were collected from infested rice fields of districts, viz. Ludhiana (30.9010°N, 75.8573°E), Patiala (30.3398°N, 76.3869°E) and Kapurthala (31.3723°N, 75.4018°E) of Punjab. The individual populations of *N. lugens* collected from different test locations were reared for first generation in separate screen cages, before using them for different experiments on insecticide resistance.

Test Insecticides: The insecticides commonly used by Punjab farmers for management of rice insect pests were used in the bioassay experiments (Table 1). The test concentrations of these insecticides were prepared from the commercial formulations by adding required quantities of water. Eight to ten concentrations for each of the insecticide were prepared to work out the LC_{50} for that test insecticide.

Table 1 Common insecticides used in bioassay experiments

Active substance	Trade name
Imidacloprid	Crocodile 17.8SL
Chlorpyriphos	Dursban 20 EC
Quinalphos	Ekalux 25 EC
Buprofezin	Applaud 25EC
Lambda cyhalothrin	Metador 5CS

Determination of the insecticide resistance in N. lugens: The 'stem-dip' method of bioassay (Zhuang et al. 1999) was used to determine LC_{50} values against the 4th instar nymphs of the susceptible and field collected populations. Rice stems of test variety PR 121 sown in pots were pulled out along with the roots and thoroughly washed under tap water. The basal 10 cm long stems were cut, air dried and the excess water removed. Three such rice stems were grouped and dipped into appropriate insecticide test solution for 30 sec with gentle agitation. There were four replications for each insecticide concentration along with the control that was dipped in water only. After air drying for about an hour, these were placed in plastic cups with 2–3 cm soil and covered with Mylar film cages with muslin cloth at the top. Fifteen 4th instar BPH nymphs were introduced in each cage. Data on mortality of BPH adults were recorded after 24 and 48 h of treatment. The planthoppers were counted dead, if they did not move in a coordinated way when prodded with a fine brush. The mortality data was subjected to probit analysis using the software package POLO-PC (LeOra Software 1987) and the log concentration-mortality regression was estimated as per Finney (1971). The results were expressed as percentage mortality with correction for the untreated (control) mortality using Abbott's formula (Abbott 1925). The resistance in field collected population was calculated as per the given formula.

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

Determination of activity of enzymes imparting insecticide resistance in N. lugens: The BPH population exhibiting maximum resistance towards test insecticides was used for studying the activity of enzymes: acetylcholineesterase and esterases to ascertain their possible role in imparting resistance to test insecticides.

Acetylcholinesterase (*AChE*): The homogenate prepared by homogenizing 50 insects in 3.0 ml of ice cold 0.1M phosphate buffer (*p*H 7.5) containing 0.1% (v/v) Triton-X-100 was centrifuged and the supernatant thus obtained was used as enzyme extract for determination of enzyme activity (Ellman *et al.* 1961). The absorbance was measured at 412 nm against reagent blank after 10 min, using a spectrophotometer. The enzyme activity, expressed in µg of free thiol formed/min/mg of insect, was calculated from standard curve prepared by using different concentrations (12–68 µl) of reduced glutathione.

Esterases: The enzyme extract prepared by homogenizing 50 insects in pre-chilled Teflon homogenizer using 3.0 ml of ice cold 0.1M phosphate buffer (*p*H 6.5) having 0.1% Triton X-100, was centrifuged and supernatant thus obtained was diluted and used for determination of enzyme activity as per Wool and Greenburg (1990). The absorbance was measured at 577 nm against the reagent blank after 15 min, using a spectrophotometer. The enzyme activity, expressed in µg of β-napthol formed/min/mg of insect, was calculated from the standard curve prepared by using different concentrations (2–10 µl) of β-napthol.

Statistical analysis: The LC_{50} values for test insecticides were calculated using the software package POLO-PC (LeOra Software 1987). Analysis of Variance (ANOVA) was applied on the enzyme activity data to statistically differentiate among the resistant and susceptible populations.

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%. Imidacloprid and buprofezin with LC_{50} of 0.0007%, each were found to be the most toxic and chlorpyriphos was the least toxic (LC_{50}= 0.0010%). Based on the LC_{50}, the order of toxicity was imidacloprid = buprofezin > lambda-cyhalothrin > quinalphos > chlorpyriphos (Table 2). Basanth *et al.* (2013) also observed lower LC_{50} value (0.00033%) for imidacloprid against BPH population maintained for over 60 generations without any exposure to insecticides in contrast to chlorpyriphos, acephate and thiamethoxam which recorded LC_{50} values of 0.0010%, 0.0032% and 0.00035%, respectively. Wang *et al.* (2009) also reported higher toxicity of imidacloprid (LC_{50} = 0.0003%) and buprofezin (LC_{50} = 0.0002%) against susceptible BPH population in comparison

Table 2 Toxicity of test insecticides on the 4th instar nymphs of the susceptible and field collected *N. lugens* populations from different locations of the Punjab

Insecticide BPH Population	LC_{50} (%)	Fiducial range	χ^2	df (n-2)	Slope ± SE	Resistance Ratio
Imidacloprid						
Susceptible	0.0007	0.0002 - 0.002	0.59	4	0.961±0.253	--
Ludhiana	0.0141	0.003 - 0.256	0.31	4	0.555±0.135	19.09
Patiala	0.0099	0.002 - 0.122	0.67	4	1.699±0.236	14.14
Kapurthala	0.0077	0.002 - 0.069	0.27	4	0.614±0.140	11.00
Chlorpyriphos						
Susceptible	0.0010	0.0003 - 0.003	0.71	4	0.989±0.234	--
Ludhiana	0.0061	0.0014 - 0.0256	0.38	4	0.908±0.246	6.61
Patiala	0.0061	0.0008 - 0.0173	0.67	4	1.396±0.495	6.15
Kapurthala	0.0050	0.0007 - 0.0135	0.50	4	1.455±0.507	5.00
Buprofezin						
Susceptible	0.0007	0.0002 - 0.002	0.36	4	1.070±0.225	--
Ludhiana	0.0032	0.0009 - 0.011	0.59	4	0.957±0.231	4.57
Patiala	0.0036	0.0012 - 0.014	0.49	4	0.934±0.215	5.14
Kapurthala	0.0029	0.0010 - 0.010	0.31	4	0.960±0.216	4.57
Quinalphos						
Susceptible	0.0009	0.0002 - 0.003	0.16	4	0.840±0.174	--
Ludhiana	0.0043	0.001 - 0.017	0.27	4	0.789±0.171	4.77
Patiala	0.0039	0.001 - 0.013	0.26	4	0.887±0.190	4.39
Kapurthala	0.0027	0.0009 - 0.008	0.07	4	0.991±0.208	3.00
Lambda-cyhalothrin						
Susceptible	0.0008	0.0001 - 0.002	0.52	4	1.568±0.551	--
Ludhiana	0.0050	0.001 - 0.149	0.24	4	0.646±0.182	4.77
Patiala	0.0037	0.0009 - 0.071	0.46	4	0.703±0.194	4.39
Kapurthala	0.0037	0.001 - 0.014	0.53	4	1.118±0.479	3.00

to imidaclothiz, thiacloprid, acetamiprid and thiamethoxam. Similarly, Lakshmi *et al.* (2010b) recorded LC_{50} value of 0.0002% and 0.0001% for imidacloprid and buprofezin, respectively, against susceptible BPH population.

Location specific variations in toxicity of insecticides to N. lugens

Buprofezin was found to be the most toxic insecticide to BPH populations collected from Ludhiana and Patiala and quinalphos proved to be most toxic insecticide to BPH population of Kapurthala, while imidacloprid proved to be the least effective insecticide against BPH populations collected from all the three test locations. The LC_{50} values worked out for imidacloprid, chlorpyriphos, buprofezin, quinalphos and lambda-cyhalothrin were 0.0141%, 0.0061%, 0.0032%, 0.0043% and 0.0050% against BPH population of Ludhiana; 0.0099%, 0.0061%, 0.0036%, 0.0039% and 0.0037% against BPH population of Patiala; and 0.0077%, 0.0050%, 0.0029%, 0.0027% and 0.0037% against Kapurthala population (Table 2). The order of toxicity of different insecticides was buprofezin>quinalphos>lambda-cyhalothrin >chlorpyriphos>imidacloprid for BPH population from Ludhiana; buprofezin>lambda-cyhalothrin>quinalphos >chlorpyriphos>imidacloprid for Patiala population; and quinalphos>buprofezin>lambda-cyhalothrin>chlorpyriphos >imidacloprid for the Kapurthala populations of BPH. The BPH populations from all the three test locations developed relatively higher resistance to imidacloprid as compared to other insecticides ranging from 11 to 19.09-folds (Table 2). Its widespread use and systemic nature with prolonged residual activity may be one of the reasons for the resistance development. Present findings indicate that an intensive long term use of imidacloprid has resulted in development of moderate level of resistance. This resistance to imidacloprid might be due to enhanced P450 monooxygenases detoxification (Wen *et al.* 2009) or due to the direct selection pressure of this neonicotinoid to BPH (Lakshmi *et al.* 2010a). Krishnaiah *et al.* (2006) also reported neonicotinoids resistance in China, Korea, Japan and Taiwan and some parts of India.

BPH populations exhibited low levels of resistance to chlorpyriphos; the planthopper populations from Ludhiana, Patiala and Kapurthala developed 6.61, 6.15 and 5-folds resistance to chlorpyriphos, respectively (Table 2). Insecticide resistance to chlorpyriphos and other organophosphates in BPH has also been reported from China and Taiwan. Sarupa *et al.* (1998) observed 1.24 to 2.20-folds resistance in BPH to chlorpyriphos, phosphamidon,

phorate, monocrotophos and quinalphos. Resistance ratios for chlorpyriphos in different localities of Karnataka varied greatly, ranging from 1.13 to 16.82-folds (Basanth *et al.* 2013). Yang *et al.* (2014) reported the resistance ratio of chlorpyriphos in the range of 9.90-folds at F1 to 5.94-folds at F14 generation of susceptible population.

The resistance ratio for buprofezin was lowest (4.57-folds) in case of BPH population of Ludhiana as compared to other insecticides in the area (Table 2). The observed variation in resistance levels could be due to the differences in the selection pressures. Basanth *et al.* (2013) recorded low levels of buprofezin resistance in Gangavati (3.94-folds), Kathalagere (5.43-folds) and Kollegala populations (4.45-folds) of Karnataka, whereas Mandya and Saroba populations were still susceptible to buprofezin with resistance ratios of 1.06 and 1.84-folds, respectively. Lakshmi *et al.* (2010b) also reported low to moderate levels of resistance to buprofezin in whitebacked planthopper populations of Andhra Pradesh, due to its extensive use since 2004.

The resistance development in *N. lugens* to quinalphos was 4.39 and 3.00-folds in Patiala and Kapurthala populations, respectively, in comparison to Ludhiana population which exhibited 4.77- folds resistance. Similarly, *N. lugens* population exhibited low levels of insecticide resistance development to lambda-cyhalothrin at all the test locations (Table 2). Lesser use of quinalphos and widespread use of other insecticides such as imidacloprid and chlorpyriphos for the control of BPH by the farmers may be one of the reasons for high effectiveness of quinalphos in Patiala and Kapurthala. Elanchezhian *et al.* (2008) reported superiority of lambda-cyhalothrin in controlling brown planthoppers. However, the lambda-cyhalothrin exhibited reduction in the green mirid bug populations.

The present data does not reflect field efficacy of insecticides, since it is based on linear response of *N. lugens* nymphs to a variety of dosages under laboratory conditions whereas field efficacy is influenced by a number of other factors like insecticide coverage, environmental conditions etc. However, the data serves as a comparison point of relative toxicity of various insecticides against BPH and may help in resistance monitoring.

Activity of enzymes involved in imparting insecticide resistance in N. lugens

Esterases: The activity of the esterases in the susceptible and the field populations of Ludhiana, Patiala and Kapurthala was 13.0 µg, 17.34 µg, 14.89 µg and 14.55 µg of β-napthol formed/min/mg, respectively (Table 3). The resistant BPH populations from Ludhiana showed significantly higher esterase activity while the field populations from Patiala and Kapurthala did not differ significantly from the susceptible population in their esterase activity. The present findings, depicting the resistant BPH population from Ludhiana differing significantly in respect of increased esterase activity, are in line with the findings of Hung *et al.* (1990) who reported elevated levels of this activity in the planthoppers on exposure to organophosphorous, carbamate and pyrethroid insecticides. Insecticide resistance in BPH populations from Patiala and Kapurthala to organophosphates, buprofezin and lambda-cyhalothrin was very low, and this has been depicted from only a moderate but non-significant increase in the esterase activity. Also, the decline in the usage of above insecticides in the two districts with shifting to newer insecticide molecule classes belonging to pyridine azomethines and phenyl pyrazoles, might have contributed to the non-significant increase in the esterase activity.

Table 3 Activity of Esterase and Acetylcholinesterase in *N. lugens* collected from different locations of the Punjab

Population	Esterase (β-napthol formed/ min/mg)	Acetylcholinesterase (Free thiol formed/ min/mg)
Ludhiana	17.34 ± 0.42^{a}	6.01 ± 0.81^{a}
Patiala	14.89 ± 1.21 ^{ab}	5.89 ± 0.29^{b}
Kapurthala	14.55 ± 1.01 ^{ab}	5.34 ± 0.51^{abc}
Susceptible	13.00 ± 0.67 ^{b}	5.27 ± 0.66^{c}

Values with different superscripts a,b,c in a column differ significantly at P≤0.05

Acetylcholinesterases: The activity of the acetylcholinesterases in the susceptible and field populations of *N. lugens* collected from Ludhiana, Patiala and Kapurthala was 5.27 µg, 6.01 µg, 5.89 µg and 5.34 µg of free thiol formed/min/mg, respectively (Table 3). The acetylcholinesterase activity in the resistant populations of Ludhiana and Patiala varied significantly from the susceptible population. The present findings depicting that the activity of acetylcholinesterase in resistant BPH populations of Ludhiana and Patiala differed significantly from the susceptible population indicate that resistance to organophosphorous and carbamate insecticides can be at least in part, because of the insensitivity of AChE to inhibition by these insecticides. These findings are in conformity with the results of Chung and Sun (1981), who observed that AChE was 15.7 times less sensitive to isoprocarb in the resistant BPH population as compared to the susceptible population. Hama and Hosoda (1983) also correlated the low sensitivity of acetylcholinesterase to metolcarb resistance. Likewise, Yoo *et al.* (2002) reported organophosphorous and carbamates resistance due to the reduced inhibition of AChE in the insecticide resistant BPH populations.

Among various test insecticides, buprofezin (LC_{50} value ranging from 0.0029–0.0032%) and quinalphos (LC_{50} value ranging from 0.0027–0.0043%) proved to be most toxic with lowest resistance ratios ranging from 4.57 to 5.14-folds for buprofezin, and 3.00 to 4.77-folds for quinalphos followed by lambda-cyhalothrin and chlorpyriphos. Imidacloprid was found to be the least toxic insecticide with highest resistance ratio ranging from 11 to 19.09-folds against BPH populations. The esterase activity in the resistant BPH population collected from Ludhiana was significantly

higher than in the susceptible population, whereas the field populations of Patiala and Kapurthala depicted only a moderate but non-significant increase in the esterase activity. The acetylcholinesterase (AChE) activity in resistant populations of Ludhiana and Patiala was significantly higher than in the susceptible population, indicating its probable role in imparting resistance.

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