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Persistence monitoring and mechanisms of insecticide resistance in brown plant hopper (Nilaparvata lugens (Stål)), and its associated risk on mirid bugs, Cyrtorhinus lividipennis Reuter, in North India

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Article

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Abstract

Brown planthopper (BPH), *Nilaparvata lugens*, is a major rice pest in India, causing severe yield losses and threatening food security in rice-growing regions. Continuous and indiscriminate use of insecticides has led to resistance development, reducing their effectiveness and declining sustainability. The present study investigated the persistence and status of insecticide resistance in BPH populations collected from major rice-growing states of North India, examined the biochemical mechanisms underlying resistance, and assessed the toxicity of selected insecticides to the predatory mirid bug, *Cyrtorhinus lividipennis*. Resistance to imidacloprid, thiamethoxam, pymetrozine, buprofezin, and fipronil was evaluated through toxicity bioassays, while activities of detoxifying enzymes such as esterase, cytochrome P450 monooxygenase (CYP450), and glutathione-S-transferase (GST) were analyzed. Results showed moderate resistance to imidacloprid (5–16-fold) and thiamethoxam (5.77–17.37-fold), whereas low resistance was observed against pymetrozine (1.88–5.44-fold), buprofezin (1.5–8.5-fold), and fipronil (3.04–8.12-fold). Increased activities of esterase (1.67–3.67-fold), CYP450 (2.77–3.72-fold), and GST (1.0–3.47-fold) indicated their role in resistance development. Cross-resistance was detected among thiamethoxam, imidacloprid, and buprofezin. Pymetrozine 50 WS showed the highest PT value (28.88) initially at 24 h, subsequently showing the lower persistent toxicity (PT = 1161.8) at extended exposure, indicating lower environmental persistence. Toxicity studies on *C. lividipennis* revealed that thiamethoxam and imidacloprid were highly toxic (LC_{50} at 48 HAE = 5.59 & 5.09 ppm, respectively), whereas pymetrozine and buprofezin were comparatively safer (LC_{50} at 48 HAE = 72.86 & 19.00 ppm, respectively). These findings emphasize the need for continuous resistance monitoring and integrated pest management strategies that ensure effective BPH control while conserving beneficial natural enemies.

Introduction

The brown plant hopper (BPH), *Nilaparvata lugens* Stål (Delphacidae: Hemiptera), is one of the most destructive insect pests of rice, particularly in the major rice-growing regions of Asia (Zhang et al., 2016). This pest alone is responsible for nearly 60% of yield loss in rice, primarily through phloem sap feeding and transmission of viral diseases such as rice grassy stunt virus and rice ragged stunt virus (Kumar et al., 2012; Singh and Tiwari, 2020). The annual economic loss attributed to *N. lugens* in Asia is estimated to surpass $300 million (Min et al., 2014). Factors such as migratory behavior (Hu et al., 2014), high fecundity (Piyaphongkul et al., 2012), and rapid adaptation to resistant rice cultivars (Singh and Tiwari, 2021) further complicate effective management of this pest.

Historically, rice farmers have primarily depended on synthetic chemical insecticides for BPH management (Khoa et al., 2018). The overuse and indiscriminate application of these chemicals have led to enhanced production of detoxifying enzymes in BPH (Scott, 1991) and the emergence of point mutations in detoxification-related genes (Sparks and Nauen, 2015). As a consequence, BPH now exhibits resistance to almost all major insecticide groups, including organophosphates, carbamates, pyrethroids, neonicotinoids, insect growth regulators, and phenyl pyrazole, with resistance reported to 33 molecules worldwide (Wang et al., 2008; Zhang et al., 2016; APRD, 2021).

In response, newer insecticides such as Imidacloprid, Pymetrozine, Buprofezin, Thiamethoxam, and Fipronil have gained increasing popularity from 2010 to 2020. These compounds are now the preferred choice among 82% of surveyed rice farmers in the subcontinent, particularly in India and Bangladesh (Uddin et al., 2020). Nevertheless, continuous monitoring of resistance status and elucidation of resistance mechanisms in BPH to these molecules are essential for effective and sustainable pest control.

The broad adoption of insecticides has also harmed key natural enemies of BPH, notably the predatory mirid bug, *Cyrtorhinus lividipennis*. Both nymphs and adults of this mirid bug rely on BPH as prey, with a single individual capable of consuming up to 66 BPH over its 24-day lifespan (Preetha et al., 2010). Despite their biological control potential, the widespread use of broad-spectrum insecticides severely diminishes these beneficial populations, though chemical control remains necessary in many situations.

The integration of biological agents, such as the mirid bug, alongside insecticides within an Integrated Pest Management (IPM) system, represents a promising strategy for sustainable rice protection. Evaluating the compatibility of insecticides with key biocontrol agents is vital for minimizing environmental impact, avoiding ineffective chemical use, and maximizing farmer profitability. This study, therefore, addresses the resistance status and mechanisms in BPH across various insecticides, as well as the risk associated with these chemicals to the mirid bug, supporting the development of sustainable IPM strategies for rice ecosystems.

Materials and Methods

Insects collection

Field populations of brown plant hopper (BPH) were collected from four major rice-producing states in Northern and Eastern India (Fig. 1), regions where BPH causes significant crop losses. Between 300 and 500 nymphs and adults were gathered at each location using an aspirator. These insects were then maintained on 30-day-old plants of the susceptible rice variety TN1 under controlled laboratory conditions (temperature: 25–27°C; relative humidity: 70–80%; photoperiod: 16:8 h light: dark). Collected BPH individuals were mass cultured, and 5th instar nymphs from the F1 generation were utilized for subsequent bioassays. A laboratory-susceptible BPH population, maintained for over 60 generations without insecticide exposure, was obtained from the Rice Entomology Lab at GBPUA&T, Pantnagar, for comparative studies.

Figure 1: Collection sites of *Nilaparvata lugens* field populations from different states of India

Insecticides

The technical grade insecticides of imidacloprid (96.05%), thiamethoxam (96.05%), pymetrozine (98%), Buprofezin (98%), and fipronil (95.8%), were procured from Gharda chemicals Limited, India. The stock solutions (100%) of technical-grade insecticides were prepared by determining the correction factor (C.F.) according to the protocols of Heong et al. (2013).

C.F = 100% ÷ concentration of the technical grade insecticide.

Weight of the technical grade insecticide to be taken to prepare the stock solution = Concentration of insecticides × volume × CF

The stock solution was prepared by dissolving the required weight of technical insecticide calculated in the desired volume of acetone. The stock solutions were then serially diluted with acetone to 6–7 concentrations for the bioassay.

Bioassay

Early fifth instar nymphs of the F1 generation were used for bioassay following the stem dip method as outlined by the Insecticide Resistance Action Committee (IRAC Susceptibility Test Methods No. 5; IRAC, 2021). Briefly, 30–40-day-old TN1 rice plants were immersed in six to seven serially diluted concentrations of each insecticide for 30 seconds, with three replicates per dose. Treated plants were air-dried, then each was infested with ten nymphs under controlled conditions (28 ± 1°C; 70–80% RH; 16L:8D photoperiod). Mortality was assessed at 72 hours for imidacloprid, thiamethoxam, and fipronil, 120 hours for buprofezin, and 168 hours for pymetrozine.

Enzyme Assay

The activity of esterase, glutathione-S-transferase, and cytochrome P450 was determined in early fifth instar nymphs of BPH. A group of five fifth instar nymphs were homogenized in 1000 µl of pre-cooled sodium phosphate buffer (pH 7; 0.1 M) containing 0.1% triton-x-100, 1000 µl of pre-cooled sodium phosphate buffer (pH 7; 0.1 M) containing EDTA (1 mM) and PMSF (1 mM) and in 1000 µl of Tris HCl buffer (pH 8; 0.1 M) containing 10 mM reduced glutathione for the estimation of esterase, glutathione-s-transferase and cytochrome p450, respectively. The homogenized extract was centrifuged at 10,000 g for 15 mins at 4°C. The supernatant was used to determine all the enzyme activities. The blank sample taken had included everything except the enzyme extract.

The esterase activity is adopted by the method provided by Han et al. (1998). The enzyme extract was diluted 10-fold with sodium phosphate buffer (pH 7; 0.1M). The reaction was initiated by adding 300 µL of diluted enzyme to 1200 µL of a solution containing 10 mM α-naphthyl acetate and 4 mM Fast Blue RR salt. The change in optical density was recorded at 450 nm at 27°C for 10 mins at an interval of 25 seconds. The standard curve was prepared using α-naphthol with varying concentrations. The enzyme activity was expressed as µmoles of α-naphthol formed /min/ hopper.

The activity of glutathione S-transferase was estimated using the method described by Habig et al. (1974) with 1,2-dichloro-4-nitrobenzene (DCNB) as the substrate.0.5 mL of enzyme extract was pipetted out and diluted with 1 mL of Tris-HCl buffer (pH 8, 0.1 M). The diluted enzyme extract was pre-incubated for 10 min. at 25°C. The reaction was initiated by adding 20 µL of 75 mM DCNB, prepared in ethanol. The observation was recorded at 344 nm for 5 minutes, with readings taken at 1-minute intervals.

The activity of cytochrome P450 was estimated following the method given by Hansen and Hodgson (1971). 720 µL of the clear enzyme extract was added to 800 µL of p-nitroanisole (2 mM), and this mixture was incubated for 2 min. at 27 ºC. However, the reaction was initiated by adding 80 µL of NADPH (9.6 mM). The optical density was recorded at 405 nm at 2-minute intervals for 10 minutes at room temperature.

Contact acute toxicity of Mirid bugs

The dry film method was utilized to determine the contact toxicity of insecticides against the mirid bugs. For the experiment, 1 mL class vials were used, and 0.5 mL of various concentrations of insecticides was smeared and evenly rotated manually to form a film of insecticide over the vial. Ten nymphs of mirid bugs were released into the vials with their mouths covered by a muslin cloth and allowed to remain for 30 minutes. Rice tillers, on the other hand, were cut to a height of 12–15 cm and were dipped into a test tube containing solidified agar. Some early instar BPH were released onto the rice tillers, which will serve as a food source for the mirid bugs. After one hour of exposure, the treated mirid bugs were released into the test tubes containing rice tillers and BPH. Observations on the mortality of mirid bugs were taken after 24 hours and 48 hours.

Risk Assessment of Mirid Bugs

Selectivity Ratio

The selectivity ratio was calculated as per the protocols prescribed by Peertha et al. (2010).

$$\text{Selectivity Ratio} = \frac{\varvec{LC50}\ \varvec{of}\ \varvec{bemisia}\ \varvec{nymphs}\ \varvec{of}\ \varvec{fi}}{\varvec{LC50}\ \varvec{of}\ \varvec{predator}\ \varvec{species}\ \varvec{tested}\ \varv}$$

The values of less than 1 indicate that the chemical is more toxic to mirid than BPH (Non-selective). The values of 1 and more than 1 indicate that the chemical is less toxic to mirid than BPH (selective).

Probit Substitution

This method is used to determine relative toxicities of beneficial species, at particular levels of pest mortality, say the LC_{90} of the pest. Probit substitution was made as per Stark et al. (1995), Kumar and Regupathy (2005), and Stanley et al. (2010).

Y = 5 + m [x – (log LC50 of beneficial species)]

where Y=probit value; m=slope of the probit line for beneficial species; x = log of the 95% fiducial limits for LC90 of the pest species.

Solving Y gives a probit value, which is then converted to a percentage of mortality using the conversion table (Finney, 1964). The chemical is considered selective if it kills less than 90% of mirids at the dose that kills 90% of the pest

Data Analysis

The median lethal concentration (LC_{50}) and 95% confidential limits of the insecticides were determined by Probit analysis (Finney, 1971). The resistance ratio (RR) was calculated by dividing the LC_{50} of the various field populations by the LC_{50} values of the susceptible lab culture. The classification of the level of resistance (low, moderate, and high) was done according to Kim et al. (1999). Correlation and cross-resistance among insecticides and detoxifying enzymes were determined by Pearson method (Holm, 1979). All the data analyses were performed by IBM SPSS v26 software.

Results

Bioassay of Insecticides against BPH

The present study revealed substantial variation in insecticide susceptibility among brown planthopper (BPH) populations collected from Northern and Eastern India. Field populations exhibited elevated LC50 values relative to the susceptible laboratory strain for all five tested insecticides, indicating the development of resistance to varying degrees. Imidacloprid resistance was notably higher in populations from Ludhiana (16-fold), Sonepur (11-fold), and Karnal (9-fold), while Gullarbhoj and Pantnagar remained closer to the laboratory control. For thiamethoxam, Sonepur, Ludhiana, and Karnal showed moderate resistance (17.37-fold and 11.58-fold) compared to Pantnagar and Gullarbhoj, which were more susceptible. Fipronil resistance across all locations was low (3.04- to 8.12-fold), though LC50 values remained highest in Sonepur (80 ppm), followed by Karnal and Gullarbhoj (60 ppm each). Buprofezin susceptibility was highest in the laboratory strain (20 ppm), with Sonepur (8.5-fold), Ludhiana (5.5-fold), and Gularbhoj (5-fold) showing higher but not significantly different resistance, while Pantnagar remained comparable to the control. For pymetrozine, LC50 values in field populations ranged from 170 ppm to 490 ppm—markedly higher than the laboratory strain (90 ppm)—but resistance ratios remained relatively low (1.88- to 5.44-fold) across sites. These findings point to an escalating trend of resistance in BPH populations, especially to commonly deployed neonicotinoids, underscoring the importance of continued resistance surveillance and rotation of insecticides with different modes of action to preserve efficacy and sustain rice pest management.

Enzyme Assay

The involvement of metabolic enzymes in insecticide resistance was assessed by quantifying esterase (EST), glutathione S-transferase (GST), and cytochrome P450 activities in field-collected and susceptible laboratory populations of the brown planthopper (BPH). Esterase activity ranged from 0.03 to 0.11 µmol of α-naphthol formed per minute per hopper, with populations from Sonepur and Ludhiana showing 3.67- and 3-fold higher activities, respectively, than the laboratory strain (0.03 µmol α-naphthol/min/hopper) (Fig. 2). Analysis of variance revealed comparable esterase activity between the Gullarbhoj and Karnal populations, while Pantnagar exhibited the lowest level (0.04 µmol α-naphthol/min/hopper), consistent with the susceptible strain. Significant variation in cytochrome P450 activity was observed among populations, with Ludhiana (94 µmol of p-nitrophenol formed/min/hopper) and Sonepur (85.6 µmol/min/hopper) showing the highest activity, whereas Gullarbhoj (78.4 µmol/min/hopper), Pantnagar (61.1 µmol/min/hopper), and Karnal (58.6 µmol/min/hopper) exhibited lower levels. Tukey's HSD test confirmed that P450 activity in Karnal was statistically comparable to Pantnagar. GST activity showed an increasing trend of 1.38-, 2.81-, 3.36-, and 4.15-fold in Pantnagar, Karnal, Sonepur, and Ludhiana, respectively, compared to the susceptible culture, ranging from 0.181 to 0.823 µmol/min/hopper, with Ludhiana significantly differing from other locations (Fig. 2).

Multivariate analysis of insecticide resistance and enzyme activity

The individual PCA analysis (Fig. 3a) showed that all the enzymes are located on one Principal component (Dim1), indicating their exclusive role in detoxifying insecticide molecules across BPH populations. Cytochrome P450 was observed at the extreme positive end of Dim1, suggesting it is the major enzyme responsible for insecticide detoxification. On the other hand, esterase and GST are on the negative side of Dim1. This indicates that, though both enzymes contribute to detoxification, their modes of action are quite distinct from those of Cytochrome P450. However, the magnitude of GST detoxification of insecticides is weaker than that of esterase.

The correlation between the enzymes is illustrated in the variable PCA analysis (Fig. 3b), where Dim1 is the dominant axis, accounting for 84.2% of the variation. It was also observed that the axes of Esterase and GST are almost parallel, with the least angular separation. This suggests a strong positive correlation between them, i.e., BPH populations with high esterase activity tend to have high GST activity.

On the other hand, cytochrome P450 gave a different trajectory, upward tilting towards positive Dim2 (12.1%), indicating positive correlation with both esterase and GST, with some additional variations. This might reflect that the specific role of cytochrome P450 in oxidative metabolism (Phase I) is distinct from esterase hydrolysis and GST conjugation.

Correlation between insecticide toxicity and enzyme activity

The correlations between enzyme activity and the LC50 values of different insecticides (Fig. 4) were tested using field populations of BPH from various locations. The results revealed significant correlations between esterase activity and susceptibility of BPH to buprofezin (r = 0.927*) and thiamethoxam (r = 0.957*) (Fig. 4).

Moreover, a significant positive correlation was found between cytochrome P450 monooxygenase and thiamethoxam (r = 0.834*) and imidacloprid (r = 0.891*), respectively ($p < 0.01$). On the other hand, glutathione-S-transferase was insignificantly correlated with the LC50 values of all insecticides except thiamethoxam (r = 0.884).

Persistence toxicity of various insecticides against N. lugens

The highest persistent toxicity value at 24 hours was also observed in Pymetrozine 50 WS (433.2), followed by Fipronil 5 SC (416.7), Thiamethoxam 25 WG (233.25), Imidacloprid 17.8 SL (192.5), and Buprofezin 25 EC (132.0). The persistence period was the shortest for Imidacloprid 17.8 SL and Buprofezin 25 EC (11 days), while Pymetrozine 50 WS, Fipronil 5 SC, and Thiamethoxam 25 WG exhibited persistence up to 15 days after 24 hours exposure. When the feeding exposure period increased to 48 hours, Thiamethoxam 25 WG recorded the maximum persistent value (1038.2) lasting for 20 days, with a corresponding toxicity of 51.91%. The lowest persistent toxicity was noted in Buprofezin 25 EC (571.4) for the same period, with a toxicity level of 52.86%. After 72 hours of exposure, persistent toxicity values ranged from 1057.2 to 1447.6, with Fipronil 5 SC showing the highest value (1447.6), followed by Thiamethoxam 25 WG (1181.0), Pymetrozine 50 WS (1161.8), Imidacloprid 17.8 SL (1133.4), and Buprofezin 25 EC (1057.2).

Risk Assessment of Insecticides against Mirid Bug, Cyrtorhinus lividipennis Reuter

The median lethal concentration (LC_{50}) of different insecticides against *C.lividipennis* after 24 HAT represented Thiamethoxam (5.76 ppm) as the most toxic insecticide, followed by Imidacloprid (8.90 ppm), Fipronil (12.77 ppm), Buprofezin (33.32 ppm) whereas Pymetrozine (111.04 ppm) (Table 2) is comparatively the least toxic insecticide. After 48 hours of treatment, similar trends in LC_{50} values were observed. The order of toxicity after 48 HAT was Thiamethoxam (5.09 ppm) > Imidacloprid (5.59 ppm) > Fipronil (8.02 ppm) > Buprofezin (19 ppm) > Pymetrozine (72.86 ppm) (Table 3).

Table 2
Dose-response mortality of *C.lividipennis* against different insecticides at 24 HAT

Insecticide	χ2	Regression Equation	LC_{50} *(ppm)	LC_{90} *(ppm)	FL** (ppm at 95% CL)
Imidacloprid	3.449	Y = 2.304x + 7.032	8.90	31.95	7.34–10.64
Thiamethoxam	1.944	Y = 1.426x + 4.618	5.76	45.65	2.94–8.08
Fipronil	10.571	Y = 2.132x + 6.169	12.77	50.96	10.05–15.66
Buprofezin	11.219	Y = 1.609x + 3.985	33.32	208.57	25.06–42.90
Pymetrozine	2.733	Y = 1.599x + 3.126	111.04	702.66	75.42–144.78

Table 3
Dose-response mortality of *C.lividipennis* against different insecticides at 48 HAT

Insecticide	χ2	Regression Equation	LC_{50} *(ppm)	LC_{90} *(ppm)	FL** (ppm at 95% CL)
Imidacloprid	3.812	Y = 2.114x + 6.878	5.59	22.56	4.20–6.88
Thiamethoxam	1.280	Y = 2.841x + 9.358	5.09	14.37	3.48–6.29
Fipronil	3.442	Y = 2.639x + 8.170	8.02	24.52	6.15–9.75
Buprofezin	5.855	Y = 1.577x + 4.291	19.00	123.41	13.04–25.18
Pymetrozine	2.788	Y = 2.377x + 5.081	72.86	252.13	50.96–91.63

The insecticides were classified by their selectivity ratio, and it was observed that imidacloprid, thiamethoxam, and fipronil were non-selective for mirid bugs across all study locations. On the other hand, insecticides like Buprofezin and pymetrozine were non-selective in all locations except Pantnagar (Table 4). Moreover, the probit substitution method also determined that imidacloprid caused 98–100 percent mortality of mirid bugs at the LC_{90} of BPH. Similar trends were observed in thiamethoxam, fipronil, Buprofezin, and pymetrozine, which showed 91–96 percent, 97–100 percent, 89–98 percent, and 92–96 percent mortality of mirid bugs at the doses meant to kill 90 percent of BPH. It was also observed that the mirid bug population from Pantnagar showed 89, 91, and 92 percent mortality at the doses that would cause 90 percent mortality of BPH (Table 4).

Table 4
Risk assessment of different insecticides at different locations (based on LC_{50} at 24 HAT)

Insecticide	Population	Selectivity ratio	Probit substitution (% mirid mortality at LC90 of BPH)	Category
Imidacloprid	GullarBhoj	0.178	99	Non-Selective
	Karnal	0.099	99	Non-Selective
	Ludhiana	0.056	99	Non-Selective
	Sonepur	0.081	100	Non-Selective
	Pantnagar	0.223	98	Non-Selective
Thiamethoxam	GullarBhoj	0.288	96	Non-Selective
	Karnal	0.144	96	Non-Selective
	Ludhiana	0.096	97	Non-Selective
	Sonepur	0.096	96	Non-Selective
	Pantnagar	0.576	91	Non-Selective
Fipronil	GullarBhoj	0.213	99.7	Non-Selective
	Karnal	0.213	100	Non-Selective
	Ludhiana	0.255	100	Non-Selective
	Sonepur	0.160	100	Non-Selective
	Pantnagar	0.426	97	Non-Selective
Buprofezin	GullarBhoj	0.333	95	Non-Selective
	Karnal	0.417	92	Non-Selective
	Ludhiana	0.303	97	Non-Selective
	Sonepur	0.196	98	Non-Selective
	Pantnagar	1.111	89	Selective
Pymetrozine	GullarBhoj	0.529	94	Non-Selective
	Karnal	0.292	94	Non-Selective
	Ludhiana	0.227	96	Non-Selective
	Sonepur	0.505	93	Selective
	Pantnagar	0.653	92	Selective

Discussions

Brown planthopper (BPH) has demonstrated a remarkable ability to adapt to xenobiotic compounds, resulting in resistance to numerous insecticidal active ingredients. This evolving resistance has complicated effective pest management, necessitating ongoing surveillance and a thorough understanding of the underlying mechanisms of resistance. Studies on BPH insecticide resistance in India have largely focused on limited locations in South India (Sarupa et al., 1998; Krishnaiah et al., 2002; Krishnaiah et al., 2006; Jhansilakshmi et al., 2010; Sidde Gowda & Gubbaiah, 2011; Basanth et al., 2013; Malathi et al., 2015) and Punjab (Deosi and Suri, 2022). The present investigation expands this knowledge by analyzing resistance levels in *N. lugens* populations from three northern and one eastern state of India, against the five most commonly used insecticides. It was observed that Pymetrozine is the most effective insecticide, yielding the lowest resistance ratio among the surveyed populations, whereas neonicotinoids, including imidacloprid and thiamethoxam, exhibited moderate resistance. These findings also support the hypothesis that resistance levels vary with the extent of insecticide use in their respective geographic locations.

Neonicotinoids, including imidacloprid, have gained popularity among Indian farmers over the past two decades due to their selectivity and efficacy at lower doses. During increased resistance to organophosphates and carbamates, imidacloprid became a key insecticide for managing sucking pests. Historical LC50 values for imidacloprid in South-East Asian countries increased from 0.09–2.0 ppm during 1992–2002 to 16.4 ppm by 2006 (Endo & Tsurumachi, 2001; Nagata et al., 2002; Liu et al., 2003), while LC_{50} values in Japan rose from 0.16 to 98.5 µg/g over the same period (Matsumura et al., 2008), and although southern Indian states previously reported lower toxicity (1.81–45.66 ppm) (Basanth et al., 2013), the present study recorded a further increase in LC_{50} values (10–160 ppm), reflecting the continued selection pressure from sustained insecticide use. In addition, the BPH population resistance to imidacloprid across the studied locations ranged from 4-fold to 16-fold (Table 1). In contrast, BPH populations in China and Thailand experienced substantially higher resistance (233–2029-fold) attributed to indiscriminate imidacloprid application, which led to the suspension of imidacloprid use for BPH management in China from 2015–16 onwards (Garrood et al., 2015; Wu et al., 2018; Zhang et al., 2016). Similarly, during the early 2000's, negligible resistance to thiamethoxam in BPH was reported in Southern India, likely due to the insecticide's introduction at that time (Basanth et al., 2013). Over time, however,

thiamethoxam overtook imidacloprid as the most widely used neonicotinoid among Indian farmers (Malathi et al., 2015), which may have contributed to the emergence of resistance in regions such as Ludhiana, Karnal, and Sonepur (2.89-17.37-fold), as depicted in the present study. Additionally, cross-resistance from imidacloprid-exposed populations could have accelerated the development of resistance to thiamethoxam (Wen et al., 2009; Wang et al., 2009; Khoa et al., 2018). Similar trends of higher resistance levels to thiamethoxam have been reported in Asian countries, such as China and Korea, which are attributed to their respective pesticide use patterns (Wang et al., 2008; Zhang et al., 2014; Min et al., 2014).

Fipronil, a phenyl pyrazole insecticide introduced in the 1990s for the control of lepidopteran and hemipteran rice pests, initially demonstrated low median lethal doses against BPH—ranging from 0.06–0.65 ppm in Japan, Taiwan, Vietnam, and the Philippines during 2003 (Matsumura et al., 2008). However, subsequent studies reported an increase in the LC_{50} value in Japan, from 2.6 ppm in 2005 to 77.2 ppm in 2009, indicating the development of gradual resistance (Matsumura et al., 2014). In contrast, South Indian BPH populations have previously been reported to have lower resistance ratios (1.13 to 4.06-fold; Basanth et al., 2013), and no earlier reports have documented the resistance status to fipronil in North Indian populations. However, in the present study, a maximum 8.12-fold resistance with a corresponding LC_{50} of 80 ppm was observed under North Indian conditions (Table 1).

Buprofezin, an insect growth regulator with low mammalian toxicity, acts by inhibiting chitin synthase, making it effective against sucking pests (Kullik et al., 2011). In India, relatively low resistance levels were reported in southern Karnataka during earlier studies (1.06- to 5.43-fold; Basanth et al., 2013), but resistance increased to 0.94- to 18.86-fold in subsequent years (Malathi et al., 2015). However, in the present study, all locations in north India exhibited low resistance levels, ranging from 1.5- to 8.5-fold, with Sonepur, Odisha, showing the highest resistance (Table 1). These findings underscore the importance of rotating buprofezin with insecticides with different modes of action to maintain its effectiveness and economic viability for farmers in the northern states of India. A possible reason for the low resistance level of Buprofezin might be its limited use among Indian farmers.

Pymetrozine is widely regarded as a stylet blocker that disrupts feeding behavior in sucking pests. In the present study, the resistance ratio ranged from 2.44- to 5.44-fold across the surveyed states (Table 1). On the contrary, Wu et al. (2018) documented a sharp increase in BPH resistance to Pymetrozine in China from 4-fold to 1168-fold between 2012 and 2016. Conversely, Vietnam's more restricted use of pymetrozine has maintained low to moderate levels of resistance (17-fold) (Khoa et al., 2018). These observations suggest pymetrozine can be an effective insecticide that can fit into the IPM module for rice in north India. Moreover, rotating pymetrozine with insecticides with different modes of action can prevent pest resurgence and the development of ongoing resistance.

The present study highlights much lower insecticide resistance in BPH compared to countries such as China, where annual pesticide use surged from 0.76 million tons in 1991 to 1.8 million tons by 2017, with a significant portion of this used in rice cultivation (Sharma et al., 2019). This dramatic rise in pesticide application is closely linked to increased resistance in *N. lugens* in China. In contrast, India's annual pesticide consumption was just 0.056 million tons in 2021–22 (http://ppqs.gov.in/statistical-database), nearly 32 times lower than China's. Consequently, resistance levels in India remain substantially lower than in China and other South Asian countries with high pesticide application.

Detoxification enzymes in insects play a vital role in insecticide metabolism, thereby contributing to insecticide resistance. The biochemical analysis in the current study was conducted to estimate the metabolic adaptations of field populations of BPH to their respective insecticides. Esterase enzymes primarily contribute to the detoxification of organophosphate and carbamate insecticides by rapidly hydrolyzing the ester bonds present in these compounds (Karunaratne et al., 1995; Hemingway, 2000; Malathi et al., 2015). In the current study, Sonepur and Ludhiana populations displayed markedly elevated esterase activities of 3.67-fold and 3-fold higher, respectively while Pantnagar exhibited minimal esterase activity, compared to the laboratory control (Fig. 1). These findings align with established roles of esterases in degrading insecticide molecules (Karunaratne et al., 1995; Hemingway, 2000; Malathi et al., 2015), and corroborate recent reports implicating esterases in the metabolism of neonicotinoids as well (Wen et al., 2009; Zhang et al., 2016). The pattern observed in the present study is also consistent with reports of esterase-mediated fipronil detoxification following prolonged exposure, as documented in *Sogatella furcifella* (Tang et al., 2009). Cytochrome P450 is another detoxifying enzyme belonging to the group of heme-proteins that are present at the microsomes and endoplasmic reticulum of the fat body, malpighian tubules, and the midgut epithelial cells of the insects having an inherent capacity to detoxify various insecticides. In the initial phase of metabolism, the cytochrome p450 is oxidized, and it binds to the insecticide substrate, which further gets reduced by accepting an electron from NADPH. The oxygen atom introduced to the insecticide substrate reacts with hydrogen to form water, thereby facilitating the metabolism of the toxic insecticide (Kranti, 2005). Several studies have confirmed the role of cytochrome P450 monooxygenases in conferring resistance in BPH against various classes of insecticides, including organophosphates, thiadiazines, and neonicotinoids (Zhang et al., 2016). In the present study, significantly higher levels of cytochrome P450 in the insect populations of Ludhiana and Sonepur corroborated the findings of Wen et al. (2009), who documented the overexpression of the cytochrome P450 enzyme in the BPH population under continuous selection pressure from imidacloprid for 25 generations. Besides that, Zhang et al. (2016) registered a highly significant positive correlation between the expression of cytochrome p450 and resistance of BPH against imidacloprid, dinotefuran, and ethiprole.

GST was reported to play a major role in the detoxification of organophosphates (Clark et al., 1986; Motoyama and Dauterman, 1975) and pyrethroids (Ranson et al., 1997). However, in the current study, the BPH collected from Ludhiana had significantly higher GST than those from other locations (Fig. 1), which is speculated to be due to the resistance conferred by multiple insecticides to which they might have been exposed earlier. Moreover, the PCA analysis conducted in the current study also suggested that all enzymes contributed to the detoxification of insecticide molecules in BPH collected from different locations (Fig. 3a and 3b).

When the cross-resistance among insecticides in the present study was analyzed using Pearson pairwise correlation, the results indicated a significant positive correlation between thiamethoxam and imidacloprid ($r = 0.83$) (Fig. 4) ($p < 0.05$). Cross-resistance to neonicotinoid insecticides is a common phenomenon (Zhang et al., 2016; Wang et al., 2009; Foster et al., 2008), as all act as agonists of nicotinic acetylcholine receptors in the insect nervous system (Bass et al., 2015). Additionally, the correlation studies indicated that resistance to buprofezin was significantly associated with resistance to

thiamethoxam (r = 0.86*) and fipronil (r = 0.923*). Such a relationship suggests that resistance in the *N. lugens* population might arise from multiple factors, including cross-resistance. Similar findings were reported by Zhang et al. (2015), who found a correlation between resistance to Imidacloprid and to many insecticides from different groups, including dinotefuran, buprofezin, and etofenprox. The results also revealed significant correlations between esterase activity and susceptibility of BPH to buprofezin (r = 0.927*) and thiamethoxam (r = 0.957*) (Fig. 4). Moreover, significant positive correlations were found between cytochrome P450 monooxygenase and thiamethoxam (r = 0.834*) and imidacloprid (r = 0.891*) ($p < 0.01$). On the other hand, glutathione-S-transferase was insignificantly correlated with the LC50 values of all insecticides except thiamethoxam (r = 0.884). These results corroborate the findings of Wu et al. (2022), who also reported a significant increase in esterase and cytochrome P450 in acetamiprid-resistant species, whereas GST showed no significant difference. These results suggest that resistance is likely polygenic and mechanistically complex, involving the simultaneous contributions of multiple detoxification pathways rather than a single enzyme system. This interpretation is consistent with recent reviews describing P450s, esterases and GSTs as major metabolic resistance mechanisms in *N. lugens*.

In the present experiments regarding the determination of persistent toxicity, the initial toxicity after 24 hours of exposure was highest in Pymetrozine 50 WS (28.88%), which was statistically *at par* with Fipronil 5 SC (27.78%). On the contrary, the experiments conducted by Lakshmi *et al.* (2010b) concluded that pymetrozine at 125 g a.i./ha had lower toxicity (14%) initially at 24 hours after exposure (HAE), but the toxicity gradually increased to 71% after 72 hours of exposure. However, Imidacloprid 17.8 SL, Thiamethoxam 25 WG, and Buprofezin 25 EC exhibited comparatively lower mortality rates of 17.50%, 15.55%, and 12.00%, respectively. With longer feeding exposure (48–72 hours), Persistent toxicity values increased, peaking at 72 hours with Fipronil 5 SC showing the greatest persistence and toxicity among all tested insecticides (Fig. 5). However, the extended persistence and safety for natural enemies observed in the rice ecosystem (Sato *et al.*, 1996) have advocated pymetrozine as the best alternative to highly toxic insecticides in the present study.

In the current era, when the world strives for sustainability, the importance of IPM cannot be overlooked. One of the major components of IPM is biological control. The Mirid Bug, *Cyrtorhinus lividipennis*, is one of the major predators of BPH, which feeds on as many as 10.5 eggs and 4.5 adult BPH for a continuous period of 15 days (Reyes and Gabriel, 1975). However, the insecticides used to manage BPH pose serious risks to mirid bugs. On the other hand, chemical insecticides are the most popular means of controlling BPH among farmers, which cannot be excluded due to their efficacy. So, the only way to achieve sustainability is to use insecticides that pose lower environmental risks, including those for mirid bugs.

The current study aims to assess the risks posed by the most commonly used insecticides for managing BPH, with the goal of identifying a suitable insecticide to recommend for IPM in rice. In the present study, insecticides such as imidacloprid, thiamethoxam, and fipronil were considered broad-spectrum insecticides, with high mortality rates (91–100%) against mirid bugs. The selectivity ratio of the above insecticides across all locations ranged from 0.056 to 0.576. These results corroborate many previous studies. The studies by Tanaka et al. (2000) and Lakshmi et al. (2001) evaluated the acute toxicity of imidacloprid at recommended doses against mirid bugs. Similarly, Katti et al. (2007) reported that imidacloprid at 25 mg a.i/L is highly toxic to *C. lividipennis.* Thiamethoxam and fipronil, on the other hand, had risk levels similar to those of imidacloprid at recommended doses (Jhansi et al., 2014).

In contrast, insecticides such as buprofezin and pymetrozine exhibited location-specific partial selectivity, being selective against mirid bug populations only in Pantnagar and Sonepur, whereas the other locations were non-selective. Buprofezin, an insect growth regulator, has previously been shown to have fewer adverse effects on *C. lividipennis*. Studies have reported that buprofezin did not exert considerable adverse effects on *C. lividipennis* populations, and its application resulted in a lower reduction of mirid bug abundance compared to other insecticides, such as imidacloprid and deltamethrin (Jhansi et al., 2014). This aligns with the current findings, positioning buprofezin as a potentially safer option. Pymetrozine, an azomethine pyridine, has also been recognized for its selectivity against various predatory arthropods. Laboratory tests have shown that pymetrozine is selective against predatory Coleoptera, Heteroptera, Neuroptera, and predatory mites (Sechser et al., 2002). Field studies on cotton have also indicated that pymetrozine led to predator population development similar to that in untreated control plots, demonstrating its compatibility with natural enemies (Preetha et al., 2010). The observed enhanced selectivity of pymetrozine in this study further supports its potential as a relatively safer alternative for IPM programs. The enhanced selectivity of pymetrozine may be due to its unique mode of action. Pymetrozine acts on mechanosensory neurons to block chordotonal organs, which regulate feeding behavior (Xu et al., 2018). The selectivity of pymetrozine to mirid bugs might be due to two possible reasons. Firstly, the receptors on which pymetrozine acts may be underdeveloped or absent in predators, rendering pymetrozine ineffective against them. Secondly, there is a possibility of avoiding the exposure route with mirid bugs, since they are not phloem feeders.

Conclusion

The study provides an overview of insecticide resistance in BPH populations across northern India and Odisha, along with the associated mechanisms. The resistance status varied across locations, with Sonepur, Odisha, and Ludhiana, Punjab, showing considerably more resistance than other surveyed locations, supporting the hypothesis of differential selection pressure. Pymetrozine remained the most effective insecticide across populations, whereas imidacloprid and thiamethoxam showed higher resistance ratios. Elevated Cytochrome P450 and esterase activity were responsible for the metabolic detoxification of the insecticide molecules in BPH populations. Moreover, the comparative selectivity of Pymetrozine towards mirid bugs and its lower persistent toxicity make it an ideal insecticide for integration into IPM Programs. However, future research should focus on the molecular pathways of detoxifying enzymes and the evolution of resistance through mutation in identified resistant genes to support sustainable management of BPH.

Abbreviations

BPH
Brown Plant Hopper
CYP450
Cytochrome P450 Monooxygenase

GST
Glutathione-S-Transferase
LC_{50}
Median Lethal Concentration
HAE
Hours After Exposure
RR
Resistance Ratio

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors agree to the publication.

Competing interests

The authors declare no competing interests

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Author Contribution

SS: conceptualization, data curation, Formal analysis, investigation, Methodology, Software, Visualization, Writing – original draft; AKP: Writing – review & editing, Supervision, Validation; SNT: Visualization, Supervision, Software; PN: Formal analysis, software, data curation. RJ: Supervision and Formal analysis

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Data Availability

All data supporting the findings of this study are available within the paper. Any other data (if required) will be made available by the corresponding author upon request.

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Figures

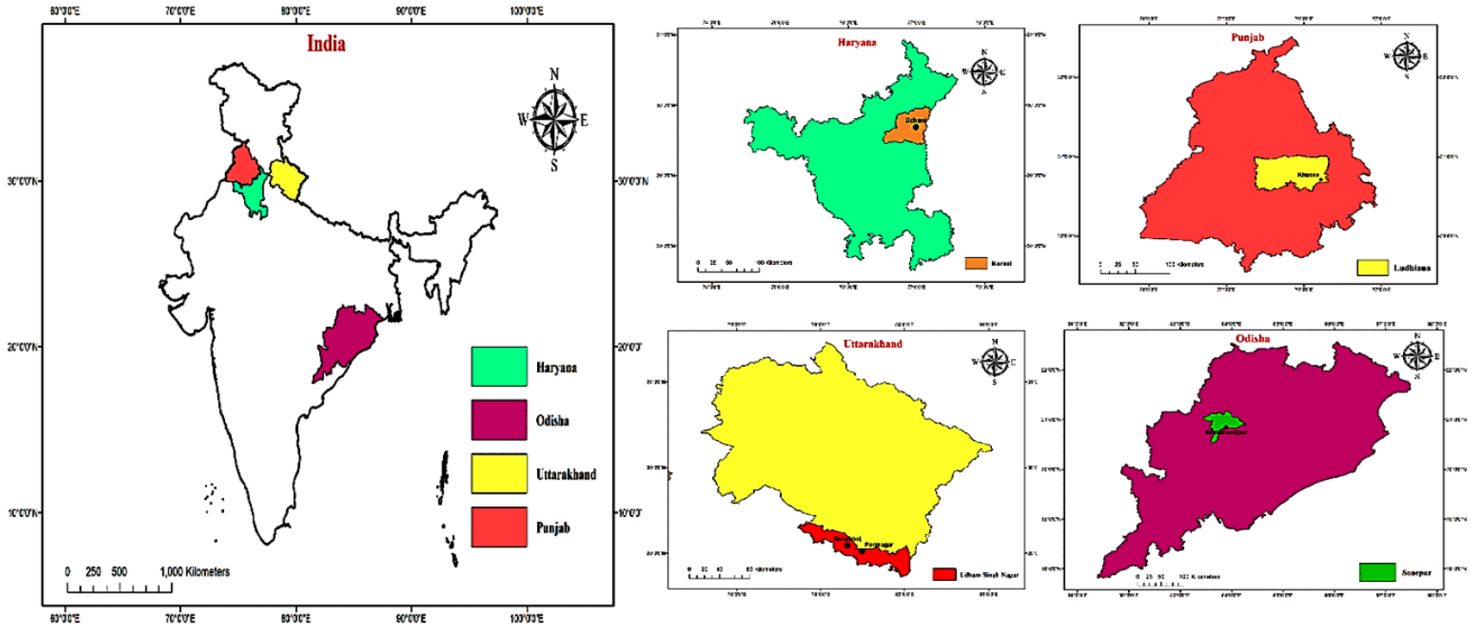


Figure 1

Collection sites of *Nilaparvata lugens* field populations from different states of India

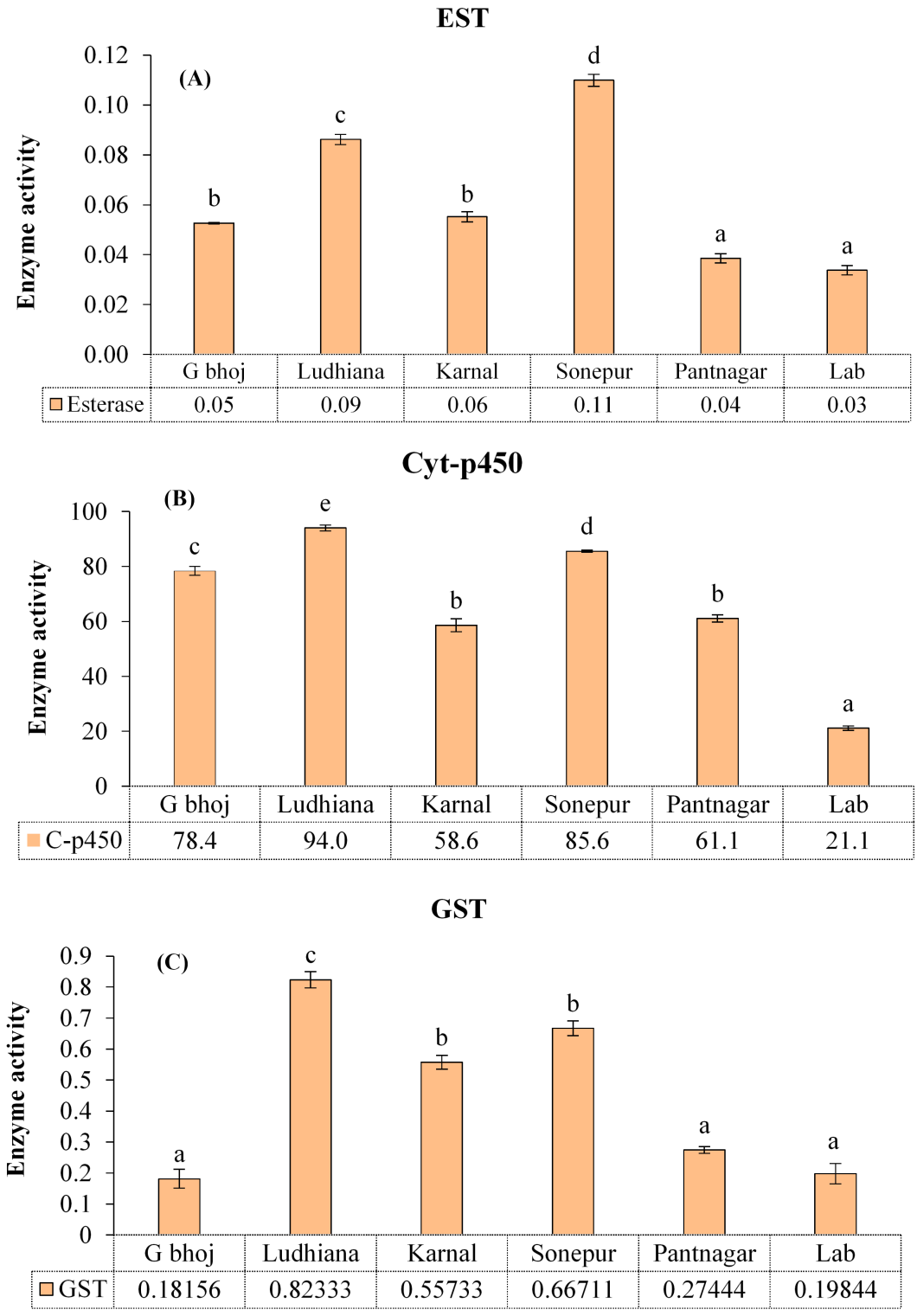


Figure 2

(**A**) Esterase activity (EST); (**B**) cytochrome p-450 monooxygenase activity (Cyt-p450), and (**C**) glutathione S-transferase activity (GST) of different field populations of brown plant hopper.

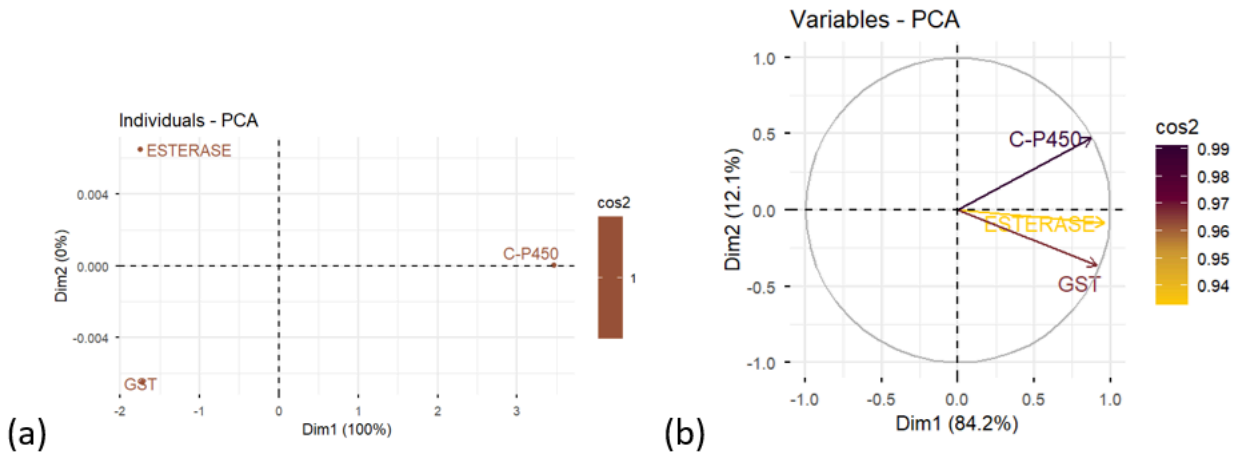


Figure 3

(a) Individual PCA showing the distribution of detoxifying enzymes and their contribution **(b)** variable plot showing correlation between detoxifying enzymes in detoxifying insecticides across different locations.

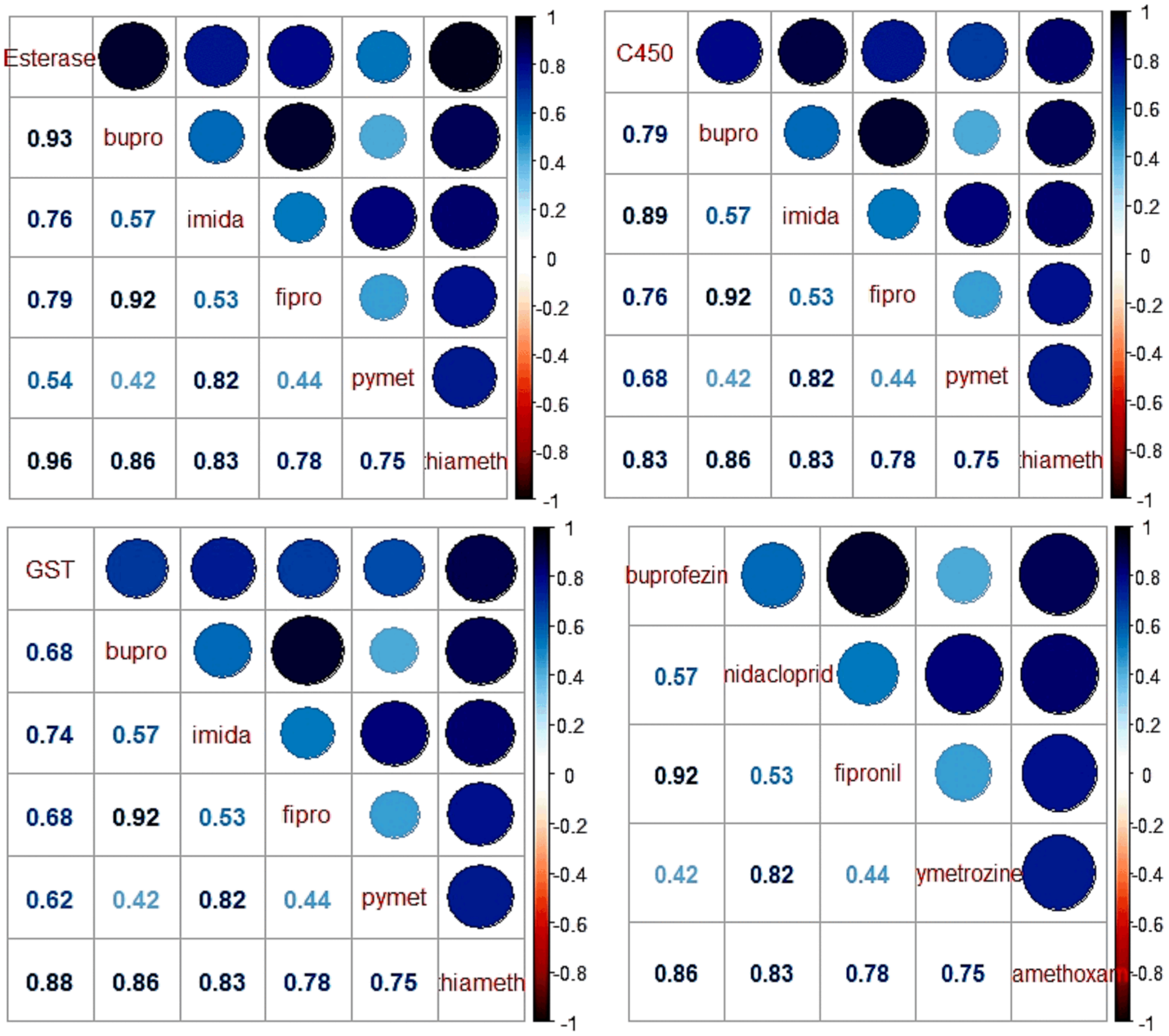
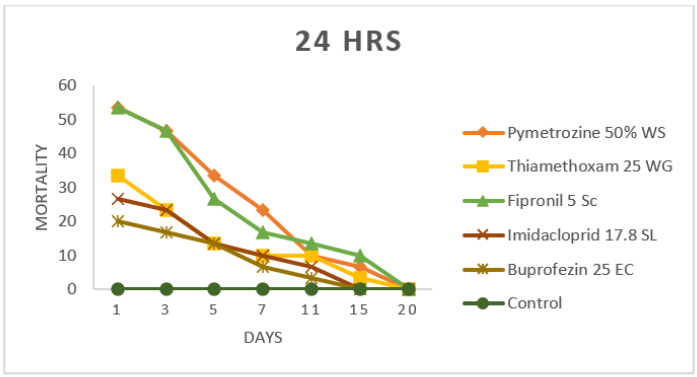
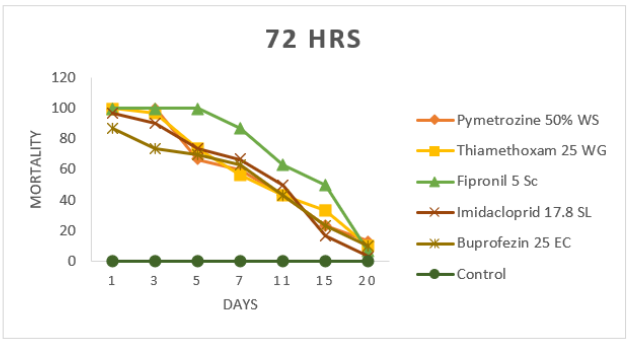
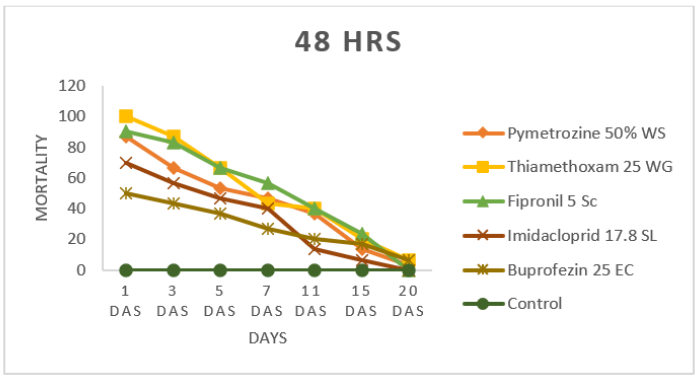


Figure 4

Correlation coefficients (r) between enzyme activity and insecticide toxicity.







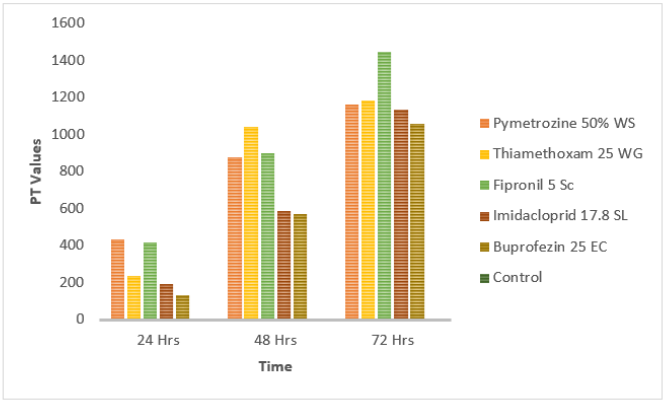


Figure 5

The data show increasing mortality rates from 24h to 72h across all insecticides, with effectiveness declining over days after spraying (DAS).