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STUDIES ON BIOASSAY OF CERTAIN INSECTICIDES AGAINST RICE BROWN PLANTHOPPER, *Nilaparvata lugens* (Stal.) IN MARUTERU REGION

G. RAMYA RISHITHA*, I. PARAMASIVA, M.S.V. CHALAM AND P. MADHUSUDAN

Department of Entomology, S.V. Agricultural College, ANGRAU, Tirupati-517 502.

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ABSTRACT

The level of insecticide resistance acquired by field collected brown plant hopper populations, *Nilaparvatha lugens* after 24 hours and 72 hours exposure to four insecticides thiamethoxam, pymetrozine, dinotefuran, and acephate was studied in comparison with the laboratory population maintained in IIRR laboratory. Compared with the laboratory population/reference population, Maruteru population was recorded higher LC_{50} and LC_{90} values against thiamethoxam, pymetrozine, dinotefuran and acephate and based on resistant ratios lower levels of resistance development was noticed in Maruteru population to all the tested insecticides. The LC_{50} values for Maruteru BPH populations were 0.129, 0.053, 0.079 and 0.149 ppm for thiamethoxam, pymetrozine, dinotefuran and acephate, respectively at 72 hrs after exposure. Whereas in laboratory BPH populations LC_{50} values were 0.031, 0.038, 0.054 and 0.75 for thiamethoxam, pymetrozine, dinotefuran and acephate, respectively at 72 hrs after exposure. The resistance ratios for Maruteru BPH populations at LC_{50} (72 hours after exposure) were 4.161, 1.463, 1.395, and 0.193 fold for thiamethoxam, pymetrozine, dinotefuran, and acephate, respectively.

KEYWORDS: Resistance ratios, insecticide resistance, *Nilaparvata lugen*s, Bioassay, LC_{50}.

INTRODUCTION

Rice (*Oryza sativa* L.) is extensively cultivated in diverse ecosystems ranging from tropical to temperate climate conditions throughout the world. More than half of the world's population relies on rice as a staple food, making it the most significant food crop in India. In India, area under cultivation of rice was 47.58 million ha with annual production of 136.70 million tonnes and productivity of 2873 Kg per hectare (Agricultural statistics at a glance, DES, 2024). After China, India holds the position of the world's second largest rice producer in India. The major five rice producing states are West Bengal, Uttar Pradesh, Andhra Pradesh, Punjab and Tamil Nadu. Andhra Pradesh is in the 3rd position in rice production with 128.95 lakh tonnes of rice in India. It is a leading rice producer with a production of 12 per cent of the total amount of rice produced in the country.

The constraints that limit the production of rice include both abiotic and biotic factors. Warm and humid climate which is essential for rice cultivation, also conducive to the survival and proliferation of insect pests and diseases. Rice is infested by more than 100 species of insects and mites and about 20 of them are considered to be major economic significance. Among the insect pests of rice, the economically significant pest of rice in tropical and temperate regions of Asia is the Brown planthopper (BPH) *Nilaparvata lugens* (Stal) (Hemiptera: Delphacidae). The BPH has become a major insect pest of rice in almost all rice growing tracts of India (Sain and Prakash, 2008; Krishnaiah and Jhansi Lakshmi, 2012). The high population density of BPH may result up to 60 per cent yield loss (Panda and Khush, 1995).

BPH is a monophagous herbivore that feeds mainly on rice sap, and it causes substantial rice yield losses in Asia and even worldwide by causing direct and indirect losses in tropical regions, where it is a migratory pest, it can complete up to 12 generations in a single year. Both nymphs and adults reside at the base of the rice plant, sucking sap from the phloem and xylem, which results in yellowing, wilting, drying up, and ultimately, the death of the rice plant. In field conditions, the damage spreads circularly, a phenomenon known as "hopper-burn."

Farmers rely solely on the use of synthetic insecticides for controlling of planthoppers. The use of insecticides as a sole tool in the management of insect pests has potential drawbacks such as the development of insecticide resistance, persistence of pesticide residues on crop produce, outbreak of secondary insect pests and pest resurgence (Togbe *et al*., 2014).

*Corresponding author, E-mail: paramasiva.reddy@gmail.com

There is an emerging need to assess the toxicity of insecticides against BPH and to monitor the development of insecticides resistance in field populations. This study aimed to assess the insecticide resistance in BPH populations collected from Maruteru (West Godavari district) region to four insecticides thiamethoxam, pymetrozine, dinotefuran and acephate.

MATERIAL AND METHODS

Maintenance of Brown planthopper population

The Brown planthopper, *N. lugens* nymphs and adults were collected with the help of aspirator from rice fields of Maruteru (West Godavari district), where the farmer were taken up excessive sprayings of insecticides on rice crop. Collected insects were mass reared under controlled conditions in rearing cages of 70 cm x 62 cm x 75 cm dimension with glass panels on one side and wire mesh on all other sides with clean potted plants of susceptible variety TN1. The F_1 population obtained was used to assess the levels of insecticide resistance. Baseline population (insecticide susceptible) was obtained from ICAR-Indian institute of rice research, Hyderabad where laboratory population was maintained without exposure to insecticides for 10 generations.

Toxicological bioassay and statistical analysis

The technical grade control insecticides of thiamethoxam, pymetrozine, dinotefuran and acephate were used for the study. The rice-stem dipping method described by Zhang and Shen (2000) was used to assay the dose-responses of BPH to different insecticides. Initially, bracketing was done using 5-6 broad dilutions of insecticides to fix the appropriate dosage range giving different levels of mortality of BPH. The individual working concentrations for each of the test insecticides were prepared from the 1.0 per cent solution through serial dilution technique starts from the highest to the lowest concentration using distilled water as solvent .

The basal 10 cm stems, including the roots, of 50-60 day old rice plants (TN1) were thoroughly washed and air-dried to remove excess water. The stems were then dipped into appropriate insecticide dilutions for 30 seconds. Three replicates were used for each dose, with five different dilutions of each chemical, along with a control (distilled water). After air-drying the treated rice stems for a few minutes, individual stems were placed in 500 ml plastic cups, with the root portion submerged in a mixture of vermicompost, sand, and water.

Fifteen early fifth-instar nymphs were introduced into each plastic cup using an aspirator and cups were secured with muslin cloth. The treated insects were maintained at room temperature. Mortality was recorded at 24 hours, 72 hours and 120 hours after the treatment. The tested 5th instar nymphs that fell on their back and unable to recover normal posture were counted as dead (Krishnaiah *et al*., 2002).

The treatment mortality data were corrected using Abbott's formula (Abbott, 1925) whenever mortality was recorded in control. The data of each test insecticide in the range of 5 to 90 per cent mortality were subjected to standard probit analysis (Finney, 1971) using the SPSS 13.0 Software for obtaining LC_{50}, LC_{90}, heterogeneity (χ^2), intercept (a), slope of regression line (b), fiducial limits and log concentration probit lines (lcp).The relative degree of resistance acquired by BPH populations was assessed adopting standard formula (FAO, 1979).

Resistance Factor =LC_{50} of the field collected population/LC_{50} of the laboratory reared population

The classification of the levels of resistance to tested insecticides was done on the base of resistance factor (RF) value at the LC_{50} level,

RF value < 10-fold: Low resistance;

RF Value = 10–40-fold: Moderate resistance;

RF value = 40–160-fold: High resistance;

RF Value > 160-fold: Extremely high resistance,

Table 1. Resistance ratios at LC_{50} and LC_{90} of brown planthopper population collected from Maruteru 2023-24

Insecticides used	At LC_{50}		At LC_{90}	
	24 hrs	72 hrs	24 hrs	72 hrs
Thiamethoxam	3.395	4.161	5.405	5.189
Pymetrozine	2.750	1.463	2.052	3.872
Dinotefuran	2.032	1.395	1.849	5.241
Acephate	3.456	0.193	6.192	2.669

Table 2. Log-dose Probit response of brown planthopper populations collected from Maruteru at 24hrs and 72 hrs after exposure during 2023-24

S. No	Insecticide	LC_{50} (95% FL)		LC_{90} (95% FL)		Slope ± S.E		Heterogeneity (x^2)	
		24 Hrs	72 Hrs	24 Hrs	72 Hrs	24 Hrs	72 Hrs	24 Hrs	72 Hrs
1	Thiamethoxam	0.163 (0.050-0.316)	0.129 (0.046-0.223)	1.389 (0.574-63.291)	0.768 (0.391-7.043)	1.377±0.470	1.657±0.510	0.094	0.257
2	Pymetrozine	0.130 (0.023-0.255)	0.053 (0.000-0.142)	1.256 (0.514-102.493)	1.127 (0.378-89.79)	1.302±0.470	0.963±0.480	0.180	0.592
3	Dinotefuran	0.165 (0.013-0.337)	0.079 (0.000-0.183)	1.881 (0.725-765.991)	0.887 (0.391-649.87)	1.214±0.474	1.218±0.525	0.870	0.233
4	Acephate	0.515 (0.006-0.300)	0.145 (0.001-0.315)	8.278 (2.032-425.62)	1.743 (0.715-141.31)	1.062±0.445	1.188±0.493	2.179	0.624

FL-Fiducial Limits ; LC_{50} – Lethal Concentration 50 ; LC_{90} – Lethal Concentration 90

Table 3. Log-dose Probit response of brown plant hopper populations collected from ICAR -IIRR at 24 hrs and 72 hrs after exposure during 2023-24

S. No	Insecticide	LC_{50} (95% FL)		LC_{90} (95% FL)		Slope ± S.E		Heterogeneity (x^2)	
		24 Hrs	72 Hrs	24 Hrs	72 Hrs	24 Hrs	72 Hrs	24 Hrs	72 Hrs
1	Thiamethoxam	0.048 (0.001-0.095)	0.031 (0.000-0.071)	0.257 (0.139-3.140)	0.148 (0.055-2.42)	1.762±0.702	1.894±0.953	0.244	0.287
2	Pymetrozine	0.064 (0.000-0.139)	0.038 (0.000-0.083)	0.679 (0.299-247.5)	0.215 (0.109-8.605)	1.252±0.520	1.706±0.759	1.651	1.001
3	Dinotefuran	0.060 (0.000-0.140)	0.054 (0.000-0.112)	0.464 (0.227-32.37)	0.229 (0.108-51.11)	1.446±0.627	2.031±0.975	0.424	0.261
4	Acephate	0.149 (0.006-0.300)	0.75 (0.000-0.184)	1.337 (0.615-96.989)	0.653 (0.308-131.6)	1.345±0.0522	1.362±0.607	1.513	0.515

FL-Fiducial Limits ; LC_{50} – Lethal Concentration 50 ; LC_{90} – Lethal Concentration 90

RESULTS AND DISCUSSION

Resistance status of BPH in Field populations of Maruteru (West Godavari)

Thiamethoxam

Toxicity of thiamethoxam in terms of LC_{50} values against susceptible laboratory population of Brown planthopper at 24 hrs and 72 hrs was 0.048 ppm and 0.031 ppm, respectively and 0.257 ppm and 0.14 8ppm at LC_{90}, respectively (Table 3). BPH population was considered to be homogenous by way of chi-square test. The log concentration probit line recorded slopes (b) were 1.762 ± 1.894 at 24 hrs and 72 hrs, respectively.

BPH populations collected from Maruteru, West Godavari district during 2023-24 has recorded LC_{50} values of 0.163 ppm and 0.129 ppm at 24 hrs and 72 hrs after exposure, respectively and the LC_{90} Values were 1.389 ppm and 0.768 ppm at 24 hrs and 72hrs after exposure. The populations were considered to be homogeneous by way of chi-square test and the slopes of log concentration probit line were 1.377 ± 1.657 at 24 hrs and 72hrs, respectively.

The LC_{50} values of Maruteru population were comparable with those of a laboratory population at 24 hours and 72 hours after exposure and results indicated that moderate levels of resistance were developed to thiamethoxam with resistance factor of 3.395 and 4.162 fold at 24 hrs and 72 hrs, respectively. Similarly, when LC_{90} Values were compared 5.404 and 5.189 folds resistance acquired in Maruteru population at 24 hrs and 72 hrs after exposure, respectively.

The results represented in the present investigation revealed that Maruteru BPH population has developed low to moderate level of resistance to thiamethoxam recording resistance ratios ranging from 3.395 and 4.162 fold.

These results are in conformity with the findings of Jhansi Lakshmi *et al.* (2010) who have reported development of moderate to high level of resistance to thiamethoxam by recording 10.78 and 10.20 fold resistance in East Godavari district population to thiamethoxam during 2004 and 2006 years, respectively. Similarly, Rao *et al.* (2012) also observed moderate to high level of resistance to thiamethoxam i.e. 13.11 and 12.94 fold resistance during 2008-09 and 2009-10, respectively in BPH population of West Godavari district. About 1.01 to 2.19 fold resistance to thiamethoxam in BPH was observed in different regions of Karnataka even though thiamethoxam was not frequently used by the farmers which might be due to the cross resistance from imidacloprid that was extensively used in those areas (Basanth *et al.*, 2013) confirming the present results.

Wen *et al.,* (2018) reported that resistance to thiamethoxam might be due to intensive use of neonicotinoids since last 10 years that resulted in the development of cross resistance to thiamethoxam in BPH populations with high level of imidacloprid resistance, and also due to direct selection pressure of thiamethoxam which was deployed for BPH management along with imidacloprid (Matsumura *et al.,* 2008) Malathi *et al.* (2017) reported 4.52 to 14.99 folds of variation in resistance in Karnataka and Andhra Pradesh populations.

Pymetrozine

Toxicity of Pymetrozine in terms of LC_{50} values against susceptible laboratory population of Brown planthopper at 24 hrs and 72 hrs was 0.064 ppm and 0.038 ppm, respectively and 0.679 ppm and 0.215 ppm at LC90, respectively (Table 3). BPH population was considered to be homogenous by way of chi-square test. The log concentration probit line recorded slopes (b) were 1.252 ± 1.706 at 24 hrs and 72hrs, respectively.

BPH populations collected from Maruteru, West Godavari district during 2023-24 has recorded LC_{50} values of 0.130 ppm and 0.053 ppm at 24 hrs and 72hrs after exposure, respectively and the LC_{90} Values were 1.256 ppm and 1.127 ppm at 24 hrs and 72hrs after exposure, respectively (Table 2). The populations were considered to be homogeneous by way of chi-square test and the slopes (b) of log concentration probit line were 1.302 ± 0.963 at 24 hrs and 72 hrs, respectively.

The LC_{50} values of Maruteru were comparable with those of a susceptible laboratory BPH population at 24 hours and 72 hours after exposure and results indicated that low levels of resistance was developed to pymetrozine with resistance factor of 2.032 and 1.395 fold LC_{50} values at 24 hrs and 72 hrs respectively. Similarly, When LC_{90} Values were compared where 1.849 fold and 5.242 fold resistance acquired in Maruteru population at 24 hrs and 72 hrs after exposure, respectively (Table 1). The present findings reveal that there was low resistance development to pymetrozine in Maruteru region with resistance factor of 2.032 and 1.395 fold .

Pymetrozine does not have a knockdown effect and acts as an inhibitor for mouth parts action (Surahmat*et al*., 2016 Similar kind of results were also documented by several authors, reporting variations in the susceptibility of different populations from various locations. For instance, Mohan *et al.* (2019) recorded resistance ratios (RR) ranging from 1.19 to 1.59 in *Nilaparvata lugens* field populations collected from the Nalgonda district of Telangana. Khoa *et al*. (2018) found 2.4-fold variance in resistance to pymetrozine in BPH populations from Vietnam.

Yang*et al.* (2016) reported BPH resistance to pymetrozine from 2010 to 2014, the resistance ratios were 2.136 and 25.72 respectively in these five years, from the susceptible status in 2010 to the medium resistance level in 2014. Wang Peng *et al.* (2013) reported that pymetrozine developed low level resistance (1.9 to 5.1 fold) in 2010, while the resistance increased to moderate level (15.7 to 25.4 fold) in the next year.

Dinotefuran

Toxicity of dinotefuran in terms of LC_{50} values against susceptible laboratory population of Brown planthopper at 24 hrs and 72 hrs was 0.060 ppm and 0.054 ppm, respectively and 0.464 ppm and 0.229 ppm at LC_{90}, respectively (Table 3). BPH population was considered to be homogenous by way of chi-square test. The log concentration probit line recorded slopes (b) were 1.446 ± 2.031 at 24 hrs and 7 2hrs, respectively.

BPH populations collected from Maruteru, West Godavari district during 2023-24, has recorded LC_{50} values of 0.165 ppm and 0.079 ppm at 24 hrs and 72 hrs after exposure, respectively and the LC_{90} Values were 1.881 ppm and 0.887 ppm at 24 hrs and 72hrs after exposure, The populations were considered to be homogeneous by way of chi-square test and the slopes (b) log concentration probit line were 24 hrs and 72 hrs, they were 1.377 ± 1.657 at 24 hrs and 72 hrs, respectively (Table 2).

The LC_{50} values of Maruteru were comparable with those of laboratory BPH population at 24 hours and 72 hours after exposure and indicated that low level resistance factor of 2.750 fold and 1.463 fold for LC_{90} values at 24 hrs and 72 hrs respectively. Similarly, the LC_{90} Values were compared 2.052 fold and 3.88 fold resistance acquired in Maruteru populations at 24 hrs and 72 hrs after exposure respectively (Table 1).

Present findings were similar to the earlier authors like Mohan *et al.* (2019) recorded resistance ratios of Nalgonda population of BPH which has developed low level of resistance to dinotefuran with resistance ratios ranging from 0.7 to 3.6 fold .Liao *et al.* (2021), where they reported that the average LC_{50} of 21.27 mg/L in *Nilaparvata lugens* field populations of China. Datta *et al.* (2021) found varied responses *Nilaparvata lugens* to dinotefuran from China and Bangladesh populations. 2.5 folds of RR was documented by Khoa *et al.* (2018) in nine *Nilaparvata lugens* populations from Vietnam. Zhang *et al.* (2016) found varied responses to dinotefuran in *Nilaparvata lugens* populations from China with RR of 6.4 to 29.1 folds. Basanth *et al.* (2013) reported 0.82 to 2.22 folds of resistance to dinotefuran in *Nilaparvata lugens* population of Karnataka with LC_{50} of 2.16 to 5.83 mg/kg.

Wang *et al.* (2008) found variation in susceptibility to dinotefuran in field collected *Nilaparvata lugens* populations of Nanjing (Jiangsu, China), the resistance strains selected against imidacloprid showed substantial cross resistance to imidacloprid, thiacloprid and acetamiprid and slight level of cross resistance to dinotefuran and thiamethoxam.

Acephate

Toxicity of acephate in terms of LC_{50} values against susceptible laboratory population of Brown planthopper at 24 hrs and 72 hrs was 0.149 ppm and 0.75 ppm, respectively and 1.337 ppm and 0.653 ppm at LC_{90} values respectively (Table 3). BPH population was considered to be homogenous by way of chi-square test. The log concentration probit line recorded slopes (b) were 1.345 ±1.362 at 24 hrs and 72 hrs, respectively.

BPH populations collected from Maruteru, West Godavari district during 2023-24 has recorded LC_{50} values of 0.515 ppm and 0.145 ppm at 24 hrs and 72 hrs after exposure respectively and the LC_{90} Values were 8.278 ppm and 1.743 ppm at 24 hrs and 72 hrs after exposure. The populations were considered to be homogeneous by way of chi-square test and the slope (b) of log concentration probit line were 1.062 ± 1.188 at 24 hrs and 72 hrs after respectively (Table 2).

The LC_{50} values of Maruteru population were compared with those of a laboratory BPH population at 24 hours and 72 hours after exposure and results indicated that low levels of resistance was developed to

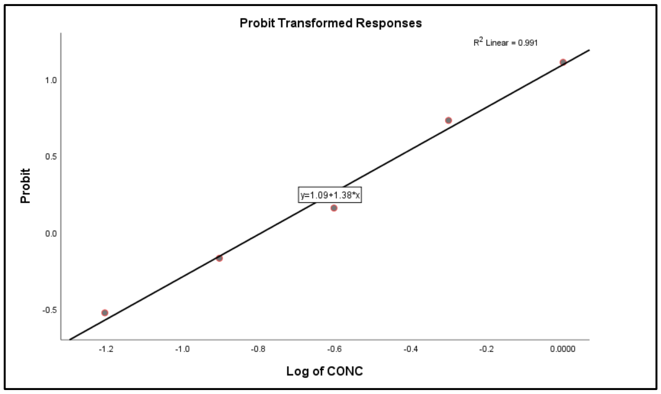

Fig. 1. **Dose Mortality response graph of BPH populations from Maruteru to thiamethoxam at 24 hours.**

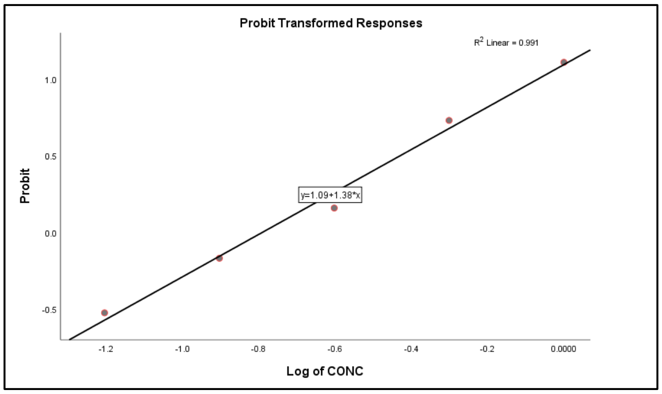

Fig. 2. **Dose Mortality response graph of BPH populations from Maruteru to thiamethoxam at 72 hours.**

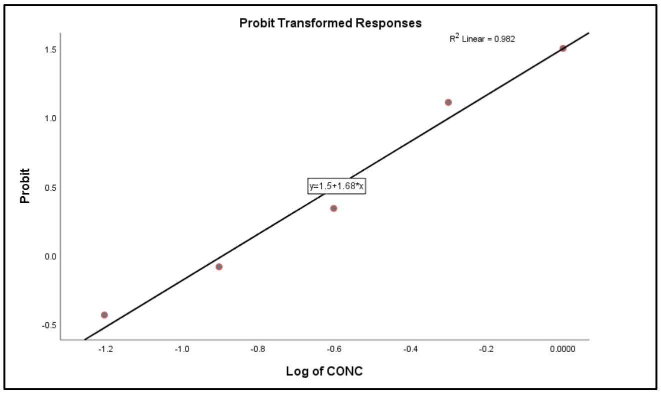

Fig. 3. **Dose Mortality response graph of BPH populations from Maruteru to pymetrozine at 24 hours.**

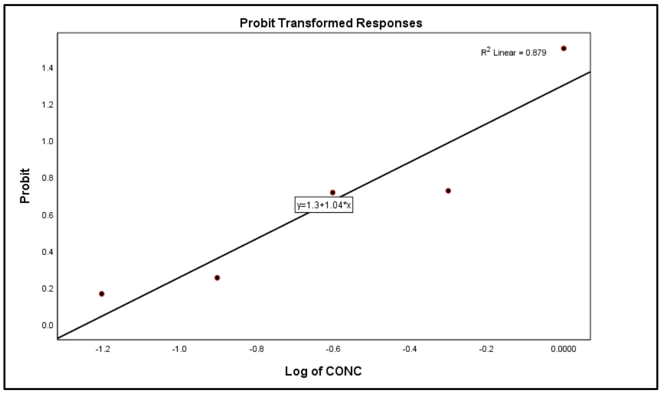

Fig. 4. **Dose Mortality response graph of BPH populations from Maruteru to pymetrozine at 72 hours.**

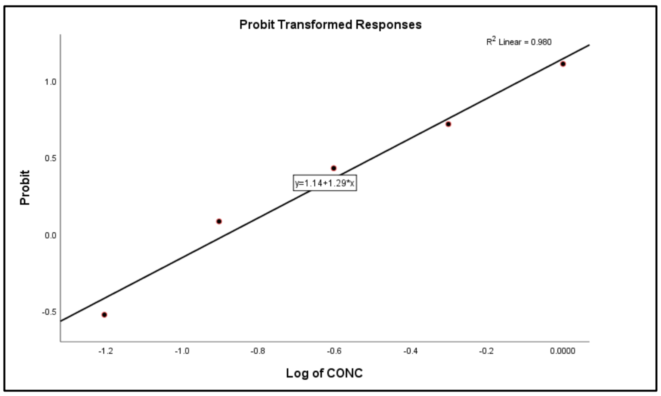

Fig. 5. **Dose mortality response graph of BPH populations from Maruteru to dinotefuranat 24 hours**

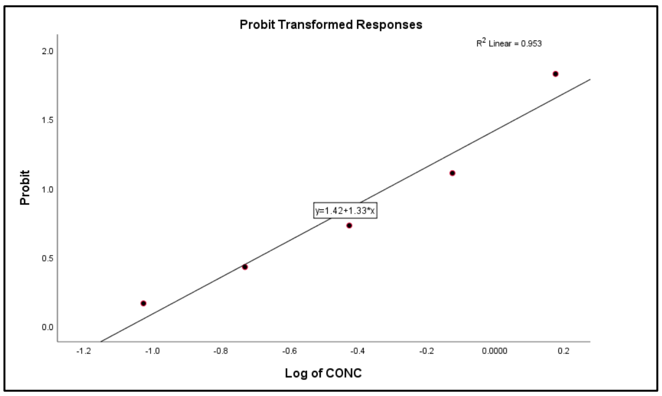

Fig. 6. **Dose mortality response graph of BPH population Maruteru to dinotefuran at 72 hours**

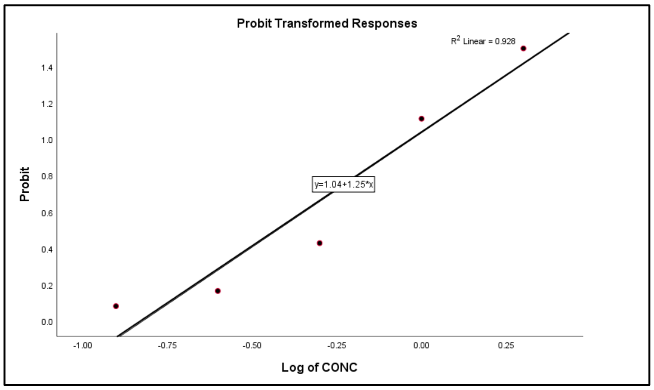

Fig. 7. Dose mortality response graph of BPH populations from Maruteru to acephate at 24 hours

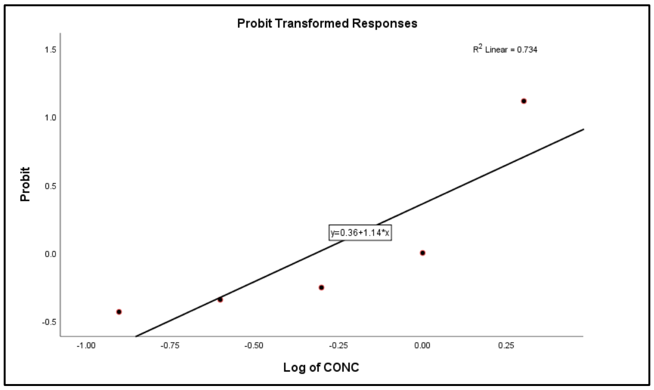

Fig. 8. Dose mortality response graph of BPH populations from Maruteru to acephate at 72 hours

acephate with resistance factor of 3.456 fold and 0.194 fold at 24 hrs and 72 hrs respectively. Similarly, when LC_{90} Values were compared factor 6.192 fold and 2.67 folds resistance acquired in Maruteru population at 24 and 72 hrs after exposure, respectively (Table 1).

The study revealed that pymetrozine exhibited the highest toxicity among the insecticides tested, as indicated by its low LC_{50} values, while acephate was the least toxic, showing the highest LC_{50} values. The Maruteru population demonstrated moderate resistance to thiamethoxam and pymetrozine, with resistance factors of 4.161-fold and 1.463-fold, respectively. In contrast, low resistance was observed to dinotefuran and acephate, with resistance factors of 1.395 fold and 0.193-fold, respectively, at 72 hours

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