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Documentation of Pyrethroid Resistance against Brinjal Shoot and Fruit Borer, *Leucinodes orbonalis* Guenee (Pyralidae: Lepidoptera)

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A B S T R A C T

In the present investigation, differential response was observed in different populations of *L. orbonalis* with respect to six different pyrethroids, *viz.*, alphamethrin, cypermethrin, deltamethrin, fenvalerate, λ-cyhalothrin, and fenpropathrin. The Kolar population was found to be most susceptible to alphamethrin, deltamethrin and λ-cyhalothrin with LC_{50} values of 0.01, 0.02 and 0.02 ml/l, respectively, while Belagavi and Bengaluru and Koppal population were recorded most susceptible to cypermethrin, fenvalerate and fenpropathrin, LC_{50} values ranged from 0.04 to 0.13 ml/l. The results also suggested different pyrethroids use pattern in different places, among different populations, Koppal population was found 24-fold resistant to alphamethrin, Belagavi population was 12-fold resistant to deltamethrin, Bengaluru population was 10.5-fold resistant to λ-cyhalothrin and Belagavi population was 2.5-fold more resistant to fenpropathrin. However, all populations were found to be highly susceptible to cypermethrin and highly resistant to fenvalerate as LC_{50} values ranged from 0.13 to 0.26 ml/l for different populations.

Introduction

Study of insecticide resistance is one of the key components to devise control measures, by altering the use of different groups of insecticides. There are several studies that suggest most of the insecticides do not provide satisfactory control of brinjal shoot and fruit borer (BSFB), thereby resulting in crop losses, leading to indiscriminate use in many parts of the country and elsewhere. Such scenario often results in development of resistance, which leads to misuse of insecticides and threaten environmental safety and cause health hazards. Although the resistance is a natural mechanism for survival, its development has been accelerated in recent year due to excessive dependence upon chemical pesticides. Insecticides resistance in BSFB especially to pyrethroids is now widespread in many brinjal-producing countries. Due to resistance, pest management program is likely to suffer and to some extent would be ineffective resulting serious reduction of crop production. It is therefore, necessary to undertake further studies towards recognition and monitoring of resistance in BSFB and other insects that are exposed very frequently to high selection pressure of insecticides.

The genetic plasticity of *L. orbonalis* is apparent in its ability to detoxify many synthetic insecticides in addition to the secondary plant metabolites present in its narrow array of host plant species. In response to the stresses, the populations of *L. orbonalis* have adapted to the insecticide applications by changing their eco-behavioural pattern, feeding physiology and reproduction (Isahaque and Chaudhuri, 1984). This in turn led to excessive and indiscriminate use of insecticides (spraying upwards of 40 times per season) by desperate farmers (Shelton, 2010). Pesticide resistance is emerging as one of the key constraints to successful crop protection and public health problems worldwide (Dover and Croft, 1984). Insecticides resistance in *L. orbonalis* especially to pyrethroids is now widespread in many brinjal-producing countries, which is evident from reports of great variations in the requirements of pesticides application in different areas. Resistance detection is the vital components of pesticide resistance management. It aims to identify the initial presence of resistant individuals in a pest population.

Materials and Methods

Field population

Leucinodes orbonalis larvae collected from 5 different places of Karnataka and reared under laboratory condition on naturally available potato to obtain healthy culture, and F_2 generation larvae were used for measuring insecticide resistance. The bioassay methods should closely simulate field-conditions to ensure predictability of control efficacy in the field from data obtained through lab-measured resistance. Over the past two decades, bioassay methods based on leaf-dip, larval dip, topical application, leaf spraying and vial-residue were developed as viable alternatives to simulated field conditions (Kranthi, 2005). The leaf/foliar spray method was used under laboratory condition for concentration - mortality test of BSFB. Each insecticide tested was diluted to get six concentrations for each of the selected insecticides.

Procedure of testing resistance

Five BSFB populations from Bengaluru, Doddaballapur, Kolar, Koppal and Belagavi were used for detection of insecticide resistance. The experiment was conducted at NBAIR, Bengaluru, from May to September, 2014. The neonates were used for testing of resistance and the observations were taken at 24 h. Before standardizing this time, mortality was observed for different time intervals from 1h to up to 48 h. At 48 h, all the larvae were dead in all the different concentrations, which were used for detecting resistance and even in control more than 90 per cent mortality was recorded, therefore, the observation was restricted at 24 h only. For conducting bioassay, neonates were used because, after hatching, larvae bore into the tender midribs of leaves or shoots or twigs or fruits and feeds inside up to final instar and come out only for pupation on calyx of fruits or on leaves or debris at base of the plant.

Insecticide treatments

The brinjal leaves were always collected from plants raised in earthen pots and such plants were not sprayed throughout experiment. The stalk of the leaves were firmly covered with finely teased wet cotton to maintain tenderness of the leaves and then the leaves were sprayed in discriminating concentration (2 ml, 0.5 ml, 0.125 ml, 0.0312 ml, 0.008 ml and 0.002 ml (4 insecticides, *viz*., cypermethrin 25 % EC, deltamethrin 2.8 % EC, alphamethrin 10 % EC fenpropathrin 20 % EC) and 4 ml, 1 ml, 0.25 ml, 0.062 ml, 0.016 ml, 0.004 ml (2 insecticides, *viz*., lambda cyhalothrin 5 % EC, fenvalerate 20 % EC) of the each insecticides and air dried. Thus, the leaves of the brinjal were made ready for use. The leaves were sprayed with the water only to serve as control. Ten larvae were released in each concentration thus, 70 larvae were used for one replication and each experiment was replicated 5 times. There were six different dilutions of each chemical for bioassay with five replications. Initial screening were done with only ten number of neonate larvae from field collected population, using six broad range of dilutions to decide on the dilution range to be used and it is called bracketing. So, initial bracketing was done and the final dilution of each insecticide was done. The different concentrations of field recommended insecticides were prepared by using water and water is used as control.

Statistical method for LC_{50} calculation

Log dose probit analysis was carried out to obtain LC_{50} and LC_{90} values, fiducial limits and regression equation that enable the calculation of the dose / concentration required for any particular per cent mortality that they cause in the test population developed on the basis of Finney's (1971) method. Once the bioassay results were found to confirm to a graded response depending on the concentration of the toxicant, they were then subjected to probit analysis through a series of manual calculations or on computer-aided programs, for this study, the data were analyzed using SPSS 16 Version of Statistical Programme.

Results and Discussion

Alphamethrin

The susceptibility of *L. orbonalis* populations for alphamethrin varied greatly (Table 1). Among the five populations, Kolar populations were more susceptible to the insecticide (LC_{50}:0.01 ml/l), followed by Bengaluru (0.04 ml/l), Doddaballapur (0.05 ml/l), Belagavi (0.12 ml/l) and Koppal (0.24 ml/l). The Koppal populations were more resistant when compared to the other field populations. The non-overlapping method of statistical analysis showed that the Kolar populations were highly distinct, it was separated from other populations having fiducial limit ranging from 0.01-0.02, followed by Bengaluru and Doddaballapur populations, which overlapped with each other having fiducial limits that ranged from 0.03-0.07, while population from Belagavi and Koppal formed a distinct cluster because they overlapped with each other but were distinct from other populations (Table 1; Fig. 1).

Cypermethrin

The median lethal dose concentrations of cypermethrin for five field populations of *L. orbonalis* ranged from 0.04 to 0.07 ml/l (Table 2). Belagavi populations were more susceptible (LC_{50}:0.04 ml/l), followed by Koppal (0.05 ml/l), Doddaballapur (0.05 ml/l) and Bengaluru (0.06 ml/l). The Kolar population (0.07 ml/l) was found to be relatively more tolerant to the insecticide. However, there was no statistical difference between the populations estimated by non-overlap of fiducial limit (Fig. 2).

Deltamethrin

The median lethal dose concentrations of cypermethrin for five field populations of *L. orbonalis* ranged from 0.02 to 0.24 ml/l (Table 3). Kolar populations were most susceptible to the insecticide (LC_{50}:0.02 ml), followed by Bengaluru (0.03 ml/l), Koppal (0.04 ml/l), Doddaballapur (0.15 ml/l) and Belagavi (0.24 ml/l). The Belagavi populations were more resistant to this insecticide (Table 3). The non-overlapping method of statistical analysis revealed that the populations from Bengaluru, Kolar and Koppal were statistically *on par* with each other, however, Doddaballapur and Belagavi populations were distinct, therefore, highly resistant (Fig. 3).

Fenvalerate

The bioassays revealed that Bengaluru and Koppal field populations were relatively susceptible to insecticide (LC_{50}:0.13 ml/l), followed by Belagavi (0.16 ml/l), Doddaballapur (0.24 ml/l) and Kolar (0.26 ml/l). The Kolar population was recorded as most tolerant to this insecticide (Table 4). Comparison of statistical analysis based on non-overlap method indicated that all populations were *on par* with each other in terms of their tolerance to this insecticide (Fig. 4).

Lambda cyhalothrin

The susceptibility of *L. orbonalis* populations to lambda cyhalothrin varied greatly (Table 5). Among the five populations, Kolar populations were most susceptible to the insecticide (LC_{50}:0.02 ml/l), followed by Doddaballapur (0.10 ml/l), Belagavi (0.12 ml/l) and Bengaluru, Koppal populations were resistant to the insecticide (0.21 ml/l) (Table 5). The non-overlapping method of statistical analysis revealed that Kolar population was a distinct one and all other populations were *on par* with each other (Fig. 5).

Fenpropathrin

The median lethal dose concentrations of fenpropathrin to five field populations of *L. orbonalis* ranged from 0.08 to 0.20 ml (Table 6). The results indicated that Bengaluru and Koppal populations were most susceptible to the insecticide (LC_{50}:0.08 ml/l), followed by Kolar (0.14 ml/l), Doddaballapur (0.19 ml/l) and Belagavi populations which were more resistant to insecticide (0.20 ml/l). Among all the populations collected from the five locations, Bengaluru and Koppal populations had similar susceptibility as indicated by non-overlap method of population, whereas Bengaluru and Kolar populations were similar as their fiducial limits overlapped and Doddaballapur, Kolar and Belagavi were not significantly different from each other (Table 6; Fig. 6).

In the present investigation, differential response was observed in different populations of *L. orbonalis* with respect to six different pyrethroids, *viz.*, alphamethrin, cypermethrin, deltamethrin, fenvalerate, λ-cyhalothrin, and fenpropathrin. Kolar population was found to be most susceptible to alphamethrin, deltamethrin and λ-cyhalothrin with LC_{50} values of 0.01, 0.02 and 0.02 ml/l, respectively, while Belagavi and Bengaluru & Koppal population were recorded most susceptible to cypermethrin, fenvalerate & fenpropathrin, LC_{50} values ranged from 0.04 to 0.13 ml/l. The results also suggested different pyrethroids use pattern in different places. Among different populations, Koppal population was found 24-fold resistant to alphamethrin, Belagavi population was 12-fold resistant to deltamethrin, Bengaluru population was 10.5-fold resistant to λ-cyhalothrin and Belagavi population was 2.5-fold more resistant to fenpropathrin. However, all populations were found to be highly susceptible to cypermethrin and highly resistant to fenvalerate as LC_{50} values ranged from 0.13 to 0.26 ml/l for different populations. In a earlier study, a high degree of resistance to pyrethroids (cypermethrin, fenvalerate and deltamethrin) and an organophosphate (quinalphos) was recorded for *L. orbonalis* from different parts of India (Saxena *et al.*, 1989).

Table.1 The probit analysis of dosage mortality response of field populations of *L. orbonalis* to alphamethrin

Population	LC_{50} (ml/l)	Fiducial limit		LC_{90} (ml/l)	Fiducial limit		Regression equation	Chi-square value	df	Probability
		Lower	Upper		Lower	Upper	Y=a+bx			
Bengaluru	0.04	0.031	0.07	1.19	0.63	2.92	1.21+0.91x	29.64	28	0.38
Doddaballapur	0.05	0.03	0.07	0.79	0.46	1.63	1.39+1.09x	14.97	28	0.97
Kolar	0.01	0.01	0.02	0.74	0.36	2.07	1.38+0.78x	19.32	28	0.88
Belagavi	0.12	0.08	0.18	2.65	1.34	6.93	0.87+0.95x	28.23	28	0.45
Koppal	0.24	0.15	0.41	6.85	3.01	22.72	0.54+0.88x	32.27	28	0.26

Table.2 The probit analysis of dosage mortality response of field populations of *L. orbonalis* to cypermethrin

Population	LC_{50} (ml/l)	Fiducial limit		LC_{90} (ml/l)	Fiducial limit		Regression equation	Chi-square value	df	Probability
		Lower	Upper		Lower	Upper	Y=a+bx			
Bengaluru	0.06	0.045	0.08	0.57	0.36	1.05	1.60+1.33x	21.71	28	0.79
Doddaballapur	0.05	0.03	0.08	3.62	1.44	14.70	0.89+0.69x	14.96	28	0.97
Kolar	0.07	0.04	0.10	1.02	0.59	2.13	1.27+1.10x	23.39	28	0.71
Belagavi	0.04	0.03	0.06	0.61	0.36	1.23	1.51+1.11x	26.00	28	0.57
Koppal	0.05	0.03	0.08	1.53	0.78	3.99	1.11+0.88x	23.14	28	0.72

Table.3 The probit analysis of dosage mortality response of field populations of *L. orbonalis* to deltamethrin

Population	LC_{50} (ml/l)	Fiducial limit		LC_{90} (ml/l)	Fiducial limit		Regression equation	Chi-square value	df	Probability
		Lower	Upper		Lower	Upper	Y=a+bx			
Bengaluru	0.03	0.021	0.04	0.81	0.43	1.92	1.36+0.91x	17.58	28	0.93
Doddaballapur	0.15	0.13	0.30	2.18	1.22	4.90	0.90+1.11x	21.12	28	0.82
Kolar	0.02	0.01	0.03	0.53	0.28	1.25	1.53+0.90x	17.87	28	0.92
Belagavi	0.24	0.15	0.41	6.85	3.01	22.72	0.54+0.88x	32.27	28	0.26
Koppal	0.04	0.03	0.07	1.53	0.75	4.16	1.12+0.85x	27.13	28	0.51

Table.4 The probit analysis of dosage mortality response of field populations of *L. orbonalis* to fenvalerate

Population	LC_{50} (ml/l)	Fiducial limit		LC_{90} (ml/l)	Fiducial limit		Regression equation	Chi-square value	df	Probability
		Lower	Upper		Lower	Upper	Y=a+bx			
Bengaluru	0.13	0.076	0.24	13.95	5.13	66.86	0.55+0.63x	32.98	28	0.23
Doddaballapur	0.24	0.16	0.37	5.40	2.73	14.21	0.58+0.94x	14.28	28	0.98
Kolar	0.26	0.17	0.39	5.32	2.73	13.55	0.57+0.97x	19.23	28	0.89
Belagavi	0.16	0.11	0.24	2.72	1.53	6.05	0.82+1.05x	19.24	28	0.89
Koppal	0.13	0.09	0.19	1.93	1.12	4.05	0.96+1.10x	15.62	28	0.97

Table.5 The probit analysis of dosage mortality responses of field populations of *L. orbonalis* to lambda cyhalothrin

Population	LC_{50} (ml/l)	Fiducial limit		LC_{90} (ml/l)	Fiducial limit		Regression equation	Chi-square value	df	Probability
		Lower	Upper		Lower	Upper	Y=a+bx			
Bengaluru	0.21	0.146	0.31	3.82	2.16	8.37	0.68+1.02x	30.25	28	0.35
Doddaballapur	0.10	0.07	0.14	1.48	0.87	3.04	1.09+1.10x	14.50	28	0.98
Kolar	0.02	0.01	0.04	0.88	0.47	2.17	1.32+0.84x	26.44	28	0.54
Belagavi	0.12	0.08	0.19	3.71	1.84	10.10	0.78+0.86x	19.06	28	0.89
Koppal	0.21	0.13	0.334	8.09	3.77	24.64	0.54+0.80x	33.97	28	0.20

Table.6 The probit analysis of dosage mortality response of field populations of *L. orbonalis* to fenpropathrin

Population	LC_{50} (ml/l)	Fiducial limit		LC_{90} (ml/l)	Fiducial limit		Regression equation	Chi-square value	df	Probability
		Lower	Upper		Lower	Upper	Y=a+bx			
Bengaluru	0.08	0.04	0.14	7.38	2.78	34.40	0.95+0.77x	25.28	28	0.58
Doddaballapur	0.19	0.14	0.33	3.79	1.97	9.55	0.74+1.02x	17.70	28	0.90
Kolar	0.14	0.09	0.21	2.92	1.50	7.40	1.05+0.93x	18.55	28	0.90
Belagavi	0.20	0.13	0.32	4.78	2.27	14.38	0.68+0.96x	25.75	28	0.57
Koppal	0.08	0.06	0.13	1.73	0.93	4.10	1.04+0.97x	21.37	28	0.74

Fig.1 The probit analysis of dosage mortality response of field populations of *L. orbonalis* to alphamethrin

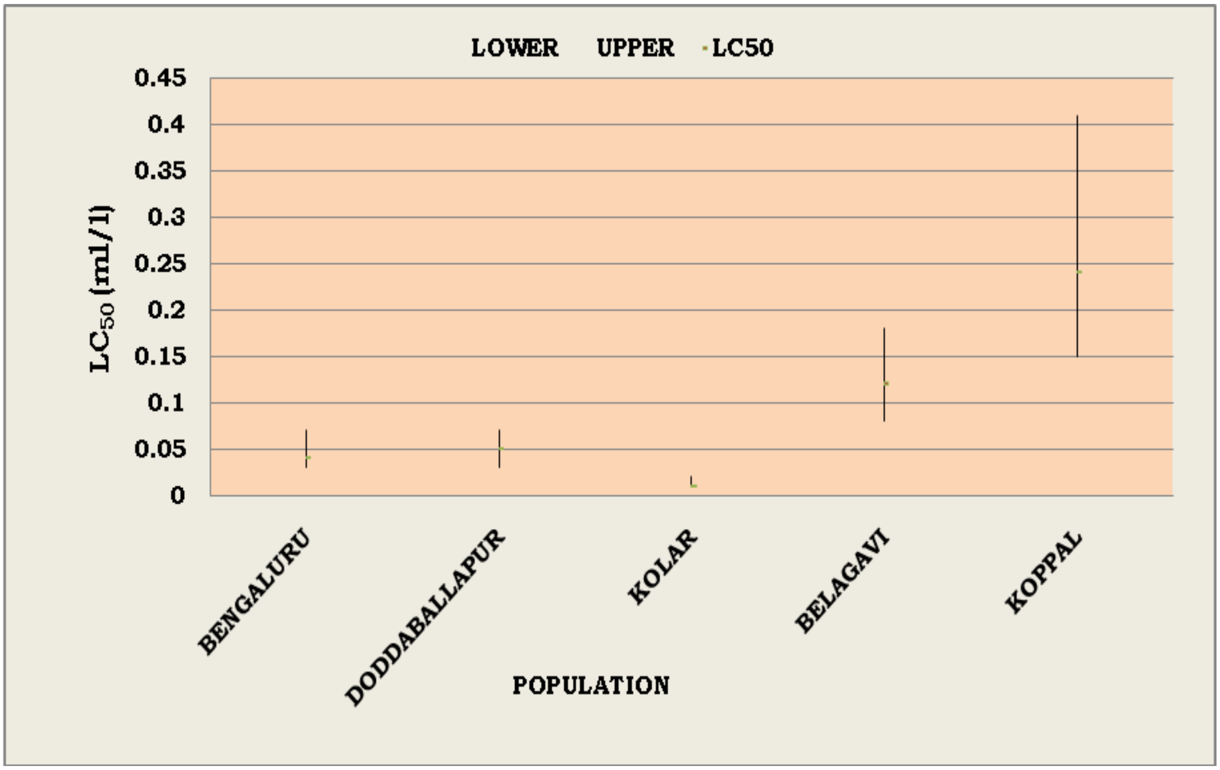


Fig.2 The probit analysis of dosage mortality response of field populations of *L. orbonalis* to cypermethrin

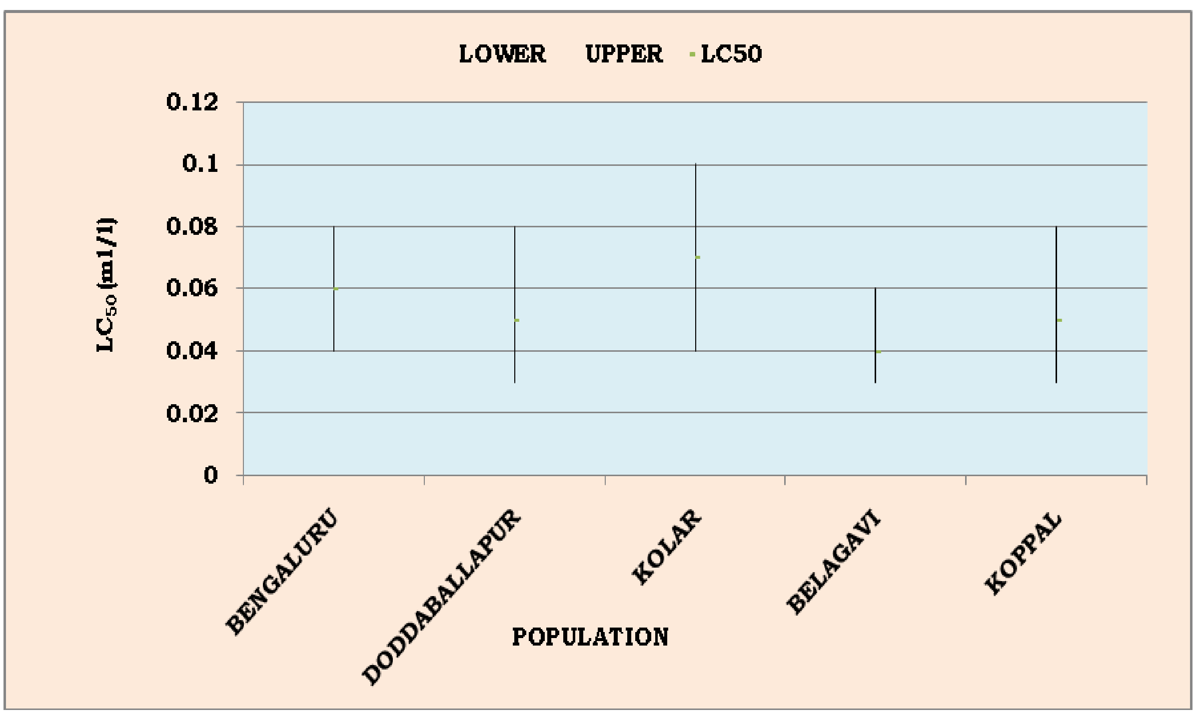


Fig.3 The probit analysis of dosage mortality response of field populations of *L. orbonalis* to deltamethrin

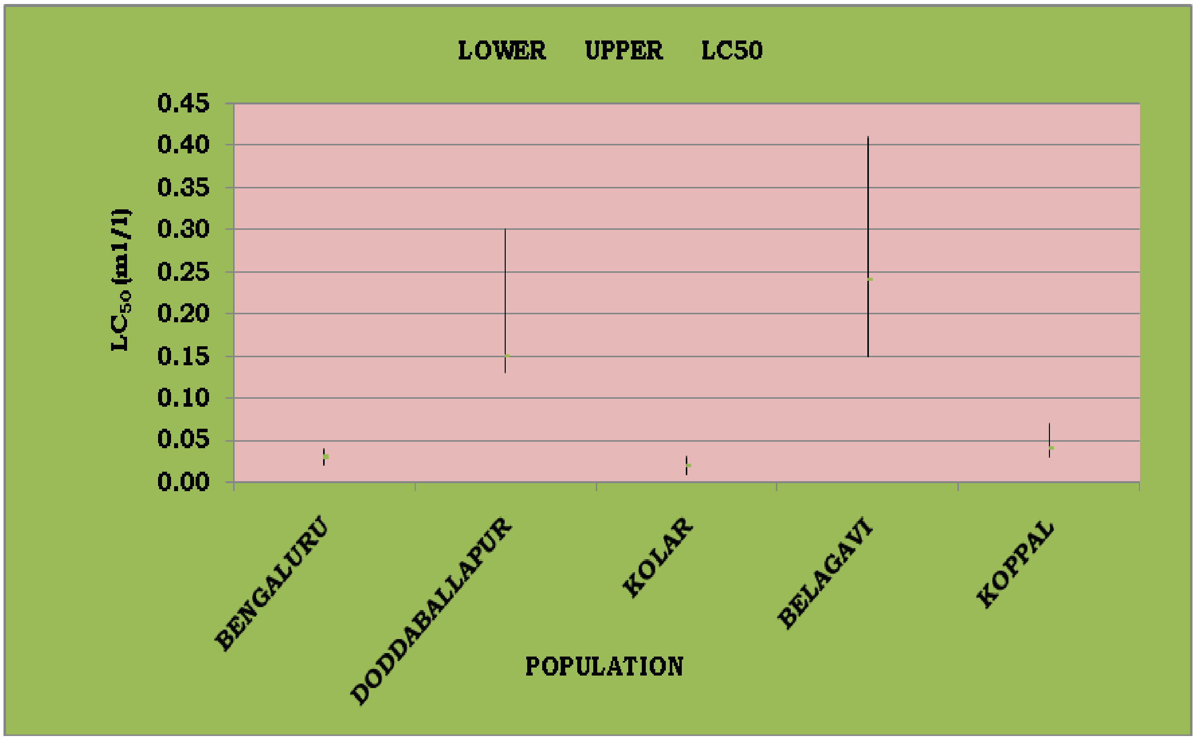


Fig.4 The probit analysis of dosage mortality response of field populations of *L. orbonalis* to fenvalerate

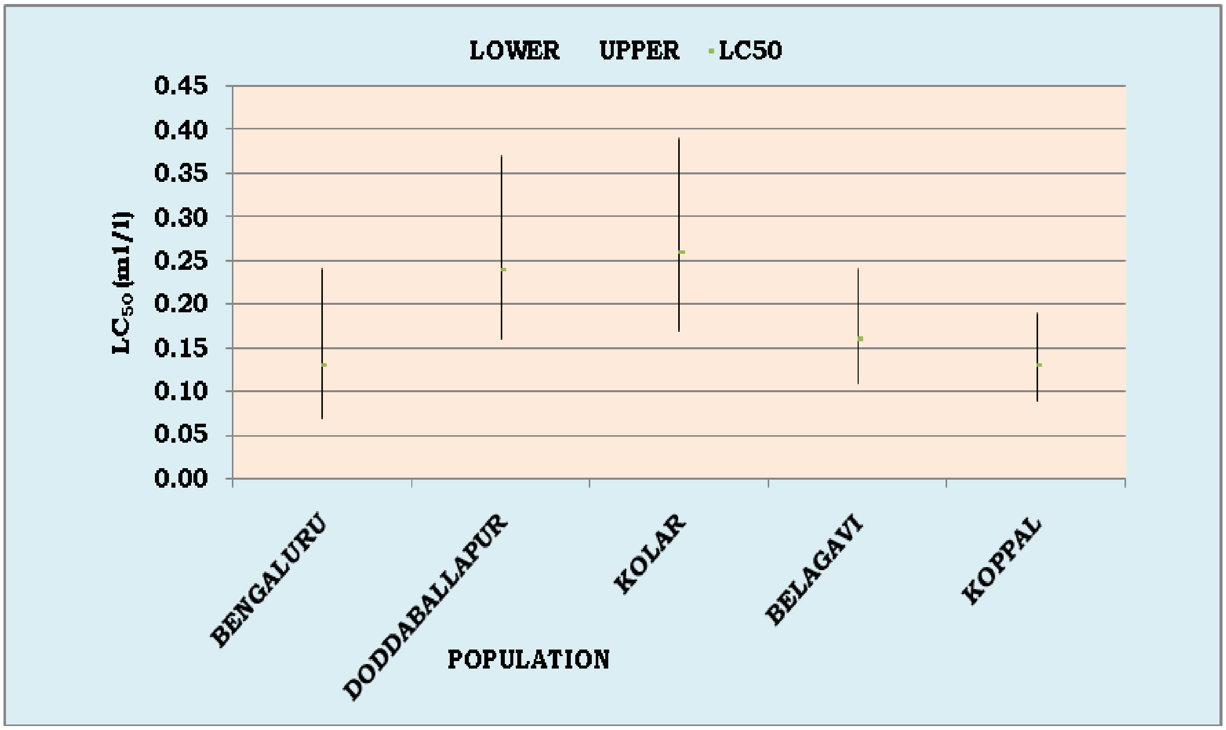


Fig.5 The probit analysis of dosage mortality responses of field populations of *L. orbonalis* to lambda cyhalothrin

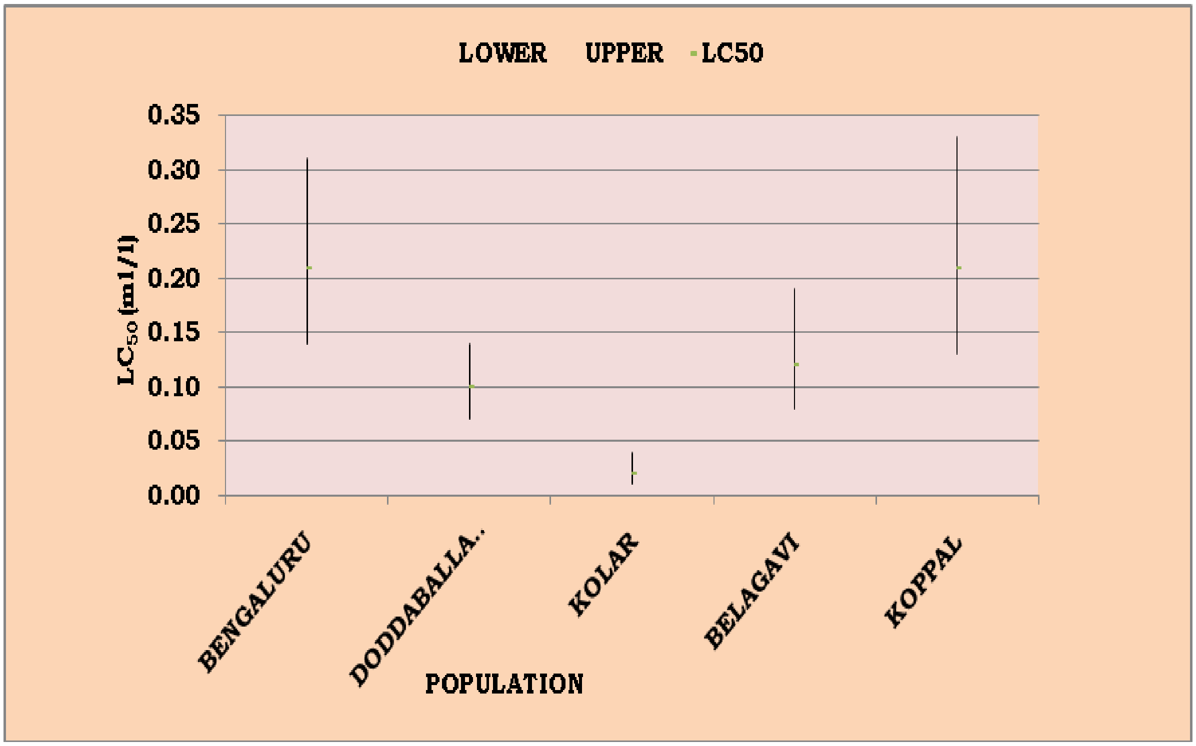
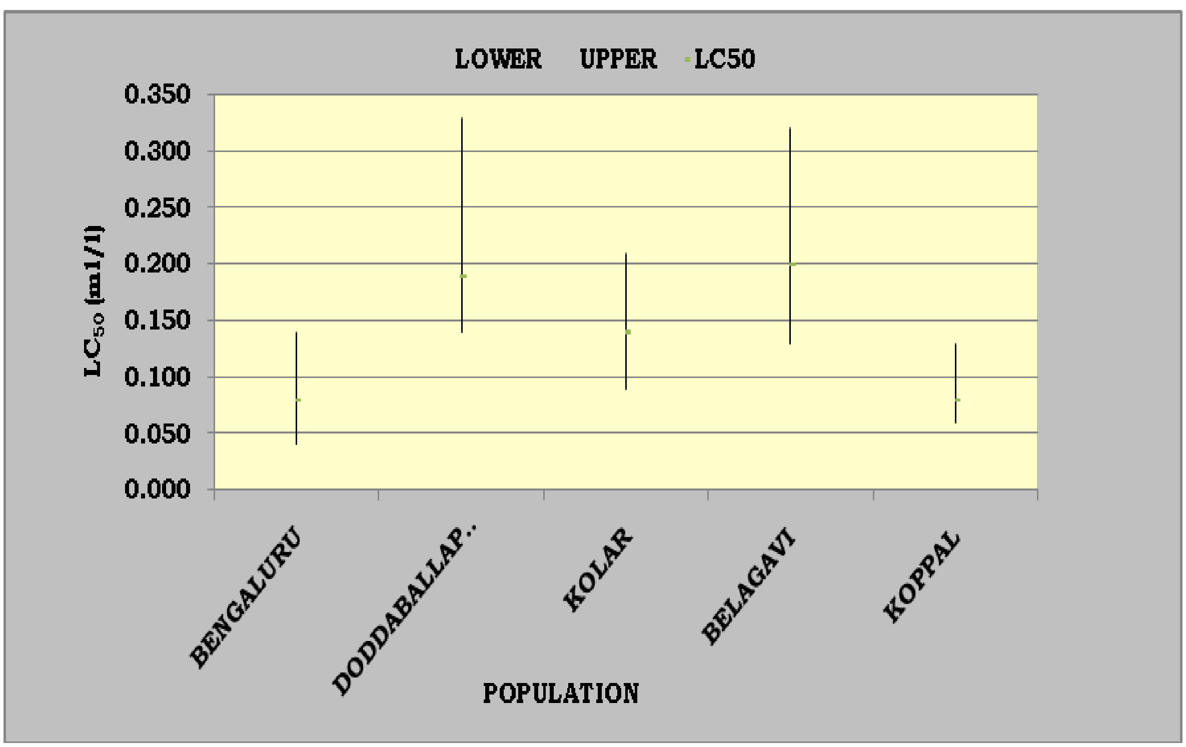


Fig.6 The probit analysis of dosage mortality responses of field populations of *L. orbonalis* to fenpropathrin



The results of the present work is in agreement with the findings of Ali (1994) who reported that *L. orbonalis* development of resistance to pyrethroid in Bangladesh and observed that in summer season, the farmers of Bangladesh spray almost daily for control of *L. orbonalis*, whereas in West Bengal, (India) frequency of application exceeds three sprays per week. The presence of such cross or multiple resistances for Jessore (Bangadesh) population was likely since farmers of Jessore area sprayed brinjal fields every day or even twice a day with a variety of insecticides (Kabir *et al.*, 1996). Later on, Rahman and Rahman (2009) reported that 7.28 to 18.19 per cent resistance to three insecticides belonging to carbamate and pyrethroid population was found in Jessore and Gazipur populations of Bangladesh.

From the above research findings were supported by Rahman and Rahman (2009), who reported the variation of mortality estimates for *L. orbonalis*, the lowest LC_{50} value was found for Marshal 20 EC, Ripcord 10 EC and Basathrin 10 EC for Jessore and Gazipur region and reported mortalities of 73.3, 80.0, 73.3, 73.3 and 73.3 and 80.0 per cent, respectively for Marshal, Ripcord and Basathrin in Jessore and Gazipur districts. The percentage of resistance was higher (18.19 %) to Marshal 20 EC in Jessore population than in Gazipur (11.52 %). Abrol and Singh (2003) in Jammu evaluated six insecticides and their eight combinations for their efficacy against *L. orbonalis* and reported the combination of endosulfan + deltamethrin and and endosulfan + fenvalerate were highly effective, the other promising treatments were carbaryl + fenvalerate = dichlorvos + fenvalerate > malathion + fenvalerate > fenvalerate + deltamethrin > dichlorvos = carbaryl + deltamethrin = malathion = dichlorvos + deltamethrin = malathion + deltamethrin > endosulfan > carbaryl, respectively. In a study in Punjab, three populations collected from Amritsar, Malerkotla and Hoshiarpur, LC_{50} values ranged from 0.06 to 1.6 ppm for five different populations with emamectin benzoate showed highest toxicity for Amritsar and Malerkotla populations, while no appreciable differences was observed for Hoshiarpur population for any of insecticides tested (Kaur *et al.,* 2014).

Due to resistance development, it can be assumed that pest management programme may result in reduced levels of control, thereby reduction in crop production. It is therefore, necessary to further take up studies on insecticide resistance management and further monitoring of the resistance levels.

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