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Baseline Susceptibility and Resistance Monitoring of Pyridalyl 10 EC against *Plutella xylostella* L. (Lepidoptera: Plutellidae) in Tamil Nadu, India

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

In vitro studies were conducted to assess the baseline toxicity of pyridalyl 10 EC against Diamondback moth, *Plutella xylostella* collected from four major cabbage and cauliflower growing tracks in Tamil Nadu. The LC_{50} and LC_{95} values of Pyridalyl 10 EC from F_1 to F_{15} generations declined from 2.528 to 0.447 ppm and 14.978 to 2.235 ppm respectively. The susceptibility index to pyridalyl was 5.655 based on LC_{50} and 6.702 based on LC_{95}. With regard to number of generation required for ten-fold decrease in LC_{50} was 19.934. Considering the F_{15} population of *P. xylostella* as the most susceptible, the tentative discriminating dose arrived was 2.235 ppm. Resistance monitoring studies of *P. xylostella* across locations *viz.* Coimbatore, Hosur, Ooty, and Oddanchatram indicated that the per cent resistance ranged from the lowest of 2.008 ppm in Oddanchatram to the highest of 3.696 ppm in Hosur. The Pyridalyl 10 EC reflected the highest resistance ratio of 8.268 fold in Hosur field population and the lowest resistance ratio of 4.492 fold in Oddanchatram field population.

Keywords: Baseline susceptibility; discriminating dose; P. xylostella; Pyridalyl 10 EC; resistance ratio; resistance monitoring; susceptibility index.

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1. INTRODUCTION

The Diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae) is one of the most devastating insect pests of cruciferous vegetables, *viz.,* cabbage, cauliflower, broccoli, brussels sprouts, and turnips all over the world. With a productivity of 22.92 MT ha^{-1} and an area of 3.72 lakh hectares, India is the second-largest producer of cabbage in the world, after China [1]. *P. xylostella* is a globally important pest, causing serious yield losses to crucifers. It was originally reported in India in 1914 [2]. Worldwide, it causes around 90% yield loss by feeding on the foliage of the crops and the damage might reach up to 4–5 billion USD per year. The expense of managing the pest was estimated to be one billion USD per year [3]. Commercial venture of this crop unfortunately has compelled the farmers to make more frequent treatments of different pesticides at higher doses than recommended dose for controlling this pest. The judicious use of chemicals with novel mode of action needs to be implemented to manage this insect pest [4]. Totally 25 insecticides representing various chemical groups are registered in India for the control of Diamondback moth. The field populations of *P. xylostella* have developed resistance to approximately 101 common pesticides due to frequent application of insecticides, high fecundity, genetic flexibility, and rapid generation times [5]. In India, the first report of insecticide resistance development in the diamondback moth was in 1966 around Ludhiana, Punjab against DDT and Parathion [6]. Pyridalyl is a novel insecticide with uncertain mode of action and efficient against wide range of pests including Lepidoptera [7, 8], Thysanoptera [9] and Diptera [10]. Pyridalyl was first registered in 2004 as an agricultural chemical in Japan and Korea and has been commercialized for Diamondback moth control [11]. The ever challenging *P. xylostella* showed resistance against Pyridalyl 10% EC around the World, including China [11, 12] and Japan [13]. Sakamoto [14] reported that Lepidopteran pests with resistance to pyridalyl show little cross-resistance to organophosphates, benzoylureas and pyrethroids and also pose little toxicity to a variety of helpful insects and mammals. Despite the advantages of pyridalyl, the excessive spraying of pyridalyl in field might lead to development of resistance in DBM. In this evolving scenario, generating baseline data of Pyridalyl 10 EC against *P. xylostella* was taken up in the context of pest management system support.

2. MATERIALS AND METHODS

2.1 Maintenance of Insect Culture

Field populations of *P. xylostella* were collected from four different geographical locations, *viz.* Coimbatore, Hosur, Ooty, and Oddanchatram in Tamil Nadu, India (Fig. 1 and Table 1). Fourth instar larvae and pupae were collected using fine brush and forceps from different crops *viz.,* cabbage and cauliflower, belongs to the Brassicaceae family. Collected larvae were mass reared on insecticide free *Brassica oleracea* var. botrytis leaves which were cultivated under maintained conditions in plastic pots in glass house. The larvae that pupated on different days were collected and stored in refrigerator at 4 to 5ºC to enhance uniform adult emergence. Then the pupae were taken out from refrigerator and kept in adult emergence cage. The emerged adults were fed with 10 per cent sugar solution enriched with multivitamin tablets and allowed to lay eggs on mustard seedlings raised in paper cups. The populations were maintained separately at 26 ± 1 ºC, and photoperiod of 14:10 (L:D) h. The Coimbatore population was continuously reared up to F_n generation under laboratory conditions by providing insecticide free cauliflower leaves as feed and bioassay was conducted for subsequent generations.

Table 1. Background data for field populations of *P. xylostella* collected from different sites

Collected Location	Coordinates	Map Reference no.	Host Plant
Coimbatore, Tamil Nadu	10.99º N, 76.75º E	1	*Brassica oleracea* var. botrytis
Hosur, Tamil Nadu	12.75º N, 77.89º E	2	*Brassica oleracea* var. botrytis
Ooty, Tamil Nadu	11.39º N, 76.69º E	3	*Brassica oleracea* var. capitata
Oddanchatram, Tamil Nadu	11.51º N, 77.74º E	4	*Brassica oleracea* var. botrytis

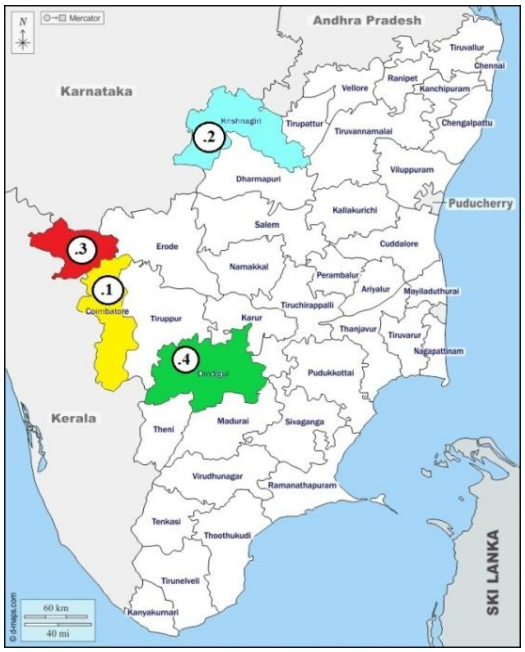

Fig. 1. Sampling sites of *P. xylostella* field populations in Tamil Nadu

2.2 Leaf Dip Bioassay

Certified Reference Material (CRM) of pyridalyl with 96.2 per cent purity was obtained from M/s. Sigma Aldrich (Bangalore, India). The CRM was diluted to 1000 ppm with acetonitrile (C_2H_3N) and further serial dilutions for different treatments were made with distilled water. Field collected larvae (*P. xylostella*) were cultured to establish a population in their natural host and leaf dip bioassay (IRAC, 018) [15] was used to determine resistance using insecticide-free Brassica oleracea var. botrytis leaves. The insecticide dilutions required for bioassay were prepared by dissolving the insecticide in distilled water containing 0.5% Triton X-100, and distilled water containing 0.5% Triton X-100 only was used as control. In each concentration, three replicates were conducted and the insecticide free leaves of *Brassica oleracea* var. botrytis were cut into discs (diameter 6.0 cm), immersed in each concentration for 10 sec then shade dried for 1h. Leaf discs were transferred to bioassay container (10 cm in diameter, 4.0 cm in depth) lined with slightly moistened filter paper. Ten individuals of 3rd instar larvae measuring 1.83 ± 0.28 mg in weight and 0.5 ± 0.12 cm long were used for each replicate and the bioassay containers were sealed with a lid. Mortality was recorded at 24, 48 and 72 h after treatment and the final assessment was made at 72 h. All bioassay data were analysed using POLOPLUS software.

2.3 Discriminating Dose Fixation

Mortality data was generated from bioassay and the median lethal concentration (LC_{50}) of the field-collected F_1 population was determined. Then the field-collected insects were continually cultured without any selection pressure (or exposure to insecticides) up to F_n generation. Based on the doses computed by the preliminary range finding test, bioassays were carried out to create the log concentration probit mortality line (lcpm) for the susceptible population. A Discriminating dose was tentatively fixed based on the LC_{95} value obtained for 'n' generation of population maintained under insecticide free conditions.

2.4 Statistical Analysis

The median lethal concentrations (LC_{50}) of the insecticide used were determined by Finney's probit analysis and confirmed in POLOPLUS software version 2.0. Susceptibility indices were worked out based on LC_{50} and LC_{95} values obtained for the final generation maintained without exposure of insecticides. The Susceptibility Index (SI) is the ratio of LC_{50} or LC_{95} of first generation to the LC_{50} or LC_{95} of last generation. Rate of resistance decline (R) and number of generations required for ten-fold decrease in LC_{50} value (G) were calculated as per Regupathy and Dhamu [16].

$$\text{Slope function increase/decrease \%} = \frac{\text{Slope of Last generation}}{\text{Slope of First generation}} - 1 \times 100$$

Resistance factors (RF) or Resistance Ratio (RR) were estimated at the LC_{50} level as RF= LC_{50} of field strains/LC_{50} of the susceptible strain.

2.5 Insecticide Resistance Monitoring

The diluted insecticide based on concentration of discriminating dose (2.25 ppm) was applied to the insecticide free leaves using leaf dip bioassay method against the larval population collected from the fields of four locations *viz.* Coimbatore, Hosur, Ooty and Oddanchatram.

Resistance Percentage (RP) = (100-CM) ± SE. The corrected mortality (CM) and Standard Error (SE) was worked out using the method as described by Abbott [17].

3. RESULTS AND DISCUSSION

The log concentration probit mortality lines (lcpm) were constructed for the population of diamondback moth collected from cauliflower field and reared up to F_{15} generations without exposure to insecticides and baseline data for test insecticide pyridalyl 10 EC was generated. The LC_{50} and LC_{95} values of pyridalyl 10 EC against *P. xylostella* by leaf dip bioassay method determined for F_1, F_3, F_5, F_{10}, F_{14} and F_{15} generations, given in Table 2.

3.1 Baseline Susceptibility

The median LC_{50} and LC_{95} value for F_1 population was 2.528 ppm and 14.978 ppm, respectively. Similarly, the median LC_{50} and LC_{95} value for F_{15} population was 0.447 ppm and 2.235 ppm, respectively. The LC_{50} and LC_{95} value were found to be decreasing with succeeding generations and stabilized for F_{14} and F_{15} generations, which indicated that the susceptibility increased with succeeding generations.

The computed LC_{50} and LC_{95} values indicated that the susceptibility gradually increased with succeeding generations from F_1 to F_{15} (2.528 ppm to 0.447 ppm) and similarly, LC_{95} values from F_1 to F_{15} decreased from 14.978 ppm to 2.235 ppm. The susceptibility index based on LC_{50} and LC_{95} was 5.655 and 6.702 ppm respectively, after F_{15} generation. The rate of resistance decline (R) was -0.050. Negative R value indicated that the susceptibility increased with succeeding generations. The number of generations required for 10-fold decrease in LC_{50} was 20 generation (Table 3).

Considering the baseline toxicity values obtained for F_{15} generation of diamondback moth maintained under insecticide free condition, a tentative discriminating dose (DD) of 2.25 ppm was arrived based on LC_{95} value of 2.235 ppm. The tentative discriminating dose of 2.25 ppm obtained from the present

Table 2. Baseline susceptibility of *P. xylostella* to Pyridalyl 10 EC by leaf dip method

Generation	Chi square (Σ^2)	Slope ± SE	LC_{50} (ppm)	Fiducial Limit		LC_{95} (ppm)	Fiducial Limit	
				LL	UL		LL	UL
F_1	1.560	2.129 ± 0.518	2.528	1.974	3.125	14.978	8.968	47.565
F_3	2.293	2.038 ± 0.470	1.905	1.397	2.351	12.219	7.667	32.963
F_5	1.681	2.118 ± 0.460	1.440	0.993	1.803	8.609	5.881	18.353
F_{10}	0.329	2.046 ± 0.449	0.955	0.717	1.170	6.084	3.875	15.292
F_{14}	0.847	2.386 ± 0.461	0.458	0.357	0.549	2.240	1.583	4.230
F_{15}	0.384	2.355 ± 0.460	0.447	0.346	0.538	2.235	1.574	4.269

SE – Standard Error; LL – Lower Limit; UL – Upper Limit

Table 3. Susceptibility Index of *P. xylostella* to Pyridalyl 10 EC

Generation	LC_{50}	LC_{95}	Susceptibility Index		Rate of Resistance Decline		Slope function I/D %
			LC_{50}	LC_{95}	R	G	
F_1	2.528	14.978	5.655	6.702	- 0.050	19.934	10.615
F_{15}	0.447	2.235	1.000	1.000			

R= Log (final LC_{50}) - Log (initial LC_{50})/n; G = 1/R; I/D- Increase or Decrease Percentage

Table 4. Resistance Ratio of Pyridalyl 10 EC to different locations of *P. xylostella*

Location	N[a]	Σ^{2} [b]	Regression Equation	LC_{50}	Fiducial Limit		LC_{50} of susceptible Population (ppm)	Resistance Ratio (RR)
					LL	UL		
Coimbatore	180	1.131	y = 3.887 + 2.476x	2.566	1.955	3.099	0.447	5.740
Hosur	180	2.207	y = 3.757 + 2.102x	3.696	2.943	4.548	0.447	8.268
Oddanchatram	180	1.636	y = 4.234 + 2.374x	2.008	1.408	2.499	0.447	4.492
Ooty	180	2.803	y = 3.792 + 2.451x	2.963	2.352	3.536	0.447	6.629

[a] *Number of larvae used in bioassay*
[b] *Chi Square (P > 0.05).*

Table 5. Pyridalyl resistance monitoring of *P. xylostella* in four locations of Tamil Nadu

Location	No. of insects dosed (n)	No. of dead insect	Corrected Mortality	P	RP ± SE
Coimbatore	60	38	62.712	36.667	37.29 ± 6.27
Hosur	60	27	44.068	55.000	55.93 ± 6.48
Oddanchatram	60	41	67.797	31.667	32.20 ± 6.05
Ooty	60	32	52.542	46.667	47.46 ± 6.49

P- Per cent larvae surviving discriminative dose
RP – Resistance Percentage, SE- Standard Error

base line data was used for detection of pyridalyl 10 EC resistance in field populations of Coimbatore, Hosur, Ooty and Oddanchatram of Tamil Nadu, India.

Wang et al. (2021) reported that the LC_{50} value of Pyridalyl 10 EC against *P. xylostella* susceptible population (IVF-S strain) in China was 1.27 ppm [18]. Similarly, the LC_{50} value of Pyridalyl 10 EC against *Spodoptera exigua* susceptible strain in China was 0.68 ppm [19]. Chandrasekaran and Regupathy (1996) have established discriminating doses for cartap hydrochloride (10 ppm) and carbosulfan (15 ppm) against *P. xylostella* [20]. Abbasi-Mojdehi *et al*. (2019) reported that LC_{50} value of pyridalyl against *Bactrocera oleae* was 0.517 ppm [21]. Based on LC_{95}, discriminating doses for *P. xylostella* were fixed at 2 and 10 ppm for new molecules emamectin benzoate and Spinosad, respectively [22].

3.2 Resistance Ratio

The field populations of *P. xylostella* collected from Coimbatore, Hosur, Ooty, and Oddanchatram locations of Tamil Nadu were subjected to bioassay to determine the intensity of resistance to pyridalyl 10 EC. The Log concentration probit mortality (lcpm) lines were fitted for test insecticide (Pyridalyl 10 EC) against resistance populations collected across locations. The median lethal concentration (LC_{50}) values were computed for F_1 of generation of *P. xylostella* from each location.

The LC_{50} values in ppm were 2.566, 3.696, 2.008 and 2.963 for Coimbatore, Hosur, Oddanchatram and Ooty populations respectively. The Resistance ratios (RRs) were worked out by taking into account the LC_{50} of susceptible population (0.447 ppm) and they exhibited 5.740 (Coimbatore), 8.268 (Hosur), 4.492 (Oddanchatram) and 6.629 (Ooty) fold increase in resistance as compared to the susceptible population (Table 4).

Similar studies were carried out by Yin and co-workers (2019) in China. The findings showed that resistance ratio for field populations of *P. xylostella* in Hunan, China was 3.50 fold in May, 2016 and in Hubei, China was 12.10 fold in October, 2016 which is nearly in line with the findings of current investigation [11]. The slight variations on fold of resistance developed in Diamondback moth may be due to various reasons such as temporal variation, geographical variation, differential toxicity, dosage used and usage pattern of the test insecticide. Tamilselvan *et al*. (2021) reported that the resistance ratio of spinetoram and novaluron against field populations of *P. xylostella* in Tamil Nadu ranges from 1.89 to 13.85 fold and 5.01 to 16.93 fold, respectively, compared to a susceptible laboratory population [23].

3.3 Pyridalyl Resistance Monitoring

Monitoring was done as a one-time survey in cabbage and cauliflower fields of Coimbatore, Hosur, Ooty and Oddanchatram regions in Tamil Nadu. The resistance in the field population of *P. xylostella* to pyridalyl 10 EC was monitored using discriminating doses (DD) (2.25 ppm). The level of resistance of diamondback moth varied from 32.20 to 55.93 per cent. The larval population of Hosur registered the highest per cent resistance of 55.99, followed by Ooty (47.46), Coimbatore (37.29) and Oddanchatram (32.20) (Table 5).

Senguttuvan et al. earlier reported that the level of resistance of lufenuron 5.4 EC varied from 6.12 to 24.49 per cent against diamondback moth populations of major cauliflower growing areas in Tamil Nadu [24]. Muralitharan et al. (2013) recorded the level of resistance of chlorfenapyr, profenofos and indoxacarb against field population of *P. xylostella* as 6.67, 33.33 and 10.00 per cent, respectively [25]. Chakraborty and Somchoudhury, (2011) concluded that pyridalyl @ 25 -50 g a.i ha^{-1} gave sufficient control of the DBM and had a lower impact on *Apanteles plutelle* [26].

4. CONCLUSION

The present investigation revealed that the field populations of *P. xylostella* collected from different cabbage and cauliflower growing areas of Tamil Nadu *viz.* Coimbatore, Hosur, Ooty, and Oddanchatram differed in their susceptibility to pyridalyl for various reasons such as temporal variation, geographical variation, differential toxicity, dosage used and usage pattern of the test insecticide. Among them, Hosur population exhibited higher resistance to Pyridalyl 10 EC when compared with Coimbatore, Ooty, and Oddanchatram populations.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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