



## VARIATION IN THE SUSCEPTIBILITY OF BRINJAL SHOOT AND FRUIT BORER *LEUCINODES ORBONALIS* GUENEE TO DIAMIDE INSECTICIDES AND THE ROLE OF DETOXIFICATION ENZYMES

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### ABSTRACT

The susceptibility status of two field populations of brinjal shoot and fruit borer, *Leucinodes orbonalis* (Guenee) (Lepidoptera: Crambidae) collected from major vegetable growing regions of Kerala (Palakkad and Kollam) was determined during 2022-23 against diamide insecticides viz., flubendiamide 39.35% SC, chlorantraniliprole 18.5% SC, and cyantraniliprole 10.26% OD in comparison to the susceptible population. Palakkad and Kollam populations showed a shift in susceptibility to flubendiamide with an LC<sub>50</sub> value of 110.29 ppm and 23.987 ppm, respectively, as compared to that of the susceptible population with an LC<sub>50</sub> of 0.504 ppm. Similar trends were observed for chlorantraniliprole and cyantraniliprole in Palakkad population, with an LC<sub>50</sub> of 29.194 ppm and 3.399 ppm, respectively. Kollam population also showed a shift in susceptibility to chlorantraniliprole and cyantraniliprole with an LC<sub>50</sub> of 2.174 ppm and 0.23 ppm, respectively, as compared to that of the susceptible strain with an LC<sub>50</sub> of 0.119 ppm and 0.081 ppm, respectively. The wide range of variation among the field populations' vulnerability to diamides might be attributed to the differential usage of these insecticides. The increased enzymatic activities of carboxylesterase, glutathione-S-transferase, and cytochrome P-450 monooxygenase in both populations emphasize the importance of detoxification enzymes in the metabolism of xenobiotics. These findings call for the judicious use of diamide insecticides to manage brinjal fruit and shoot borer.

**Key words:** Insecticide resistance, flubendiamide, chlorantraniliprole, cyantraniliprole, LC<sub>50</sub>, carboxylesterase, glutathione-s-transferase, cytochrome P450 monooxygenase, diamide insecticides, susceptibility

Brinjal, scientifically known as *Solanum melongena*, is a popular and versatile vegetable widely cultivated in south and southeast Asia. The diverse agroclimatic conditions across the country make it conducive for brinjal cultivation with a productivity of nearly 19.10 mt ha (Press Information Bureau, 2022). The success of brinjal cultivation is often challenged by more than 53 insect pests, with *Leucinodes orbonalis* Guenee, known as the "brinjal shoot and fruit borer" (BSFB), being one of the most destructive (Alam et al. 2003). In severe infestations, yield loss of between 70 and 92% has been documented. (Chakraborti and Sarkar, 2011; Mishra et al., 2014). Farmers resort to calendar-based prophylactic insecticide spraying to eliminate the larvae before they enter into the fruit. Among vegetables, the average amount of pesticide applied to brinjal alone in India is 4.6 kg a.i ha<sup>-1</sup>, the second-highest amount after chilli, according to Koodandaram et al. (2013). According to Krishna Kumar et al. (2010), the farmers spray insecticides up to 84 times during the 6-7 month cropping season. Generally, conventional insecticides were used to control *L. orbonalis*. However, a novel

class of insecticides with a novel mode of action called diamide compounds, (Ryanodine receptor modulator), replaced these. The diamides, viz., flubendiamide, chlorantraniliprole, and cyantraniliprole have been registered. Diamide-resistant populations of BSFB were documented in countries like Philippines and Thailand due to inadequate product rotation and excessive reliance on diamide chemistry in 2011 itself (IRAC, 2014). Eventually, BSFB adapted to lessen the impact of these insecticides by developing resistance (Kodandaram et al., 2015; Kariyanna et al., 2019). The present study documents the susceptibility status of two field populations of BSFB, collected from major brinjal growing tracts of Kerala, against diamide compounds and the possible role of different detoxification enzymes in conferring resistance.

### MATERIALS AND METHODS

The field populations of *L. orbonalis* were collected from brinjal growing areas of Kerala: Kullarayanpalayam (10045'28.2"N, 760 49'43.7"E) belonging to Palakkad district and Anchal (8°57'47.6"N,

76° 52' 14.6"E), belonging to Kollam district during 2022-23. The pesticide usage pattern of farmers from these localities were also collected. The iso-female line (Lo-S) procured from ICAR- NBAIR, Bengaluru (13°5'48.912" N, 77°33'59.83"E) was maintained as a susceptible population. The collected populations were maintained under laboratory conditions at 27± 2°C, 60-70% RH, and a photoperiod of 14:10h (L : D). The larvae were reared on a natural diet (potato), and adults were fed with 10 per cent honey solution. The three days old F<sub>1</sub> larvae were used for bioassay studies. The following commercial grade insecticides were used for insecticide bioassays: flubendiamide 39.35% SC, chlorantraniliprole 18.5% SC, and cyantraniliprole 10.26% OD. A preliminary bioassay was conducted to determine the concentrations yielding 20-80% mortality. A fruit dip bioassay with slight modification was carried out (Kodandaram et al., 2015). Healthy potatoes were thoroughly washed in deionized water, air dried, and cut into small cubes of 2x 2x 0.5 cm dimension. The cubes were dipped for 30 sec in the treatment concentrations and were air dried for 30 min at room temperature on filter paper. The treated cubes were transferred individually into sample bottles of size 40 ml, and larvae pre-starved for one hr were introduced into each bottle at the rate of one larva/ bottle. A total of 40 insects were used for each concentration, and a minimum of six concentrations, including control, were used. The treated test insects were maintained at 27± 2°C, 60-70% RH, and at a photoperiod of 14:10h (L: D). Larval mortality at 48 hr after treatment was observed and recorded. The corrected mortality rate was calculated using Abbotts formula (Abbott, 1925). The mortality data were subjected to probit analysis using POLO-PC (Finney, 1971). The median lethal concentration (LC<sub>50</sub>), fiducial limit (at 95% confidence limit), and heterogeneity factor were calculated and the resistance ratio (RR) (LC<sub>50</sub> of field population/ LC<sub>50</sub> of susceptible population) was worked out.

The enzymatic extract was prepared by grinding 16 mg of insect sample in 700 µl of sodium phosphate buffer with a pH 7.4. Total protein was estimated as per the procedure given by Lowry et al. (1951) using bovine serum albumin (BSA) solution as the standard. The activity of the enzyme carboxylesterase (EST) was determined as per the method described by van Asperen (1962) with α-naphthyl acetate as the substrate. The assay mixture composed of 50 µl of enzyme extract (7 larvae), 1 ml of 30 mM α-naphthyl acetate, 2 ml of sodium phosphate buffer (pH 7.4), and 50 µl staining solution. The final mixture was incubated at 37 °C for

color development for five min. The intensity of the red color developed was measured in a spectrophotometer (Agilent Cary 60) at 600 nm, and the enzyme activity was computed from the α-naphthol standard curve. Glutathione S transferase (GST) activity was determined by the method described by Kao et al. (1989) using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. The enzyme assay sample was prepared by adding 2.75 ml of sodium phosphate buffer (pH 6.5), 50 µl of 50 mM CDNB, 150 µl reduced glutathione (GSH) and 50 µl of enzyme extract sequentially and the change in absorbance was measured at 340nm for five min at 30 sec intervals. The enzyme activity in terms of micromoles of CDNB conjugate/ min/ mg protein was calculated using the extinction coefficient of 9.6 mM/ cm.

The cytochrome P450 (MFO) assay was carried out according to the method of Brogdon et al. (1997) with slight modifications. The reaction mixture was prepared by adding 50 µl of enzyme extract, 500 µl of 3,3',5,5'-tetramethylbenzidine (TMBZ), 200 µl potassium phosphate buffer (pH 7.2), and 62.5 µl hydrogen peroxide (3%) and were incubated for 30 min. The absorbance was recorded at 630 nm in a spectrophotometer, and cytochrome P450 activity was computed from the standard graph and was expressed in pmol/ min/ mg protein. The EST, MFO, and GST enzymatic activity were analyzed by Grapes software (Gopinath, 2021) and the enzymatic activity ratio (EAR) was calculated by dividing the mean enzymatic activities of the field populations by those of susceptible strain.

## RESULTS AND DISCUSSION

The insecticides used by the farmers in the collected locations were diverse. Among the two locations surveyed, Palakkad showed an insecticide usage history with acetamiprid, thiamethoxam, chlorantraniliprole, flubendiamide, imidacloprid, emamectin benzoate, and fipronil. Insecticide application was the sole method of management, and spraying was carried out every 15 days interval after sowing throughout the six-month cropping season at doses above the recommended ones. A need based application, strictly as per the recommended dose, was practiced in Kollam with insecticides, flubendiamide, chlorantraniliprole, and spinetoram. The LC<sub>50</sub> value and resistance ratio for the test insecticides viz., flubendiamide, chlorantraniliprole, and cyantraniliprole on two field populations of *L. orbonalis* collected from brinjal growing tracts of Palakkad and Kollam districts of Kerala are presented in Table 1. LC<sub>50</sub> value of flubendiamide in Palakkad,

Table 1. Susceptibility of populations of *L. orbonalis* in Kerala to diamide insecticides

Insecticides	Population	LC <sub>50</sub> (ppm)	Slope± SE	F. limits		χ <sup>2</sup> Heterogeneity (DF)	Resistance ratio
				Lower	Upper		
Flubendiamide	Palakkad	110.293	0.808± 0.235	58.350	182.49	0.815 (4)	218.84
	Kollam	23.987	0.848± 0.251	13.062	42.532	0.915 (3)	47.59
	Lo-S	0.504	0.912± 0.247	0.319	0.838	1.916 (4)	-
Chlorantraniliprole	Palakkad	29.194	1.371± 0.253	9.869	48.045	6.23 (4)	245.33
	Kollam	2.174	0.814± 0.271	1.298	5.862	0.333 (3)	18.27
	Lo-S	0.119	1.504± 0.369	0.09	0.159	0.315 (4)	-
Cyantraniliprole	Palakkad	3.399	0.980± 0.245	1.916	4.989	2.693 (4)	41.96
	Kollam	0.23	1.110± 0.276	0.159	0.418	1.941 (3)	2.84
	Lo-S	0.081	1.110± 0.355	0.044	0.12	0.309 (3)	-

Kollam, and the susceptible population were 110.293 ppm, 23.987 ppm, and 0.504 ppm, respectively. An LC<sub>50</sub> value of 29.194 ppm, 2.174 ppm, and 0.119 ppm was shown by Palakkad, Kollam, and the susceptible population to chlorantraniliprole. The field population collected from Palakkad showed the least susceptibility to cyantraniliprole with an LC<sub>50</sub> of 3.399 ppm, followed by the Kollam population (0.23 ppm) as compared to that of the LC<sub>50</sub> value of 0.081 ppm in the susceptible population.

The LC<sub>50</sub> values indicated a reduced susceptibility of the field populations compared to the laboratory population. Palakkad population showed a RR of 218.84 fold, 245.33 fold, and 41.96 fold to flubendiamide, chlorantraniliprole, and cyantraniliprole, respectively, compared to the laboratory susceptible population. The Kollam population showed 47.59 fold resistance to flubendiamide and 18.27 fold and 2.84 fold resistance to chlorantraniliprole and cyantraniliprole, respectively. Our study is the first report of resistance to diamide insecticides from Kerala in *L. orbonalis*. The higher levels of resistance in the Palakkad population may be due to the frequent and indiscriminate use of diamides, especially flubendiamide, and chlorantraniliprole, as compared to that of Kollam, where need based application at the recommended dose was carried out. Field failure of diamide insecticides was first reported in cabbage against diamondback moth, *Plutella xylostella*, two years after the launch of flubendiamide in the Philippines and Thailand (Trocza

et al., 2012). Later on, low to high levels of resistance development were reported all over the world against diamides in other lepidopteran pests like tomato leaf miner, *Tuta absoluta* (Roditakis et al., 2015), rice stem borer, *Chilo suppressalis*, oriental leaf worm, *Spodoptera exigua* (Che et al., 2013), and rice leaf folder, *Cnaphalocrocis medinalis* (Zhang et al., 2014). In India, by 2011, diamide resistant population of *L. orbonalis* was reported from Uttar Pradesh due to inadequate product rotation and over-dependence on diamide insecticides (IRAC, 2014). Later, Kariyanna et al. (2020) reported 31.9 - 363.9 fold resistance development in *L. orbonalis* against flubendiamide and 1.6 - 8.6 fold in chlorantraniliprole from different parts of the country. Recently, cyantraniliprole (5.47 fold) and chlorantraniliprole (9.15 fold) resistant populations of *P. xylostella* were reported from Krishnagiri and Kotagiri districts of Tamilnadu by Pasupathi et al. (2022). Development of cross resistance to cyantraniliprole was observed in Palakkad and Kollam population even if the chemical was not used by the farmers. This can be substantiated by the findings of Silva et al. (2016).

The activity of detoxifying enzymes, such as glutathione S-transferase (GST), microsomal P-450 monooxygenase (Cyt P-450), and carboxylesterase (CE) in the field populations of *L. orbonalis* are given in Table 2. A relatively higher Cyt-P450 activity (5.15 fold) was observed in the Palakkad population compared to the Kollam population (3.14). The given data shows the correlation between the cytochrome P450 activity and

Table 2. Activity of detoxifying enzymes of field populations and Lo-S strain of *L. orbonalis*

Population	Carboxylesterase ( $\mu\text{mol}/\text{min}/\text{mg}$ )	Fold variation as compared to LoS	GST ( $\mu\text{mol}/\text{min}/\text{mg}$ )	Fold variation as compared to LoS	Cytochrome P450 activity ( $\text{pmol}/\text{min}/\text{mg}$ protein)	Fold variation as compared to LoS
Palakkad	13.10 <sup>a</sup>	1.47	0.50 <sup>a</sup>	4.16	2.94 <sup>a</sup>	5.15
Kollam	10.45 <sup>b</sup>	1.17	0.17 <sup>b</sup>	1.4	1.79 <sup>b</sup>	3.14
LoS	8.91 <sup>c</sup>		0.12 <sup>c</sup>		0.57 <sup>c</sup>	
SE(d)	0.01		0.006		0.052	

SE(d)-Standard error of deviation

variation in diamide susceptibility in both populations. Nauen and Steinbach (2016) also stated the role of cytochrome P450-mediated detoxification of diamides in Lepidopteran pests. Likewise, significantly elevated activities (5.46 to 18.5 fold) of O-demethylase in field-collected populations over the susceptible Lo-S population of *L. orbonalis* were reported by Kariyanna et al. (2021). Elevated levels of Cyt P450 activity in the Palakkad population also signify the role of monooxygenase in the metabolism of neonicotinoids. The reports of Wang et al. (2018) also state the importance of CytP450 isoenzymes in neonicotinoid metabolism.

GSTs are involved in the resistance of insects to organophosphate (OPs), chlorine, and pyrethroid insecticides (Ketterman et al., 2011). The function of GSTs in the metabolism of fipronil was also described by You et al. (2015), where 12 genes associated with GST showed a significantly higher level of expression in fipronil resistant strains of *P. xylostella*. Hence, these reports further validate the association of increased activity of GST (4.16 fold) in the Palakkad population with an increased exposure to fipronil compared to the Kollam population with reduced exposure to fipronil. On the other hand, Ismail (2020) reported a higher activity level of GST in *Spodoptera littoralis* larvae when treated with spinetoram, which substantiates the GST activity (1.4 fold) in the Kollam population that was exposed to spinetoram. Li et al. (2021) and Shi et al. (2022) have reported carboxylesterase in the metabolism of pesticides containing esters, such as organophosphates (OPs), synthetic pyrethroids (SPs), and carbamates. Enzyme tests carried out by Kariyanna et al. (2020) revealed higher levels of detoxifying enzymes, specifically EST and GST, in the field collected populations of *L. orbonalis* with high level of resistance development against fenvalerate (48.2-160-fold), phosalone (94-534.6-fold), emamectin benzoate (7.2-55-fold), thiodicarb (9.64-22.7-fold), flubendiamide

(187.4-303.0-fold), and chlorantraniliprole (1.6-8.6-fold) as compared to laboratory-reared susceptible iso-female colony (Lo-S). In the present study, the activity level of EST was significantly different in Kollam (1.17 fold) and Palakkad (1.47 fold) populations as compared with that of susceptible iso-female colony (Lo-S). The repeated use of pesticides belonging to diamide, neonicotinoid, avermectin, and phenyl pyrazole groups can be a reason for the significantly higher level of all three detoxification enzymes in the Palakkad population.

The present study indicates the development of resistance against diamides in field populations of *L. orbonalis* collected from different localities of Kerala, which may be due to the heavy reliance on diamides in brinjal growing tracts for managing BFSB. Also, pesticide use pattern in various localities determine the level of resistance and detoxifying enzymes in various populations. Strategies should be devised to manage diamide resistance in Palakkad and Kollam by rationalising the use of pesticides with varying modes of action.

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#### AUTHOR CONTRIBUTION STATEMENT

Conceived and designed the analysis (AT, SMS, and BP); Performed work (AT); contributed reagents (BP); wrote the manuscript (AT); Corrected the paper (SMS, BP, and MC). All authors read and approved the manuscript.



## CONFLICT OF INTEREST

No conflict of interest.

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