LR-5443
[1-9]

RESEARCH ARTICLE Legume Research- An International Journal

Field Evolved Insecticide Resistance in Legume Pod Borer, *Maruca vitrata* F. (Lepidoptera: Crambidae)

N. Vijayakumari[1,3], M. Shanthi[2], Nitta Anusha[3], M. Murugan[3], S. Paripoorani[3], S. Varanavasiappan[4], M. Jayakanthan[4], R. Gandhi Gracy[1], M. Mohan[1]

10.18805/LR-5443

ABSTRACT

Background: *Maruca vitrata*, commonly known as the legume pod borer, is a significant pest affecting leguminous crops like cowpea, pigeon pea, lab lab, beans, mung bean and others across tropical and subtropical regions. It severely reduces yields by boring into flowers, pods and seeds, causing significant damage. Field control failures with insecticides have been experienced by farmers due to development of insecticide resistance upon over reliance on insecticides. Hence, the present study attempted to quantify the levels of insecticide resistance and their detoxification routes in field collected populations of *M. vitrata* from intensive legume growing regions of India.

Methods: In this study, a survey was conducted in intensive legume-growing regions of India to collect field populations of *M. vitrata* in 2024. Dose mortality bioassays were conducted against five commonly used insecticides (quinolphos, chlorantraniliprole, chlorpyrifos, flubendimide, indoxacarb) and resistance ratios were determined by comparing the LC_{50} values of field populations with the susceptible laboratory population. Enzyme assays were performed to evaluate the role of detoxifying enzymes like cytochrome p 450s, glutathione S-transferases and carboxylesterases in conferring resistance.

Result: The resistance ratios varied from 56.48 to 311-fold for quinolphos, 3.84 to 97-fold for chlorantriniliprole, 15.76 to 92.088-fold for chlorpyrifos, 30.86 to 75.26-fold for flubendimide and 33.21 to 82.95-fold for indoxacarb. The activities of detoxification enzymes were significantly higher in the midgut of field collected *M. vitrata* larvae. The data suggests rational use of various insecticides in rotation so as to delay the further development of resistance in *M. vitrata.*

Key words: Detoxification enzymes, Insecticide resistance, LC_{50}, *Maruca vitrata*.

INTRODUCTION

Insecticide resistance is a major challenge in modern agriculture, threatening the effectiveness of pest control strategies and the sustainability of food production systems. *M. vitrata* commonly known as legume pod borer causes extensive damage on 73 wild and cultivated legume host plant species (Srinivasan *et al*., 2021). Legumes such as pigeonpea, field and garden beans and cowpea are severely damaged. The larvae damage the flowers, flower buds and developing pods Around 20-60% reduction in legume grain yield is evident across the legume growing countries (Tamo *et al*., 2002; Jat *et al*., 2024; Margam *et al*., 2011; Regmi *et al*., 2016; Aryal *et al*., 2021; Mahalakshmi *et al*., 2016).

In India, the farmers traditionally relied on chemical insecticides such as organophosphates, carbamates and synthetic pyrethroids are being used extensively to protect the yield of grain and vegetable legumes from insect pests including *M. vitrata* (Sharma *et al*., 2010). Many newer insecticides such as indoxacarb, methoxyfenozide, chlorantraniliprole, flubendiamide and cyantraniliprole are also being used in recent years (Sreelakshmi *et al*., 2016) to protect the crop yield.

Insects have the ability to develop resistance through many routes (Scharf and Gondhalekar, 2021). Metabolism mediated detoxification through cytochrome p 450 monooxygenase (CYP), carboxylesterae (CE) and

[1]Division of Genomic Resources, ICAR- National Bureau of Agricultural Insect Resources, Hebbal-560 024, Bengaluru, India. [2]Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India. [3]Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India. [4]Department of Plant Biotechnology, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.

Corresponding Author: M. Mohan, M. Shanthi; Division of Genomic Resources, ICAR- National Bureau of Agricultural Insect Resources, Hebbal-560 024, Bengaluru, India; Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore-641003, Tamil Nadu, India.

Email: mohan_iari@yahoo.com and mshanthiento@tnau.ac.in

Orcid: https://orcid.org/0009-0002-6300-9558; https://orcid.org/0000-0001-5737-4781; https://orcid.org/0000-0001-5571-3946; https://orcid.org/0000-0002-7485-1153; https://orcid.org/0009-0001-5610-5034; https://orcid.org/0000-0001-6169-7823; https://orcid.org/0000-0001-7444-9343; https://orcid.org/0000-0002-6764-5167; https://orcid.org/0000-0002-2327-6988.

How to cite this article: Vijayakumari, N., Shanthi, M., Anusha, N., Murugan, M., Paripoorani, S., Varanavasiappan, S., Jayakanthan, M., Gracy, R.G. and Mohan, M. (2024). Field Evolved Insecticide Resistance in Legume Pod Borer, *Maruca vitrata* F. (Lepidoptera: Crambidae). Legume Research. 1-9. doi: 10.18805/LR-5443.

Submitted: 07-11-2024 **Accepted:** 02-01-2025 **Online**: 25-01-2025

glutathione S-transferase (GST) is the most more common mechanism in many insects (Mohan and Gujar, 2003; Khan *et al*., 2020; Panini *et al*., 2016). Quantification of insecticide resistance in field collected populations of *M. vitrata* and the role played by various detoxification enzymes are essential to device suitable resistance management strategies. Thus, the present study, determined the resistance status of field collected populations of *M. vitrata* to flubendimide, chlorantraniliprole, indoxacarb, chlorpyriphos and quinolphos using a diet overlay bioassay. The activities of various metabolic enzymes were also quantified for their role in insecticide resistance.

MATERIALS AND METHODS

Insect collection and maintenance

The experimental study was carried out in Division of Genomic Resources, ICAR- National Bureau of Agricultural Resources, Bangalore during the year 2024. Field populations of *M. vitrata* were collected intensive legume growing areas of Karnataka (Basavanapalli; Kolar Dt), Kerala (Vellayanikara; Thrissur Dt), Tamil Nadu (Kariamangalam; Dharmapuri Dt) andhra Pradesh (Vengalayapalem ; Guntur Dt) and Telangana (Patancheru; Hyderabad Dt) states of India (Table 1). Larvae collected from the field were fed with modified semi synthetic diet. The emerged adults were transferred to oviposition jars and provided with 10 % sucrose solution fortified with multivitamins. The insecticide susceptible iso-female line (National Accession number: NBAIR-IS-CRA-02), designated as Lo-S (70th generation) was originally derived from *M. vitrata* collected near Bengaluru (12.97160N,77. 54960E) and maintained at insect genomic resources laboratory at ICAR-NBAIR, Bengaluru. The species identity of the *M. vitrata* was confirmed by amplification of its COI region and the sequence was deposited in the NCBI -GenBank and BOLD databases (Accession number: PP112191.1).

Dose-mortality bioassays

Insecticides approved by the Central Insecticide Board and Registration Committee (CIB and RC) for the management of *M. vitrata* on legume crops were selected for conducting dose-mortality bioassays. Commercial formulations of flubendimide 39.35% SC, indoxacarb 14.5% SC, chlorpyrifos 20% EC, quinolphos 20% EC and chlorantriniliprole 18.5% SC that are being commonly used by farmers were obtained from local retailer shop. Bioassays were performed on newly emerged third-instar larvae, according to the IRAC susceptibility test series method No: 020., diet overlay method was adopted. Appropriate dilutions of the commercial formulations were prepared in distilled water. A preliminary range-finding bioassay was conducted with five widely spaced concentrations to determine the range in such a way to get larval mortality between 5% and 95 per cent. One ml of diet was poured into each bioassay tray well and allowed for drying under laminar airflow. After drying, 30 µl of the insecticidal solution was applied uniformly on the diet surface and individual third instar larva was transferred to each well. A self-adhesive pull-and-peel tabs (BIO-CV-16, Pitman, N.J., USA, 609-582-2392) was used to seal the bioassay trays. An untreated diet was used as a control and all the bioassays were replicated three times. The bioassay trays were incubated at 24±1°C and 70±5% relative humidity, 12:12 (L:D) photoperiod and the mortality count were observed after 48 hours of insecticide exposure.

Detoxification enzymes

Preparation of midgut homogenate

The pre-starved one day old fourth instar larvae were used for the preparation of midgut homogenate and Midguts were dissected and homogenized with homogenization buffer (50 mM sodium phosphate buffer pH 7.8). The content was centrifuged at 12000 rpm for 20 min and the clear supernatant was used as an enzyme source for estimating the titers of cytochrome p 450 monooxygenase, glutathione s-transferase and carboxylesterase.

Protein estimation

Total protein was estimated by the Bradford method using bovine serum albumin (BSA) as a protein standard (Bradford, 1976). To obtain a standard curve, concentrations of 0 to 20 µg BSA were prepared in eppendorf tubes and 100 µl distilled water was added and the blank contained 100 µl distilled water. To which 1 ml of Bradford's dye was added and left undisturbed for 5 minutes to develop a blue color. The amount of protein in the samples was determined by adding 1 ml of Bradford dye (Coomassie brilliant blue G-250) to 2.5 µl of the enzyme extract. After a time interval of 5 minutes, the absorbance was recorded spectrophotometrically at 595 nm in a BioSpectrometer® (Eppendorf India Private Ltd., Pune, India).

Quantification of glutathione S- transferase

Glutathione S-transferase activity was determined using 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate (Kao *et al*., 1989). Thirty microliters of midgut homogenate and

Table 1: Sampling sites for collection of field populations of *M. vitrata in legume* crop cultivated areas of India.

Location	District (State)	Host plant	Latitude longitude
Kariamangalam	Dharmapuri (Tamil Nadu)	*Lablab purpureus*	12.229615°N 78.1543°E
Basavanapalli	Kolar (Karnataka)	*Lab purpureus*	13.543357°N 78.333298°E
Vellayanikara	Thrissur (Kerala)	*Vigna unguiculata*	8.4316°N, 76.9860°E
Patancheru	Hyderabad (Telangana)	*Cajanus cajan*	17.5111°N, 78.2752°E
Vengalayapalem	Guntur (Andhra Pradesh)	*Cajanus cajan*	16.2874°N, 80.3707°E

ten microliters of 50 mM CDNB and L-glutathione reduced were used in the test mixture. The change in absorbance was monitored at 340 nm for a duration of 5 minutes with measurements taken every 30 thirds.

Quantification of carboxylesterase

Carboxylesterase activity was quantified using the method described by Van Asperen (1962) and α-naphthyl acetate as substrate. Using a 96-well microplate (iMark, Biorad microplate reader), the assay mixture was prepared with 800 µL of 0.3 mM α-naphthyl acetate, 0.1 M sodium phosphate buffer, pH 7.4 and 15 µL of midgut homogenate. The mixture was incubated for 20 minutes at 30°C in the dark. The sample was then incubated for 20 minutes at room temperature in the dark using 200 µl of staining solution containing 1% Fast Blue B and 5% sodium dodecyl sulfate. The production of α-naphthol was measured at 595 nm and the standard curve of α-naphthol was used to calculate enzyme activity.

Quantification of cytochrome P 450 monooxygenase

O-demethylase activity, a marker enzyme for measuring cytochrome p 450 monooxygenase was determined spectrophotometrically by analyzing p-nitrophenol production. The mixture contain 100 ul midgut homogenate, 75 ul phosphate buffer and 175 µl of p-nitroanisole. The mixture was incubated for 60 minutes at 30°C. To this, 700 µL of ice-cold acetone and 0.2 mL of 0.5 M glycine-NaOH buffer (pH 9.5) were added to stop the reaction. Post centrifugation, the supernatant's absorbance was measured at 410 nm and a p-nitrophenol standard curve was used to calculate the enzyme activity (Kinoshita *et al*., 1966).

Statistical analysis

Probit analysis was done by Polo Plus (LeOra Software, 2003) to calculate fiducial limits, slopes, chi square values and lethal concentrations. Prior to analysis, Abbott's formula was used to adjust larval mortality for control survival (Abbott, 1925). The LC_{50} of each individual field population was divided by the LC_{50} of a susceptible population in order to calculate the resistance ratio (RR) (Tong *et al*., 2013). Using Pearson's correlation analysis, pairwise correlation coefficients between LC_{50} values of field populations of insecticides were estimated for cross-resistance and visualized using GraphPad prism software. Based on the RR values (RR<3.0, RR =3.1-5, RR= 5.1-10.0, RR =10.1-40.0, RR = 40.1-160.0, RR >160.0) obtained, the insect population tested was categorized as susceptible, slightly susceptible, showing low resistance, exhibiting moderate resistance, showing high resistance and demonstrating very high resistance respectively (Jiang *et al*., 2015). Data on enzyme activities were assessed using IBM SPSS® Statistics 22.0, utilizing one-way analysis of variance (ANOVA) (P<0.05), followed by Tukey's test (Tukey, 1977) for comparing means.

RESULTS AND DISCUSSION

Insecticide resistance monitoring in *M. vitrata* populations

Diet overlay bioassay revealed that the laboratory population is highly susceptible. The LC_{50} values of the laboratory population were 0.386 ppm for flubendiamide, 0.551 ppm for indoxacarb, 3.031 ppm for chlorpyrifos, 0.062 ppm for quinolphos and 0.019 ppm for chlorantraniliprole (Table 2). *M. vitrata* population collected from Dharmapuri showed highest LC_{50} value of 28.60 ppm against flubendimide followed by Kolar (23.07 ppm), Vellayanikara (11.73 ppm) compared to susceptible populations (Table 2) and the observed LC_{50} range. 0.38 ppm to 28.60 ppm) (Table 2). Resistance ratio ranged from 30.868 to 75.26 times, indicating a high level of resistance (Fig 1). The statistical analysis aimed at testing the parallelism and equality hypotheses between the regression lines of the populations showed that, although the regression lines were parallel (x^2 = 8.92; df = 5; P = 0.11), suggesting that the rate of increase in resistance is similar across populations, they were not equal (x^2 = 170; df = 10; P<0.05), suggesting that the resistance levels among the populations differ significantly.

The bioassay using indoxacarb depicted highest LC_{50} value for the population collected from Dharmapuri (45.70 ppm) followed by Kolar (38.50 ppm) and Vellayanikara (18.29 ppm) (Table 2). Resistance ratios (RRs) ranging from 33.21 to 82.95-fold (Fig 1) indicating high levels of resistance in all the tested populations. Statistical analysis to test the hypotheses of equality and parallelism of the regression lines of the populations showed that the regression lines were neither equal (x^2 = 218; df = 10; P< 0.05) nor parallel (x^2 = 29.28; df = 5; p = 0.01). This indicates that there are significant differences in resistance levels between populations and that the degree of resistance does not follow a uniform pattern between them.

Regarding chlorpyriphos, the observed LC_{50} values ranged from 47.77 ppm to 279.12 ppm (Table 2) for the field populations with significant differences as compared to the susceptible strain. Among the tested populations, Kolar showed the highest resistance with an LC_{50} of 279.12 ppm, followed by Dharmapuri with 234.72 ppm, while Vellayanikara showed the lowest resistance with an LC_{50} of 47.774 ppm. The Resistance ratio varied from 15.76 to 92.088 fold, representing moderate to high resistance levels. According to the concepts of parallelism and equality of populations, the regression lines that depicted the dose-response relationships were also parallel (x^2 =59; df = 5; P = 0.12) indicating similar slopes between populations. Nevertheless, the regression lines were not identical (x^2 = 282; df = 10; P< 0.05), suggesting significant differences in susceptibility levels between populations.

M. vitrata population collected from Guntur exhibited the lowest LC_{50} (3.50 ppm) for quinolphos, whereas the population from Dharmapuri has the highest LC_{50} (19.282),

Table 2: Dose-responses of *M. vitrata* populations to different insecticides.

Insecticides	Population	LC_{50} (95% CL)	Slope±SE	Chi-square (df)	h	LCR (95% CL)
Flubendiamide	Kolar	23.070 (16.391-36.127)	1.479 ± 0.276	3.358 (4)	0.84	0.017 (0.002-0.128)
	Vellayanikara	11.730 (08.776-15.839)	1.785± 0.282	3.460 (4)	0.86	0.033 (0.004-0.246)
	Hyderabad	14.340 (11.236-18.420)	2.316 ± 0.354	1.827 (4)	0.45	0.027 (0.004-0.200)
	Dharmapuri	28.600 (20.242- 46.138)	1.539 ± 0.291	3.005 (4)	0.75	0.013 (0.002- 0.104)
	Guntur	18.310 (13.083-137.49)	1.464 ± 0.265	3.950 (4)	0.99	0.021 (0.003-0.160)
	Susceptible (Strain: NBAIR-IS-CRA-02)	0. 380 (0.386- 1.141)	0.958 ± 0.334	1.569 (4)	0.89	-
Indoxacarb	Kolar	38.507 (28.058-53.962)	1.754 ± 0.276	4.346 (4)	1.08	0.014 (0.001- 0.165)
	Vellayanikara	18.299 (13.606-22.877)	2.441 ± 0.326	4.410 (4)	1.10	0.030 (0.003-0.345)
	Hyderabad	33.646 (23.785-44.931)	2.903 ± 0.436	4.682 (4)	1.17	0.017 (0.001- 0.189)
	Dharmapuri	45.707 (32.744-67.114)	2.192 ± 0.338	6.804(4)	1.70	0.012 (0.001- 0.138)
	Guntur	27.394 (19.067-37.906)	1.739 ± 0.260	4.648 (4)	1.16	0.020 (0.002-0.231)
	Susceptible (Strain:NBAIR-IS-CRA-02)	0.551 (0.001-2.473)	0.788 ± 0.258	2.874 (4)	0.71	-
Chlorpyrifos	Kolar	279.12 (180.73-587.572)	2.679 ± 0.503	4.829 (4)	1.20	0.016 (0.003 - 0.088)
	Vellayanikara	47.774 (19.023- 86.919)	1.787 ± 0.307	5.764 (4)	1.44	0.095 (0.018 -0.518)
	Hyderabad	133.99 (85.218-227.13)	2.388 ± 0.303	4.788 (4)	1.19	0.033 (0.006- 0.175)
	Dharmapuri	234.720 (134.45-615.87)	2.867 ± 0.513	7.749 (4)	1.93	0.019 (0.004 -0.104)
	Guntur	186.35 (110.38-405.408)	2.270 ± 0.373	5.976 (4)	1.49	0.024 (0.005 - 0.131)
	Susceptible (Strain : NBAIR-IS-CRA-02)	3.031 (0.028-12.500)	1.299 ± 0.463	1.100 (4)	0.27	-
Quinolphos	Kolar	13.313 (10.418-17.457)	3.215 ± 0.605	3.136 (4)	0.78	0.005 (0.001- 0.023)
	Vellayanikara	4.420 (3.394-5.627)	3.552 ± 0.603	2.745 (4)	0.68	0.011 (0.002- 0.058)
	Hyderabad	7.620 (4.798-11.957)	3.777 ± 0.642	5.980 (4)	1.49	0.008 (0.002- 0.040)
	Dharmapuri	19.282 (13.110-38.118)	1.853 ± 0.417	3.035 (4)	0.75	0.014 (0.003- 0.070)
	Guntur	3.502 (2.439-4.720)	0.893 ± 0.154	3.867 (4)	0.96	0.018 (0.004 -0.089)
	Susceptible (Strain:NBAIR-IS-CRA-02)	0.062 (0.005- 0.206)	0.708 ± 0.154‘	3.158 (4)	0.78	-
Chlorantriniliprole	Kolar	1.843 (0.710-13.050)	1.202 ± 0.213	5.95 (4)	1.48	0.009 (0.004-0.023)
	Vellayanikara	0.073 (0.013-0.261)	0.781± 0.140	4.991 (4)	1.24	0.263 (0.096 - 0.723)
	Hyderabad	0.151 (0.047- 0.505)	0.872 ± 0.142	4.598 (4)	1.14	0.119 (0.047-0.305)
	Dharmapuri	1.387 (0.423-7.786)	1.433 ± 0.244	7.682 (4)	1.92	0.014 (0.006-0.032)
	Guntur	0.493 (0.134-2.898)	0.997 ± 0.152	7.252(4)	1.81	0.039 (0.016- 0.098)
	Susceptible (Strain:NBAIR-IS-CRA-02)	0.019 (0.009-0.034)	1.240 ± 0.255	3.158(4)	0.78	-

*h- Heterogeneity; LCR- Lethal concentration ratios at LC_{50}.

followed by Kolar (13.31 ppm) (Table 2) over the susceptible laboratory population. Analysis of the regression lines for the two populations' hypotheses of equality (of slopes and intercepts) and parallelism (of slopes) showed that they were neither parallel (x^2 = 75.95; df = 5; P =0.01) or equal (x^2 = 301; df = 10; P < 0.05). The resistance ratio showed high to extremely high resistance values, ranging from 56.48 to 311-fold.

Chlorantriniprole exhibited higher toxicity on *M. vitrata* larvae as compared to other pesticides. The LC_{50} values for the field populations ranged from 0.073 ppm (Vellayanikara) to 1.843 ppm (Kolar) (Table 2) with 3.84-97 fold variation in susceptibility among the field populations. Tests of the hypotheses of equality and parallelism of the populations showed that the regression lines were not equal (x^2 = 161; df = 10; P < 0.05) but parallel (x^2 = 8.73; df = 5; P = 0.12) for chlorantriniprole.

Assessment of cross-resistance by pairwise correlation analysis

The Pearson's correlation analysis was conducted to evaluate the cross resistance among different classes of insecticides. These coefficients can provide insights into how these chemicals are related to each other in terms of their effects and properties (Fig 2). Between flubendimide and indoxacarb there is a strong negative correlation between the two chemicals (r = -0.927, P<0.05). This indicates that when the presence or level of one chemical increases, the presence or level of another tends to decrease and vice versa. Between flubendimide and chlorpyrifos, there is a moderate positive correlation (r = 0.833, P<0.05) between the two chemicals. Quinolphos and flubendimide exhibit a highly significant positive association (r = 0.999, P<0.05) with one another. This implies that their levels have a nearly perfect linear connection. Flubendimide and chlorantriniliprole showed that these two insecticides have an extremely significant positive correlation (r = 0.999, P<0.05), which is comparable to the correlation found between flubendimide and quinolphos. The modest non-significant negative association (r = -0.565, P>0.05) between indoxacarb and chlorpyrifos has been observed. Quinolphos and indoxacarb have significant adverse association as seen by their significant negative correlation (r = -0.930, P>0.05). Indoxacarb and chlorantriniliprole have strong negative connection (r = -0.935, P<0.05), which is comparable to the correlation between indoxacarb and quinolphos. A significant relationship between their levels can be observed by the slightly positive correlations between chlorpyrifos and quinolphos (r = 0.829, P>0.05) and between chlorpyrifos and chlorantraniliprole (r = 0.816, P > 0.05). Quinolphos and chlorantraniliprole have a very significant positive correlation (r= 0.998, P>0.05) indicating a nearly perfect linear connection. The levels of these two compounds appear to be strongly related to one another based on the extraordinarily high correlation coefficient.

Detoxification enzymes

The enzyme assays quantified the activities of cytochrome p 450 monooxygenase (CYP), carboxylesterase (CE) and glutathione S-transferase (GST) in field collected populations so as to ascertain their involvement in

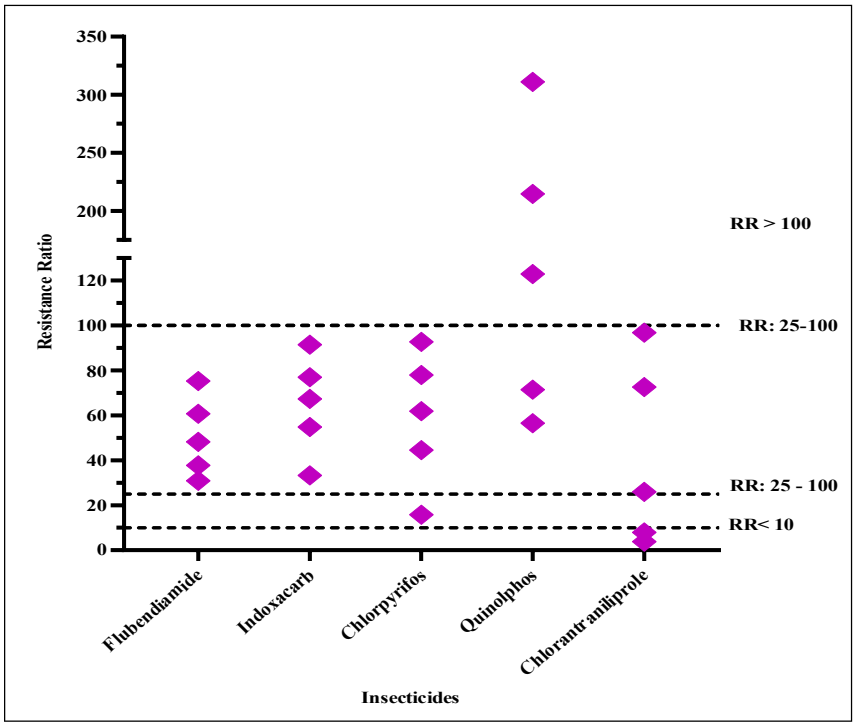
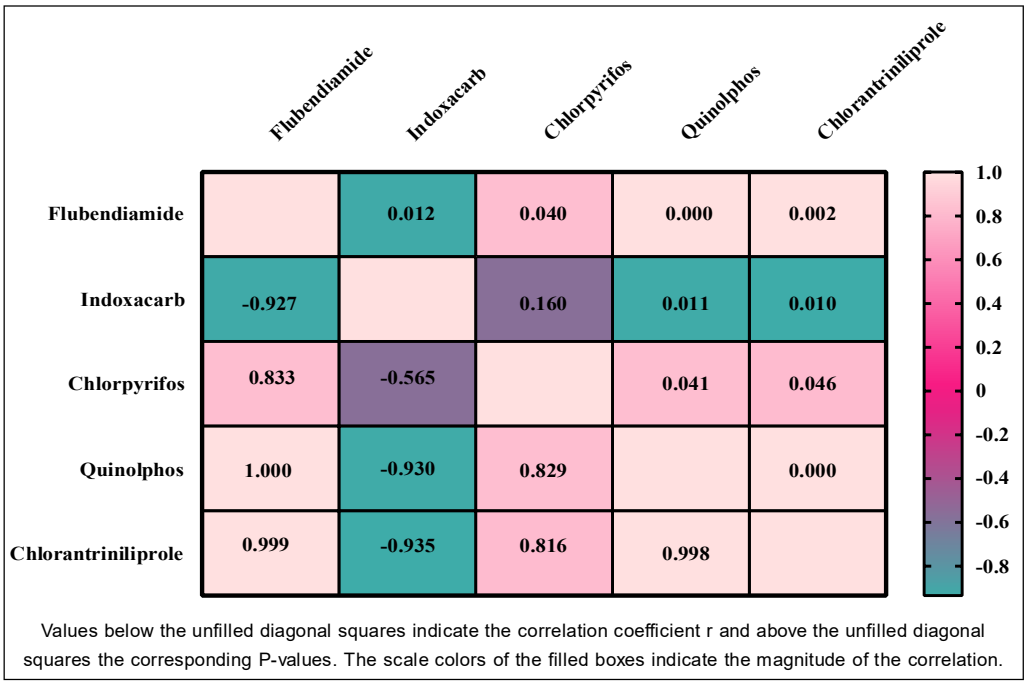


Fig 1: Proportion of resistance ratio to different insecticides for field populations of *M. vitrata* in different states of India.

insecticide resistance. The field-collected populations had 2.61 to 9.55- fold greater GST activity as compared to the laboratory reared population. All of the field-collected *M. vitrata* populations showed increased carboxylesterase titre activity (1.8 to 9.71 fold) in comparison to the susceptible laboratory population. O-demethylase, the marker enzyme for measuring the activity of p 450 monooxygenases exhibited significantly higher activity (2.95 to 8.80-fold) in all the field collected populations (Fig 3).

Populations of many insect species have developed insecticide resistance as a result of the selection pressure created by the extensive use of chemical pesticides (Zhu *et al*., 2016). Farmers commonly use many synthetic insecticides to manage the legume pod borer, *M. vitrata* (Ba *et al*., 2019). Though resistance to insecticides were reported for old generation insecticides (Ekesi, 1999; Sreelakshmi *et al*., 2015), there were no further follow up studies on resistance against newer molecules and the biochemical mechanisms of resistance development in *M. vitrata*. The advocation and use of insecticide mixtures in recent years may also exacerbate the situation if resistance monitoring is not done periodically.



Values below the unfilled diagonal squares indicate the correlation coefficient r and above the unfilled diagonal squares the corresponding P-values. The scale colors of the filled boxes indicate the magnitude of the correlation.

Fig 2: Pairwise correlation analysis of LC_{50} values of five insecticides in field populations of *M. vitrata*.

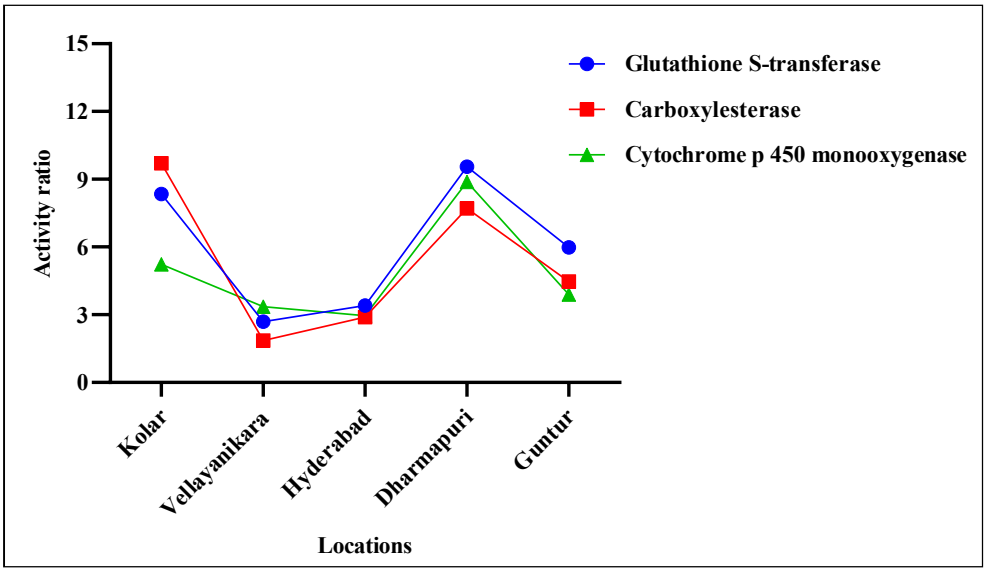


Fig 3: Activity ratio of detoxification enzymes for different field collected populations of *M. vitrata*.

Flubendimide, indoxacarb, chlorpyrifos, quinolphos and chlorantriniliprole are the commonly used insecticides for the management of *M. vitrata* in southern Indian states (Sireesha *et al*., 2024). In general, farmers adopt their own custom made strategies with reference to dose, tank mixture and time of application on various legume crops meant for vegetable and grain purposes. Hence, the reasons for variations in levels of resistance noted in different field collected populations of *M. vitrata* are obvious. The populations of *M. vitrata* occuring in India are homogenous interms of their genetic structure (Mahalle *et al*., 2022) but differs with reference to insecticide susceptibility as noted in the present investigation. It is an emerging pest of pigeonpea, cowpea and in many other food legumes not only in India but in many Asian and African countries (Mahalle and Taggar, 2017; Rathee and Dalal, 2018; Srinivasan *et al*., 2021).

However, the resistance level is moderate to very high for chlorpyrifos and quinolphos. The resistance ratio of the three populations to quinolphos varied from 122.90 to 311.00-fold. Since these insecticides have been around for a while, numerous insects, including *M. vitrata*, have become used to them (Sreelaksmi *et al*., 2016). The present results revealed that the high levels of resistance to organophosphates is partly due to higher expression of metabolic enzymes.

The filed populations of *M. vitrata* have also developed high levels of resistance against indoxacarb (33.27 to 91.44-fold) due to wide spread use of this insecticide. Studies conducted by Chen *et al*, (2023) in *Spodoptera frugiperda* also revealed resistance to indoxacarb (33.21 to 82.95-fold) in the field populations. The observed resistance levels against flubendimide (30.868 to 75.26-fold) also a matter of concern. Since flubendiamide has a novel mode of action that it attaches itself to ryanodine receptors in insect muscle cells. This results in the uncontrollable release of calcium ions, which keeps the insect's muscles contracting constantly, paralyzing it and finally killing it.

Research on cross-resistance among pesticides is crucial since changing, rotating and combining pesticides are standard ways to prevent or postpone the emergence of resistance. There was a significant positive relationship between resistance among the various insecticides, as indicated by the pairwise correlation coefficients calculated between the LC_{50} values of the investigated compounds. Other pesticides minimal cross-resistance may serve as a guidance for their rotation and sequential application in the field. Likewise, the green peach aphid (*Myzus persicae*) demonstrated that neonicotinoids and pyrethroids developed cross-resistance due to overexpression of many cytochrome p 450 genes. This suggests that resistance to many pesticide classes can be mediated by a single detoxification route (Bass *et al*., 2014). This methodology combined LCRs, their 95% CLs and the testing of parallelism and equality assumptions (Robertson, 2017). With the exception of quinolphos and indoxacarb, all other insecticides had regression lines that were parallel but not equal based on tests for equality and parallelism hypotheses. This suggests that the populations are heterogeneous interms of susceptibility to insecticides.

Many mechanisms are often implicated in insecticide resistance, the most prominent ones being elevated activity of detoxifying enzymes, changes in metabolic pathways and mutations at the target location. These enzymes include esterases (EST), including carboxylesterase, mixed-function oxidases (MFO) and glutathione S-transferases (GST). Carboxylesterases, GST and cytochrome p 450 are the major metabolic enzymes responsible for insecticide resistance. The field populations of *M. vitrata* exhibited higher levels of activity for all three enzymes *viz*., carboxylesterases, GST and cytochrome p 450 as compared to the susceptible population. Moreover, the metabolic activity of carboxylesterase, GST and Cyt P-450 was increased by 9.71-, 9.55- and 8.88-fold respectively in field collected resistant populations over the susceptible population. The findings of the present study align with those of Mohan and Gujar (2003), who reported a 2-fold increase in the activity of GSH S-transferase, a 1.8-fold increase in carboxylesterase activity and a 7.7-fold increase in microsomal monooxygenase activity in field populations of *Plutella xylostella*. The metabolic enzymes are well known for their ability to metabolise various insecticides in the resistant populations of *Leucinodes orbonalis* (Kariyenna *et al*., 2020). Similarly, elevated levels of glutathione-S-transferase and carboxylesterase are

Table 3: Metabolic enzymes activities in the midgut of *M. vitrata* populations.

Location	Glutathione S-transferase	Carboxylesterase	Cytochrome P450 monooxygenase
	Specific activity ± SE (µmol/min/mgprotein)	Specific activity ± SE (mmol/min/mgprotein)	Specific activity ± SE (µmol/min/mgprotein)
Susceptible (Strain: NBAIR-IS-CRA-02)	15.589±1.926^{a}	2.208±0.498^{a}	0.486±0.142^{a}
Kollar	130.112±16.26^{d}	21.447±2.443^{d}	2.546±0.204^{c}
Hyderabad	53.181±7.535^{b}	6.416±1.034^{ab}	1.438±0.157^{b}
Vellayanikara	42.032±4.111^{ab}	4.120±0.446^{a}	1.630±0.259^{b}
Dharmapuri	149.545±6.723^{d}	15.840±2.086^{c}	4.319±0.259^{d}
Guntur	93.280±15.170^{c}	9.875±1.082^{b}	1.891±0.120^{b}

*Numbers and means (± SE) within a column and followed by different letters are significantly different at $p<0.05$ (Tukey'test).

associated with insecticide resistance in *Pectinophora gossypiella* (Alam *et al*., 2021). Increased activity of cytochrome p 450 monooxygenase and carboxylesterase has also been observed in resistant populations of *Tuta absoluta* (Prasannakumar *et al*., 2020). These enzymes are known to confer resistance to a wide variety of insecticides, including carbamates, synthetic pyrethroids, organophosphates (OP) and insecticides with novel mode of action (Singh and Singh, 2021).

CONCLUSION

The present study generated valuable base-line data on the insecticide resistance status in *M. vitrata* populations to several insecticides collected from South Indian states where the legumes are grown extensively. The data further suggests cautious use of various insecticides for the management of *M. vitrata*.

ACKNOWLEDGEMENT

The authors extend gratitude to the Indian Council of Agricultural Research (ICAR) for funding under the Consortium Research Platform (CRP) on Genomics project. The first author pro-fusely thanks the FMC India Private Ltd for the award of the FMC Science Leaders Scholarship. The facilities provided by the Director, ICAR-National Bureau of Agricultural Insect Resources are thankfully acknowledged.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

I consent to the collection and analysis of *Maruca vitrata* field populations for insecticide resistance research by taking part in this project. I am aware that the goal is to improve leguminous crop pest control techniques and I agree to the use of the data gathered for this research.

Author contributions

All authors contributed to the study conception and design. N. Vijayakumari conducted the experiment, performed data analysis and prepared the original draft. M. Mohan contributed to framing of the study, resources supply, data curation and draft corrections.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

REFERENCES

Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. Journal of economic Entomology. 18(2): 265-267. https://doi.org/10.3109/13880209.

Alam, D.R., Sobeiha, A., Khdir, A.A. and Alsenosy, N. (2021). Detoxifying Enzymatic Activity and Insecticide-Resistance Gene Expression in Field Populations of Pink Bollworm, [*Pectinophora gossypiella* (Saund.)]. Arab Universities Journal of Agricultural Sciences. 29(3): 925-931. https://doi.org/10.21608/ajs.2021.92821.1412.

Aryal, L.N., Regmi, R., Lohani, S. and Bhusal, Y. (2021). Efficacy of commercial insecticides for cowpea pod borer (*Maruca vitrata* F.) management in Pokhara, Nepal. Journal of Agriculture and Natural Resources. 4(1): 165-175. https://doi.org/10.3126/janr.v4i1.33250.

Ba, N.M., Huesing, J.E., Dabiré-Binso, C.L., Tamò, M., Pittendrigh, B.R. and Murdock, L.L. (2019). The legume pod borer, *Maruca vitrata* Fabricius (Lepidoptera: Crambidae), an important insect pest of cowpea: A review emphasizing West Africa. International Journal of Tropical Insect Science. 39: 93-106. https://doi.org/10.1007/s42690-019-00024-7.

Bass, C., Puinean, A.M., Zimmer, C.T., Denholm, I., Field, L.M., Foster, S.P. and Williamson, M.S. (2014). The evolution of insecticide resistance in the peach potato aphid, *Myzus persicae*. Insect Biochemistry and Molecular Biology. 51: 41-51. https://doi.org/10.1016/j.ibmb.2014.05.003.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 72: 248-254.

Chen, H.L., Hasnain, A., Cheng, Q.H., Xia, L.J., Cai, Y.H., Hu, R. and Wang, X.G. (2023). Resistance monitoring and mechanism in the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) for chlorantraniliprole from Sichuan Province, China. Frontiers in Physiology. 14: 1180655. https://doi.org/10.3389/fphys.2023.1180655.

Ekesi, S. (1999). Insecticide resistance in field populations of the legume pod-borer, Maruca vitrata Fabricius (Lepidoptera: Pyralidae), on cowpea, *Vigna unguiculata* (L.), Walp in Nigeria. International Journal of Pest Management. 45(1): 57-59. https://doi.org/10.1080/096708799228058.

Jat, B.L., Sharma, H.C., Pagaria, P., Meena, A.K., Mali, G.R. and Khan, T. (2024). Legumes: Source of bioactive compounds and their potential use in legume crops improvement: A review. Legume Research: An International Journal. 47(11). doi: 10.18805/LR-4945.

Jiang, T., Wu, S., Yang, T., Zhu, C. and Gao, C. (2015). Monitoring field populations of *Plutella xylostella* (Lepidoptera: Plutellidae) for resistance to eight insecticides in China. Florida Entomologist. 1: 65-73.

Kao, C.H., Hung, C.F. and Sun, C.N. (1989). Parathion and methyl parathion resistance in diamondback moth (Lepidoptera: Plutellidae) larvae. Journal of Economic Entomology. 82(5): 1299-1304. https://doi.org/10.1093/jee/82.5.1299.

Kariyanna, B., Prabhuraj, A., Asokan, R., Agrawal, A., Gandhi Gracy, R., Jyoti, P. and Mohan, M. (2020). Genome mining and expression analysis of carboxylesterase and glutathione S-transferase genes involved in insecticide resistance in eggplant shoot and fruit borer, *Leucinodes orbonalis* (Lepidoptera: Crambidae). Frontiers in physiology. 11: 594-845. https://doi.org/10.3389/fphys.2020.594845.

Khan, S., Uddin, M.N., Rizwan, M., Khan, W., Farooq, M., Shah, A.S. and Ali, S. (2020). Mechanism of Insecticide Resistance in Insects/Pests. Polish Journal of Environmental Studies. 29(3): 2023-2030. https://doi.org/10.15244/pjoes/108 513.

Kinoshita, F.K., Frawley, J.P. and DuBois, K.P. (1966). Quantitative measurement of induction of hepatic microsomal enzymes by various dietary levels of DDT and toxaphene in rats. Toxicology and applied Pharmacology. 9(3): 505-513. https://doi.org/10.1016/0041-008X(66)90011-1.

LeOra, S.O. (2003). Poloplus, a user's guide to probit or logit analysis. LeOra Software, Berkeley, CA.

Mahalakshmi, M.S., Sreekanth, M., Adinarayana, M., Reni, Y.P., Rao, Y.K. and Narayana, E. (2016). Incidence, bionomics and management of spotted pod borer [*Maruca vitrata* (Geyer)] in major pulse crops in India-A review. Agricultural Reviews. 37(1): 19-26. doi: 10.18805/ar.v37i1.9260.

Mahalle, R.M. and Taggar, G.K. (2017). Yield loss assessment and establishment of economic threshold level of *Maruca vitrata* in pigeonpea. Journal of Food Legumes. 31(1): 36-44.

Mahalle, R.M., Chakravarty, S. and Srivastava, C.P. (2022). Population genetic differentiation and structure of *Maruca vitrata* (Lepidoptera: Crambidae) in India. Diversity. 14(7): 546. https://doi.org/10.3390/d14070546.

Margam, V.M., Coates, B.S., Ba, M.N., Sun, W., Binso-Dabire, C.L., Baoua, I. and Murdock, L.L. (2011). Geographic distribution of phylogenetically-distinct legume pod borer, *Maruca vitrata* (Lepidoptera: Pyraloidea: Crambidae). Molecular Biology Reports. 38: 893-903. doi: 10.1007/s11033-010-0182-3.

Mohan, M. and Gujar, G.T. (2003). Local variation in susceptibility of the diamondback moth, *Plutella xylostella* (Linnaeus) to insecticides and role of detoxification enzymes. Crop Protection. 22(3): 495-504. https://doi.org/10.1016/S0261-2194(02)00201-6.

Panini, M., Manicardi, G.C., Moores, G.D. and Mazzoni, E.J.I.S.J. (2016). An overview of the main pathways of metabolic resistance in insects. Invertebrate Survival Journal. 13(1): 326-335. https://doi.org/10.25431/1824-307X/isj.v13i1.326-335.

Prasannakumar, N.R., Jyothi, N., Saroja, S. and Kumar, G.R. (2021). Relative toxicity and insecticide resistance of different field population of tomato leaf miner, *Tuta absoluta* (Meyrick). International Journal of Tropical Insect Science. 41: 1397-1405. https://doi.org/10.1007/s42690-020-00334-1.

Rathee, M. and Dalal, P. (2018). Emerging insect pests in Indian agriculture. Indian Journal of Entomology. 80(2): 267-281. https://doi.org/10.5958/0974-8172.2018.00043.3.

Regmi, R., Tiwari, S., Thapa, R.B., Maharjan, R. and Poudel, K.P. (2016). Survey on management of spotted pod borer (*Maruca vitrata* Fabricius) on yardlong bean in Chitwan, Nepal. International Journal of Applied Sciences and Biotechnology. https://doi.org/10.3126/ijasbt.v4i1.14572.

Robertson, J.L., Jones, M.M., Olguin, E. and Alberts, B. (2017). Bioassays with Arthropods. CRC Press. 212 p. https://doi.org/10.1201/9781315373775.

Scharf, M.E. and Gondhalekar, A.D. (2021). Insecticide resistance: perspectives on evolution, monitoring, mechanisms and management. Boston (MA). CABI. 231-255.

Sharma, H.C., Srivastava, C.P., Durairaj, C. and Gowda, C.L.L. (2010). Pest management in grain legumes and climate change. Climate change and management of cool season grain legume crops. Springer. Dordrecht. pp. 115-139.

Singh, S.K. and Singh, P.S. (2021). Biochemical factors associated with resistance to spotted pod borer, Maruca vitrata (Fabricius) in green gram. Legume Research-An International Journal. 44(11): 1398-1401. doi: 10.18805/LR-4302.

Sreelakshmi, P., Paul, A., Antu, M., Sheela, M.S. and George, T. (2015). Assessment of insecticide resistance in field populations of spotted pod borer, *Maruca vitrata* Fabricius on vegetable cowpea. International Journal of Farm Sciences. 5(3): 159-164.

Sreelakshmi, P., Paul, A., Beevi, S.N. and Narayanan, P. (2016). Management of resistant populations of legume pod borer, *Maruca vitrata* Fabricius (Lepidoptera: Crambidae) using new generation insecticides. Environment and Ecology. 34(3): 917-21.

Sireesha, E., Singh, G., Singh, R. and Singh, B. (2024). Bioefficacy and economics of certain novel generation insecticides against spotted pod borer, *Maruca vitrata* (Geyer) in Greengram (*Vigna radiata* L.). Legume Research. 47(2): 328-331. doi: 10.18805/LR-4651.

Srinivasan, R., Tamò, M. and Malini, P. (2021). Emergence of *Maruca vitrata* as a major pest of food legumes and evolution of management practices in Asia and Africa. Annual Review of Entomology. 66(1): 141-161. https://doi.org/10.1146/annurev-ento-021220-084539.

Tamo, M., Arodokoun, D.Y., Zenz, N., Tindo, M., Agboton, C. and Adeoti, R. (2002). The importance of alternative host plants for the biological control of two key cowpea insect pests, the pod borer *Maruca vitrata* (Fabricius) and the flower thrips *Megalurothrips sjostedti* (Trybom). Challenges and Opportunities for Enhancing Sustainable Cowpea Production. 81-93 p. https://doi.org/10.1007/s10526-009-9263-4.

Tong, H., Su, Q., Zhou, X. and Bai, L. (2013). Field resistance of *Spodoptera litura* (Lepidoptera: Noctuidae) to organophosphates, pyrethroids, carbamates and four newer chemistry insecticides in Hunan, China. Journal of Pest Science. 86: 599-609. https://doi.org 10.1007/s10340-013-0505-y.

Tukey, J.W. (1977). Exploratory Data Analysis. Reading, MA. Addison-Wesley. pp. 131-160.

Van Asperen, K. (1962). A study of housefly esterases by means of a sensitive colorimetric method. Journal of Insect Physiology. 8(4): 401-416.

Zhu, F., Lavine, L., Neal, S., Lavine, M., Foss, C. and Walsh, D. (2016). Insecticide resistance and management strategies in urban ecosystems. Insects. 7(1): 2. https://doi.org/10.3390/insects7010002.