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Determination of esterase activity in susceptible and resistant population of *Helicoverpa armigera* (Hubner) Hardwick in pigeonpea from the different location of middle Gujarat

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Abstract
A study was carried out on determination of the activity of esterase (µmol/min/mg protein) of the susceptible and resistant population of pigeonpea pod borer, *Helicoverpa armigera* (Hubner) Hardwick during the year 2016-17 at B. A. College of Agriculture, Anand Agricultural University, Anand. The result indicated higher level of esterase presents in different resistant populations collected from various locations. The larvae collected from Vadodara location showed higher level of esterase (1.36 ± 0.04 µmol/min/mg protein) than other locations and it was followed by the esterase activity found in Ahmadabad population (1.28 µmol/min/mg protein). Larvae collected from Anand (1.17 ± 0.03 µmol/min/mg protein) and Dahod (1.02 ± 0.05 µmol/min/mg protein) districts showed moderate level of esterase activity, whereas low level of esterase activity was found in the susceptible population (0.14 ± 0.02 µmol/min/mg protein) of *H. armigera.*

Keywords: commonly used insecticides, esterase activity, *Helicoverpa armigera*, insecticidal resistance, middle Gujarat, pigeonpea

Introduction
The pigeonpea pod borer, *Helicoverpa armigera* (Hubner) Hardwick (Lepidoptera: Noctuidae) is the most dreaded among the insect pests associated with pigeonpea. It is widely distributed in Asia, Africa, Australia and Mediterranean Europe. This pest has been recorded in more than 181 species of cultivated and wild crops and about 45 host families, including Asteraceae, Fabaceae, Malvaceae, Poaceae and Solanaceae in India (Pawar *et al.*, 1986; Fitt, 1989; Manjunath *et al.*, 1989; Pogue, 2004) [19, 6, 18, 20]. This pest is a cosmopolitan in distribution and has gained national importance as a major destructive pest owing to its capacity to feed on many important agricultural crops (Dinsdale *et al.*, 2010) [5]. In case of pigeonpea, *Helicoverpa* caused 60 to 90 per cent loss in the grain yield under favorable conditions (Lal *et al.*, 1993; Priyadarshini *et al*., 2013; Keval *et al*., 2017) [16, 21, 11]. Further, *Helicoverpa* caused heavy losses up to 60 per cent with an annual loss estimated to be US $ 400 million in pigeonpea (Anon., 2007) [2]. It was estimated that the infestation of one larva per plant on pigeonpea caused yield loss of 1015 kg/ha (Reddy and Basavanna, 1978). In India, crop losses due to *H. armigera* were commonly more than half yield and annual losses to cotton and pulses alone have been estimated to US $ 300-500 million (King, 1994).
The distribution of *H. armigera* is ubiquitous in all the states of India including Gujarat, yet little work has been undertaken to assess the level of resistance developed by the pest. Despite of this fact, and in many occasions, control of *H. armigera* through the insecticides failed even after frequent application of recommended dosages of insecticides in various crops like tomato, pigeonpea, chickpea, sorghum and cowpea (Horowitz and Denholm, 2000) [9]. Development of resistance to various insecticides is a major problem associated with insecticidal control of insect pests. Number of factors *viz.*, indiscriminate use of insecticides, continuous use of single group insecticides, over dosing, mixing of different insecticides of various groups, sub lethal dose, improper applications etc. are responsible for the development of resistance to insecticides by *Helicoverpa*. In addition to operational factors, various biotic factors such as stage of insect, slow cuticular penetration, mixed function oxidase enzyme, glutathione S-transferases and hydrolases are also responsible for development of resistance

Against insecticides (Satpute and Sarode, 1995; Sarode, 1999 and Kranthi *et al*., 2001) [24, 22, 23]. It is, therefore, necessary to generate the toxicity data of various insecticides against *H. armigera* in Gujarat and also require to generate data on level of resistance developed by the pest against various insecticides for effective and economical management of the pest.

Materials and Methods

To determine the activity of esterase, a study was carried out at Department of Biochemistry, B. A. College of Agriculture, Anand Agricultural University, Anand during 2016-17.

For the purpose, *H. armigera* larvae were collected from different locations of middle Gujarat *viz.*, Anand, Vadodara, Dahod and Ahmedabad and maintained at a constant temperature of 27 ± 1 ^{0}C with adequate food. The extraction of esterase was carried out after 24 hrs by following standard methods (Kranthi, 2005) [14]. Thirty larvae (fifth instar) in each treatment were mass homogenized in 2 ml buffer (100 mM phosphate buffer, containing 1 mM each of EDTA, PTU, PMSF and 20% Glycerol, pH 7.0) and homogenates was subjected to centrifugation at 10,000 rpm for 20 min. The volume of supernatant obtained after centrifugation was made up to 2 ml using phosphate buffer (100 mM, pH 7.0). Hundred microliters of aliquot was taken from the supernatant in a 1.5 ml micro-centrifuge tube and the volume was made up to 1 ml.

To find out the esterase activity, the procedure given by Kranthi (2005) [14] was followed. Fifty microliters of enzyme assay solution was taken up in a 10 ml test tube and the volume was made up to 1 ml with 950u phosphate buffer (40 mM, pH 6.8). Then, five milliliters of substrate solution (1 ml of 30 mM a-NAA in 99 ml of phosphate buffer, 40 mM, pH 6.8) was added to each test tube. One milliliter of 40 mM phosphate buffer with 5 ml of substrate solution without the enzyme assay solution was kept as control. The whole set was maintained in dark for 20 min at 30 ^{0}C with occasional shaking. After incubation, 1 ml of staining solution (2 parts of 1% fast blue BB solution in 5 parts of 5% SDS) was added to each tube including in control and the tubes were kept in dark for 20 min at room temperature. 1-Naphthol produced as a product during the esterase action on the substrate (a-naphthyl acetate) was coupled with fast blue BB salt (Sigma, USA). A strong blue colour produced was measured at its absorbance maxima of 590 nm on a double beam spectrophotometer (Perkin Elmer k 3B). For the calibration of 1-naphthol, the procedure of Van as detailed by Kranthi (2005) [14] was followed. The standard error of mean was calculated for each replication and the error bars was drawn on the basis of upper and lower confidential interval.

Results and Discussion

Esterase is catalyzing the hydrolysis of a wide range of aliphatic and aromatic esters, choline esters and even organo-phosphorous compounds. Overproduction of esterase in insect body is responsible for sequestration of insecticidal compounds. Thus, elevation in the level of esterase, produced as a result of insecticidal application, could be used as biochemical markers for resistance monitoring of insect pests. To determine the activity of esterase in the susceptible and resistant population of H. armigera, a study was carried out at Department of Biochemistry, B. A. College of Agriculture, Anand Agricultural University, Anand during 2015-16 and 2016-17 and the results are presented and discussed hereunder.

The populations of H. armigera were collected from four different locations of middle Gujarat and tested for their resistance to different insecticides during the year 2015-16 and 2016-17. The data (Table 2) on comparative resistance indicated that all the populations were found to be susceptible to chlorantraniliprole 20 SC and flubendiamide 480 SC except Vadodara population which had developed very low level of resistance (RI: 1.17 & 1.21). The Vadodara population showed maximum level of resistance (29.18 folds) to chlorpyriphos among all the populations and insecticides tested, whereas rest of the populations showed 11.60 to 18.05 folds resistance to this insecticide. Dahod population showed susceptibility to indoxacarb while rest of the populations had developed moderate level of resistance (2.29 to 5.14 folds) to indoxacarb. The data in Table 6 also indicated that the populations from Anand, Ahmedabad, Dahod and Vadodara districts had developed higher level of resistance i.e. 23.08, 19.54, 7.81 and 25.21 folds, respectively against profenofos. In case of Dahod population, it showed susceptibility to profenofos 40% + cypermethrin 4%, whereas population collected from Anand, Ahmedabad and Vadodara have developed 10.93, 12.64 and 14.88 folds resistance, respectively to the same insecticide. It was found that all the tested populations i.e. Anand, Ahmedabad, Dahod and Vadodara had developed moderate resistance of 6.67, 7.34, 2.34 and 8.67 folds, respectively to lambda cyhalothrin. All the populations collected from middle Gujarat have shown high level of resistance (10.54 to 22.56 folds) to a synthetic pyrethroid, cypermethrin.

Table 1: Base-line toxicity and susceptibility of different insecticides to *H. armigera*

Insecticides	Generation	Heterogenity		Regression Equation	LC_{50}	Fiducial Limit (95%)	SI
		Chi-square*	df				
Chlorantraniliprole 20 SC	First	0.155	5	Y=3.55+1.59X	0.006	0.003-0.010	01.00
	Sixth	1.077	5	Y=4.27+1.89X	0.006	0.003-0.009	
Flubendiamide 480 SC	First	0.389	5	Y=2.73+1.53X	0.017	0.010-0.029	01.21
	Sixth	0.264	5	Y=2.63+1.43X	0.014	0.008-0.026	
Indoxacarb 15.8 EC	First	0.273	5	Y=2.69+1.34X	0.010	0.006-0.019	01.43
	Sixth	0.270	5	Y=3.08+1.44X	0.007	0.004-0.013	
Chlorpyriphos 20 EC	First	0.827	5	Y=0.37+1.39X	0.539	0.301-1.509	11.98
	Sixth	0.269	5	Y=2.24+1.66X	0.045	0.027-0.077	
Lambda cyhalothrin 5 EC	First	0.073	5	Y=3.41+1.49X	0.005	0.003-0.010	01.67
	Sixth	0.221	5	Y=3.58+1.39X	0.003	0.001-0.004	
Profenofos 50 EC	First	0.088	5	Y= 0.04+1.33X	0.928	0.518-2.219	10.20
	Sixth	0.700	5	Y= 1.63+1.56X	0.091	0.051-0.155	
Profenofos 40% + cypermethrin 4% 44 EC	First	0.072	5	Y=1.56+1.51X	0.093	0.053-0.165	02.21
	Sixth	0.066	5	Y=2.29+1.66X	0.042	0.025-0.070	
Cypermethrin 10 EC	First	0.945	5	Y=1.24+1.31X	0.113	0.062-0.032	12.56

	Sixth	0.641	5	Y=3.39+1.67X	0.009	0.006-0.016	

Notes: SI-susceptible index
In none of the case, the data were found to be significantly heterogeneous at p=0.05

Table 2: Comparative resistance of *H. armigera* to insecticides for different location of middle Gujarat

Insecticides	Resistance index			
	Anand	Ahmedabad	Dahod	Vadodara
Chlorantraniliprole 20 SC	S	S	S	01.17
Flubendiamide 480 SC	S	S	S	01.21
Indoxacarb 15.8 EC	03.29	02.29	S	05.14
Chlorpyriphos 20 EC	16.47	18.05	11.60	29.18
Lambda cyhalothrin 5 EC	06.67	07.34	02.34	08.67
Profenofos 50 EC	23.08	19.54	07.81	25.21
Profenofos 40% + cypermethrin 4% 44 EC	10.93	12.64	S	14.88
Cypermethrin 10 EC	14.34	21.45	10.54	22.56

Note: 'S' indicates the susceptibility of population towards insecticides

Activity of esterase (alpha-naphthyl acetates as substrates) of fifth instar larvae collected from different location of middle Gujarat is presented in Table 3. Results revealed the elevated activities of esterase in different four location *viz.*, Vadodara, Ahmedabad, Anand and Dahod. The data on esterase activity (µmol/min/mg protein) in fifth instar *H. armigera* larvae collected from different location showed higher level of esterase. The larvae collected from Vadodara location showed higher level of esterase (1.36 µmol/min/mg protein) than larvae of other location. However, it was followed by the esterase activity found in Ahmedabad district population (1.28 µmol/min/mg protein). Larvae collected from Anand and Dahod districts showed moderate level of esterase activity which was 1.17 and 1.02 µmol/min/mg protein, respectively, whereas low level of esterase was found in the susceptible population of *H. armigera* (0.14 µmol/min/mg protein). On the basis of esterase activity as biochemical marker, the population of different locations showed high level of esterase activity which is in the order of Vadodara (1.36 µmol/min/mg protein) > Ahmedabad (1.28) > Anand (1.17) > Dahod (1.02) > laboratory reared susceptible population (0.14).

Table 3: Activity of esterase in *H. armigera* larvae of different locations of middle Gujarat (µmol/min/mg protein)

Location	α-NA esterase activity (Mean ± SD)
Anand	1.17 ± 0.03
Ahmedabad	1.28 ± 0.04
Dahod	1.02 ± 0.05
Vadodara	1.36 ± 0.04
Susceptible (Laboratory condition)	0.14 ± 0.02
S. Em. ±	0.01
C.D. at 5%	0.03
C. V.%	3.95

Increased detoxification enzyme activity in pest population is considered one of the most important factors for development of insecticidal resistance (Denholm and Rowland, 1992; Li *et al.,* 2007). The results of present investigations are in conformity with the report of Gunning *et al.* (1996), who reported that resistance factors in *H. armigera* were positively correlated with esterase titres and that increased resistance was accompanied by increased esterase activity. Elevated esterase activity has shown to be responsible for cross-resistance in organophosphorus, carbamates and pyrethroids (Zhao *et al.* 1996) [28]. According to Kranthi (1998) [14], enhanced esterase activity also played an important role in conferring resistance to organophosphorus and pyrethroids. Esterase (EST) activities were significantly higher in profenofos resistant population than susceptible one and activities were highly correlated ($r^2 = 0.87$) with resistance to profenofos (Harold and Ottea, 2000) [8]. Several researchers suggested production of esterase which was responsible for restoration of insecticidal compounds (Small and Hemingway, 2000) [26]. While, Achaleke *et al.* (2009) [1] found that esterase activity was positively correlated with resistance to cypermethrin against *H. armigera* in cotton.
Reported that Nagpur and Delhi strain of *H. armigera* that displayed high degree of resistance towards deltamethrin, had higher esterase activity compared to a susceptible laboratory strain. Enhanced activities of mixed function oxidase and esterase enzymes are associated with pyrethroid resistance in Australian population of *H. armigera* (JouBen *et al.*, 2012; Teese *et al.*, 2013) [10, 27]. Shah (2014) [25] studied on determination of esterase activity in the susceptible and resistant populations of *S. litura* indicated higher level of esterase activity (1.18 ± 0.02 to 1.43 ± 0.02 µmol/min/mg protein) in different resistant populations collected from different locations *i.e.* Surendranagar, Sabarkantha, Anand and Amreli districts than the susceptible strain (0.19 ± 0.03 µmol/min/mg protein). The production of more amount of esterase might be due to chemical reaction of insecticides in insect body. The results of the above workers are also fall in the same line with the results of present findings.

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