Pakistan J. Zool., vol. 50(4), pp 1475-1482, 2018. DOI: http://dx.doi.org/10.17582/journal.pjz/2018.50.4.1475.1482

Deltamethrin Induced Changes in the Activities of Various Esterases in Deltamethrin-Resistant Populations of *Trogoderma granarium*

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Article Information
Received 04 July 2017
Revised 14 August 2017
Accepted 22 August 2017
Available online 22 June 2018

Authors' Contribution
FRS designed and supervised the research project. AH conducted the experimental work. AH, TR and FRS analyzed the data and wrote the article.

Key words
Khapra beetle, Total esterases, Insecticide resistance, Carboxyl esterases, Pyrethroid insecticide.

ABSTRACT

The present study was aimed to evaluate the toxic effects of deltamethrin on the level of various esterases in deltamethrin-resistant populations of *Trogoderma granarium* collected from some godowns of Punjab. The level of various esterases like total esterases, cholinesterase, acetylcholinesterase, arylesterase and carboxylesterase in 4th, 6th instar larvae and adult beetles of deltamethrin-resistant populations' *viz*., Gujranwala, Okara and D.G. Khan was significantly increased as compared to susceptible population of *T. granarium* (population never exposed to any kind of insecticide/fumigant since 2001). Different developmental stages possessed different levels of esterase activities. Based on the level of activities the adult beetles were more susceptible to deltamethrin than 4th and 6th instar larvae. The increased level of esterases contributes towards resistance against pesticide in stored grain pests.

INTRODUCTION

Wheat is a leading food grain in Pakistan (Wajid, 2004; Goyal and Parasad, 2010) and is stored at farms in heaps, pots, baskets and bags covered by either straws, plastered or mud, house type godowns, PASSCO type godowns, hexagonal bins, silos and open bulk heads (Peng *et al*., 2011). During storage one third of the potential food supply is lost every year (Duveiller *et al*., 2007) and about 10-20% post-harvest losses are mainly caused by insect pests (Khan *et al*., 2010). *Trogoderma granarium* (Everts) is the most important insect species that adversely infest wheat in tropical regions of the world (Lowe *et al*., 2000; Ahmedani *et al*., 2011). A number of control methods have been used to control the pest population which include the use of botanical insecticide (Fields, 2006; Prakash and Rao, 2006; Musa and Dike, 2009; Gandhi *et al*., 2010), synthetic insecticides like organochlorines, carbamates, organophosphates and synthetic pyrethroids (Kljajic and Peric, 2006; Ali *et al*., 2007; Athanassiou *et al*., 2007), fumigants (Daglish, 2004), biological insecticides (Nayak *et al*., 2005) and application of physical agents like heat, temperature, pressure, aeration and relative humidity (Ofuya and Reichmuth, 2002; Mbata *et al*., 2004). Synthetic insecticides are most commonly used to control agricultural insect pests all over the world (Mathewes, 1993) but excessive and unplanned use of these pesticides results in the development of resistance (Fragoso *et al*., 2003; Ribeiro *et al*., 2003). Dletamethrin a synthetic pyrethroid is widely used in grain godowns before the fumigation process but many researchers have reported resistance in *T. granarium* against deltamethrin (Irshad and Iqbal, 1994; Tarakanov *et al*., 1994; Saxena and Sinha, 1995; Kumar *et al*., 2010).

Esterases cause the hydrolysis of ester containing pyrethroids into their corresponding alcohol and acid. They can also sequester insecticides by the formation of stable compounds so toxic insecticidal molecules may not be available for chemical reactions within insect body (Devonshire and Moores 1982; Oakeshott *et al*., 2005; Wheelock *et al*., 2005). Cholinesterase, acetylcholinesterase, carboxylesterase and arylestearse are important esterases involved in the process of detoxification and causing resistance for particular insecticide (Ellman *et al*., 1961; Fournier and Mutero, 1994) and elevated levels of esterases was studied in resistant strains of insect pests (Casida, 1973; Riaz *et al*., 2017). Also different developmental stages exhibit different levels of esterases as larval stages are found to be more resistant than adults (Nakakita and Winks, 1981; Riaz *et al*., 2017). The present study was aimed to investigate the abnormalities developed in the level of esterases in deltamethrin-resistant populations of *T. granarium* and to correlate the level of esterase activities with resistance.

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0030-9923/2018/0004-1475 $ 9.00/0

MATERIALS AND METHODS

Deltamethrin resistant and susceptible populations of *T. granarium* used in this study were those used in Hafiz *et al*. (2017). The master culture of susceptible and resistant populations of Khapra beetle was reared according to Riaz *et al*. (2014), Shakoori *et al*. (2016) and Hafiz *et al*. (2017). The culture was maintained in 300ml sterilized jam jar covered with muslin cloth at 35±2ºC with 60±5% relative humidity (Riaz *et al*., 2014). Whole wheat grains, crushed wheat and wheat floor was used as feed of different larvae (FAO, 1974). From homogeneous stock of each population 4th, 6th instar larvae and adult beetles were used to record toxicological data. To calculate LC_{50} values of 4th, 6th instar larvae and adult beetles of *T. granarium* mortality data was subjected to Probit analysis by Minitab 16 software (Finney, 1971) and were expressed in ppm. The LC_{50} values of deltamethrin for these populations are presented in Hafiz *et al*. (2017).

Biochemical analysis

Biochemical analysis for a number of esterase activities including carboxylesterase (CE), acetylcholine esterase (AchE), cholinesterase (ChE), total esterase (TE) and arylesterase (AE) were carried out. Using their respective standard curves, absorbance of various enzyme activities like CE, TE and ChE were converted into activity/quantity.

For estimation of various esterases, twenty larvae (4th and 6th instar larvae) and twenty adult beetles of Khapra beetle from each population were taken in five replicates, each containing three test tubes. They were weighed and homogenized in their respective buffer (pH 7.0) by using motor driven Teflon glass homogenizer with consistent cooling in squashed ice. The activities of acetylcholinesterase and arylesteras were measured according to Devonshire (1975a) and Junge and Klees (1981), respectively. While the activities of carboxylesterase and Total esterases were determined according to the method of Devonshire (1975b). The activity of Choline esterase was measured according to Rappapot *et al*. (1959).

Statistical analysis

The biochemical data was subjected to one way ANOVA and Tukey's test to compare the significance difference between means of susceptible and resistant populations at $P< 0.05$ using Minitab 16 software. Values <0.05 were considered statistically significant.

RESULTS

The activities of various esterases *viz.,* Total esterases, Cholinesterase, Acetylcholine esterases, Aryl esterases and Carboxyl esterases in three developmental stages (4th and 6th instar larvae and adult beetles) of deltamethrin-susceptible and three deltamethrin-resistant populations (Gujranwala, Okara and D.G. Khan) of *T. granarium* are presented in Table I. The activities of TE, ChE, AChE, AE

Table I.- Activities of various esterases (IU/mg body weight) of 4th and 6th instar larvae and adult beetles of susceptible and resistant populations of *T. granarium.*

Populations	TE	ChE	AChE	AE	CE
4th instar larvae					
Susceptible	*232.62±3.83[d]	2.84±0.03[d]	220.49±1.03[d]	92.98±0.36[d]	112.37±0.79[c]
Gujranwala	437.09±2.89[a]	3.70±0.01[a]	257.88±1.04[a]	133.43±0.18[b]	210.71±0.47[a]
Okara	365.37±3.35[b]	3.30±0.01[b]	225.30±1.04[b]	101.77±0.16[c]	175.14±0.74[b]
D.G. Khan	283.11±3.75[c]	3.08±0.06[c]	203.77±0.72[c]	140.20±0.17[a]	129.85±0.66[c]
6th instar larvae					
Susceptible	274.64±.46[d]	1.86±0.07[d]	124.64±0.54[d]	68.13±0.06[c]	108.10±0.26[d]
Gujranwala	361.34±0.35[a]	2.38±0.09[a]	179.63±0.32[a]	155.48±0.01[d]	176.80±0.73[a]
Okara	326.47±0.21[b]	2.24±0.01[b]	157.34±0.28[b]	84.68±0.04[a]	139.49±0.47[b]
D.G. Khan	366.53±1.53[c]	1.97±0.02[c]	138.27±0.42[c]	69.46±0.07[b]	121.50±0.33[c]
Adult beetles					
Susceptible	229.19±3.4[c]	8.32±0.13[c]	52.79±0.19[d]	24.86±0.92[d]	48.91±0.34[d]
Gujranwala	275.73±3.1[a]	11.07±0.08[a]	70.94±0.21[a]	81.04±0.92[a]	90.97±0.23[a]
Okara	265.12±3.14[ab]	9.88±0.130[b]	63.19±0.27[b]	72.68±1.35[b]	72.51±0.21[b]
D.G. Khan	256.27±1.98[b]	8.32±0.138[c]	57.16±0.17[c]	56.33±1.03[c]	64.58±0.27[c]

*Mean±SEM; n= 5 (Mean values in each single assay derived from five replicates and each replicate contains 20 beetles). Mean values with super script letters[a,b,c,d] within each column represents the significant differences among the means of various populations while mean values with common superscript letters indicates non-significant differences with others except susceptible population at P <0.05 according to Tukey's post hoc test.

and CE in deltamethrin-resistant populations were increased significantly as compared to deltamethrin-susceptible population at P<0.05 except the activity of ChE which are not significantly different in adult beetles of Gujranwala and susceptible populations. Similarly the TE activity in adult beetles of (D.G. Khan and Okara Population) and (Okara and Gujranwala populations) were not significantly different from each other at P<0.05. Different developmental stages (4th and 6th instar larvae and adult beetles) in all resistant populations possessed significantly different levels of esterases at P<0.05. Percent change in the activities of these esterases in deltamethrin-resistant populations with reference to susceptible population is presented in Figure 1.

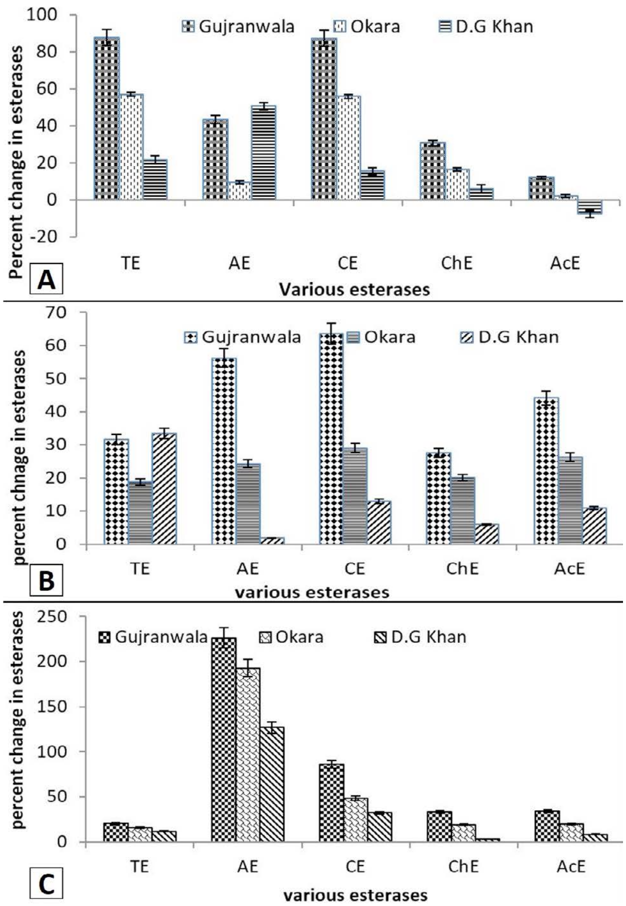

Fig. 1. Percent (%) change in various esterases of 4th instar (A), 6th instar (B) and adult beetles (C) of resistant population compared with susceptible population.

A decreasing trend was found in the activities of esterases from 4th instar larvae to 6th instar larvae and adult beetles except 6th instar larvae of D.G. Khan Population which possessed higher TE activity and 6th instar of Gujranwala Populations which possessed higher AE activity than 4th instar larvae. Similarly adult beetles of Gujranwala population exhibited higher ChE activity than 6th and 4th instar larvae.

Among resistant populations the 4th instar larvae and adult beetles of Gujranwala population possessed highest T.E activity (87.90 and 20.31%) while 4th instar larvae and adult beetles of DG Khan population possessed lowest T.E activity (21.70 and 11.82%), respectively as compared to 4th instar larvae and adult beetles of susceptible population. On other hand, 6th instar larvae of D.G. Khan population possessed highest TE activity (33.45%) and 6th instar larvae of Okara population possessed lowest TE activity (18.87%) as compared to 6th instar larvae of susceptible population. The 4th and 6th instar larvae and adult beetles of Gujranwala population possessed highest CE activity (87.51, 63.55 and 86.01%) among resistant populations and the 4th instar larvae of DG Khan population possessed lowest activity (15.55, 13.00 and 31.99%), respectively as compared to 4th, 6th instar larvae and adult beetles of susceptible population.

The AChE activity was also found significantly increased in all populations with respect to susceptible population except the 4th instar larvae of D.G. Khan population. Among resistant populations, 4th and 6th instar larvae and adult beetles of Gujranwala population possessed highest AChE (17.00, 44.13 and 34.93%) and ChE activities (36.68, 27.60 and 33.15%) while the 4th and 6th instar larvae and adult beetles of D.G. Khan population possessed lowest AChE (-7.58, 10.94 and 8.28%) and ChE activities (6.07, 6.02 and 0.02%), respectively as compared to 4th and 6th instar larvae and adult beetles of susceptible population. The 4th instar larvae of D.G. Khan population possessed highest AE activity (50.78) and the 4th instar larvae of Okara population possessed lowest AE activity (9.45) as compared to 4th instar larvae of susceptible population. The 6th instar larvae and adult beetles of Gujranwala populations possessed highest AE activity (56.17 and 226.32%) while the 6th instar larvae and adult beetles of D.G. Khan population possessed lowest activity (1.94 and 126.80%), respectively as compared to 6th instar larvae and adult beetles of susceptible population. On the basis of increased levels of activities of various esterases, the pest populations can be graded as Gujranwala > Okara > D.G. Khan > Susceptible.

DISCUSSION

The resistant populations of *T. granarium* have been collected from some stored grain godowns of the Punjab where deltamethrin has been applied on the grains prior to Phosphine application. The doses of the deltamethrin have not been calculated periodically according to level of pest resistance, so as a result of indiscriminate exposure of deltamethrin to *T. granarium* in the stored grain houses the pest has developed resistance against deltamethrin. The resistance can be measured using different resistance indicators like TE, ChE, AChE, AE, and CE activities. It was investigated that the level of all esterases tested was found significantly increased in 4th and 6th instar larvae and adult beetles of all deltamethrin-resistant populations as compared to susceptible population.

In the present study, the elevated levels of TE are in accordance to the findings of Sher *et al*. (2004) who reported that TE activity was increased in 4th instar larvae of *T. granarium* after 10 h exposure to Phosphine in Haroonabad population. Lewis and Medge (1984) investigated higher levels of TE in foliar spray resistant strains of aphid as compared to susceptible strains. Riaz *et al*. (2017) also reported increase in TE in various Phosphine tolerant populations of *T. granarium*. Cholinesterases and Acetylcholine esterase belongs to important group of enzymes that play key role in nervous system and involve in conduction of nerve impulse at neuromuscular junction (Ollis *et al*., 1992; Walsh *et al*., 2001). Due to increased ChE and AchE activities, the acetylcholine may efficiently be converted into choline and various systems of the pests may coordinate timely so insect gain protection against insecticide and develop resistance (Riaz *et al*. 2017). The increased activities of ChE and AChE in present study are in favour of the findings of Sher *et al*. (2004) who reported increased activity of ChE in 4th instar larvae of *T. granarium* after Phosphine exposure. Riaz *et al*. (2017) also reported increase in ChE and AChE in various Phosphine tolerant populations of *T. granarium*. It is also reported in literature that the role of AChE in development of resistance is correlated with the alteration in AChE binding sites in insecticide resistant pests which leads to the insensitivity of the enzyme to insecticides inhibition as studied by Siegfried and Scott (1992). Dvir *et al*. (2010) reported that there are two sub sites in active site of AChE named as esteratic and anionic. The esteratic sub site is responsible for catalytic process while anionic sub site is responsible for binding of choline. The esteratic sub site comprises of catalytic triad that consist of three amino acids as serine, histidine and glutamate. At this catalytic site, hydrolysis of acetylcholine into choline and acetic acid takes place (Soreq and Seidman, 2001). AChE insensitivity is well-known principal feature of resistant insects (Carpentier and Founeir, 2001). Karoly *et al*. (1996) reported that in resistant apple bud moths, the activity of AChE increased in each developmental stage when compared to susceptible population. Bourguet *et al*. (1996) studied that AChE gene duplication may causes overproduction of AChE and results in insecticidal resistance. Guedes *et al*. (1997) find out that higher level of AChE activity in resistant populations of *R. Dominica* were less sensitive to malaoxon inhibition than the susceptible populations. Levitin and Cohen (1998) also reported that enhanced levels of ChE activity in *Aonidiella aurantii* is due to organophosphate resistance. Zhu and Gao (1999) evaluated that resistance to organophosphates in green bugs was due to elevated levels of AChE. Similarly Charpentier and Fournier (2001) and Rumpet *et al*. (1997) also reported that increased level of ChE and AChE are responsible for development of resistance against insecticides.

Carboxyl esterases are enzymes that are involved in the hydrolysis of carboxylic esters into alcohol and free acid anion (Krisch, 1971; Junge and Krisch, 1975; Cygler *et al*., 1993; Satoh and Hosokawa, 2006; Hosokawa *et al*., 2007). The enzymes play important role in detoxification and metabolism of many compounds (Potter and Wadkins, 2006) including carbamates (Sogorb and Vilanova, 2002), organophosphates (Casida and Quistad, 2004) and pyrethroids (Stok *et al*., 2004). Byrne *et al*. (2000), Oakeshott *et al*. (2005) and Cui *et al*. (2007) investigated that elevated levels of CE are involved in the development of resistance to agrochemicals, fumigants and pesticides. Riaz *et al*. (2017) also reported an increase in CE in five Phosphine tolerant populations of *T. granarium*. Likewise all deltramethrin-resistant populations showed significant increase in AE activity. Zhu and He (2000) investigated that higher level of AE activity in *S. graminum* as compared to susceptible population. Riaz *et al*. (2017) reported increased AE activity in Phosphine tolerant populations of *T. granarium*. Sher *et al*. (2004) investigated that the level of AE activity in 4th instar larvae of *T. granarium* in Khanewal population was decreased after exposure to 0.8ppm of phosphine while elevated level of AE activity was noticed in Haroonabad population after their exposure to phosphine for 80 h. This suggests that longer periods of exposure to insecticides with their sub lethal doses leads to insecticide tolerance at first which later on results in insecticidal resistance.

In current study, it was also found that Adult beetles have significantly low TE, ChE, AChE, AE, and CE activity than 4th and 6th instar larvae. Nakakita and Winks (1981) and Riaz *et al*. (2017) reported that the level of esterase changed throughout the life cycle in different developmental stages as larvae are more tolerant to pesticides than adult beetles. Kim *et al*. (1988) suggest that it is the developmental stage of insect which determines the resistance or susceptibility of insect to particular insecticide.

ACKNOWLEDGEMENT

This research article is a part of PhD thesis of first author. FRS is grateful to University of the Punjab for providing funds for this work.

Ethical standard

This article does not contain any studies with human participants or animals performed by any of the authors.

Statement of conflict of interest

The authors AH, TR and FRS stated no conflicts of interest.

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