ESTERASE ISOZYMES DEVELOPMENT IN THE MID GUT OF THE GRASSHOPPER *SCHISTOCERCA GREGARIA*

FATMA EL ZAHRAA M. MEGAHED[1] AND EMTITHAL M. ABD-ELSAMIE[2]

[1]*National Center for Radiation Research and Technology (NCRRT) P.O.Box 29, Nasr City, Cairo, Egypt.*
[2]*Dept. of Entomol., Fac. of Science, Cairo University.*

INTRODUCTION

Locust swarms cause great damage to grassland, agricultural crops and various other plants. This damage in the invasion territories of the desert locust, *Schistocerca gregaria* (Orthoptrea: Acrididae) is not only confined to Africa, India or Asia Minor, but also affected other areas of the earth infested by other species of locusts. The desert locust is not a significant pest in its solitarious phase, in the recession areas, but gregarious phase becomes the major pest when forming swarms stimulated by the rain and improved vegetation (Nolte, 1974).

Esterases are hydrolyzing enzymes, which split ester compounds with the addition of water to yield alcohol and acids (Shaurub *et al.*, 1999). The aim of the present study was to investigate the electrophoretic variations of esterase isozymes in the gut of *Schistocerca gregaria*. This study may help in understanding the biochemical changes and the mode of development in different phases of this economic pest in Egypt.

MATERIAL AND METHODS

The stock colony of *Sch. gregaria* was maintained in the entomology laboratory, Faculty of Science, Cairo University according to Ibrahim (1971).

The tissue samples were taken from the mid gut of the 5th nymph instar and of male and female adults at three phases, *i.e.*, newly emerged, before oviposition and after oviposition of females, yellow and nonyellow males (mature and non mature).

The tissue samples were weighed, homogenized and then centrifuged at 6000 r.p.m. at 4°C. The supernatant was diluted 1:1 (vol:vol) with 5% sucrose solution in preparation of electrophoretic techniques.

Electrophoretic techniques: it was done on 1.5 mm vertical slab polyacrylamid gel (Sturdier slab gel electrophoresis unite SE 400) at 60 mA. until the tracking dye (bromophenol blue) migrated to the end of the gel. The running gel (6%) was made with 1.5 M tris buffer (pH 8.8), the stacking gel (4%) was made with 0.5 M tris buffer (pH 6.8). Both electrode chambers were filled with tris-glycine buffer (pH 8.3) (Turunen and Chippendale, 1977) and diluted 1:3 (vol/vol) with double distilled water. The temperature of the gel was maintained at 7°C be refrigerating the gel chambers during run.

After electrophoresis, the gel was soaked in 0.5 M borate buffer (pH 4.1) for 90 min at 4°C (Sims, 1965) and rinsed rapidly in two changes of double distilled water.

It was then stained for esterolytic activity by incubation at 25°C in a solution of 100 mg of α-naphthyl acetate (as substrate) and 100 mg fast blue RR salt (as diazo coupler) in 200 ml of 0.1 M phosphate buffer, pH 6.5 (Sell *et al.*, 1974). The α-naphthol, which was released on hydrolysis of the substrate, was coupled with the dye salt to produce an insoluble pigment at the site of enzyme activity. After incubation, the gel was destained in 7% acetic acid.

The effect of the known esterase inhibitor: eserine and paraoxon were examined on the tissue under investigation. Before being incubated with the substrate and coupler, the gels were placed in phosphate buffer (pH 6.5) containing inhibitor (10^{-4} M) for 30 min at 25°C.

Densitometric scanning was made to estimate the relative concentrations of the detected bands using Epson GT-8000 scanner at 570 wave length.

RESULTS AND DISCUSSION

Substrate specificity

The esterase bands or zones of esterolytic activity were detected using α-naphthyl acetate as a substrate. This substrate showed the presence of 30 bands in the mid gut of *Sch. Gregaria*. Their Rm values were shown in Table (1). Densitometric scanning of esterase bands revealed the relative concentrations of each band in a value of its percentage Table (1) Fig. (1).

79

TABLE (I)

Relative concentration of different esterase bands detected in the mid gut of nymph and adults of *Schistocerea gregaria* by using α- naphthyl acetate

Band No.	Rm value	% of different No. of esterase bands						
		Nymph	Male			Female		
			N_1	B_1	A_1	N_2	B_2	A_2
1	0.052	1	-	-	-	-	-	-
2	0.095	2	-	-	-	-	-	-
3	0.16	1	-	-	-	-	2	-
4	0.19	-	-	-	-	3	2	-
5	0.23	2	-	-	-	3	2	-
6	0.28	2	3	2	2	5	3	3
7	0.33	2	-	7	4	5	5	6
8	0.35	-	-	-	-	-	5	10
9	0.37	1	4	6	-	5	5	7
10	0.41	1	-	8	-	5	6	10
11	0.45	1	-	6	-	-	5	10
12	0.48	3	-	3	3	-	3	10
13	0.50	6	-	4	2	-	-	-
14	0.54	10	4	2	3	-	-	-
15	0.57	5	-	-	3	-	-	-
16	0.60	6	-	2	5	7	-	-
17	0.62	5	6	2	-	-	2	-
18	0.63	-	5	2	5	10	-	-
19	0.65	4	6	3	6	-	5	-
20	0.68	8	8	3	12	12	5	3
21	0.71	7	10	2	6	8	3	3
22	0.74	4	7	3	7	-	5	3
23	0.77	3	-	2	7	-	6	5
24	0.78	-	-	-	-	-	3	-
25	0.80	7	5	3	6	6	5	-
26	0.82	-	5	3	-	5	-	-
27	0.86	4	5	5	5	-	5	-
28	0.89	3	6	6	5	6	6	5
29	0.92	6	12	11	7	10	6	10
30	0.95	6	14	15	12	10	11	15

Rm: relative mobility
N_1: newly emerged males
B_1: males before yellow colour
A_1: males after yellow colour
N_2: newly emerged females.
B_2: females before oviposition
A_2: females after oviposition

According to Megahed (1996), enzyme bands that had a relative concentration of 15% or over were considered of relatively high concentration. Meanwhile, those having relative concentrations of 5% or below were considered of relatively low concentrations. The values between the two extremes (over 5% and below 15%) represented moderate concentrations. Table (1) and Fig. (1) show that α-naphthyl acetate was hydrolyzed by thirty bands (1-30).
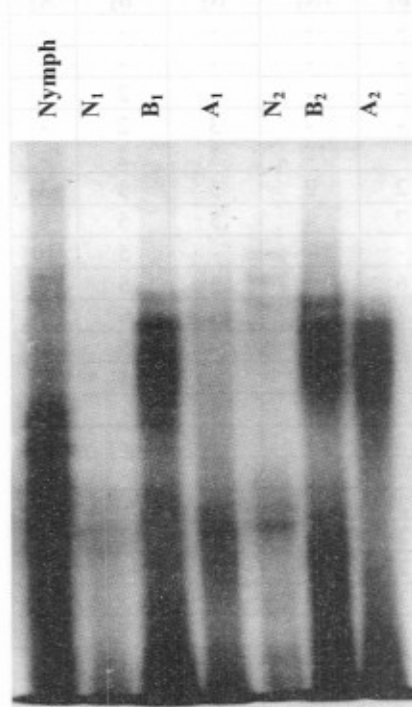

Fig. (1): Zymograph of esterase patterns in the mid gut of the nymph and adults of Sch. gregaria using α-naphthyl acetate as a substrate. Explanation of the abbreviations as in table 1.
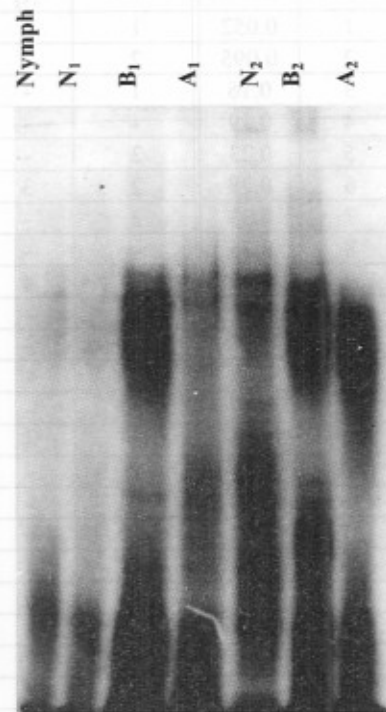
Fig. (2) : Zymograph of esterase patterns in the mid gut of the nymph and adults of Sch. gregaria using α-naphthyl acetate as a substrate and eserine as an inhibitor.

In the mid gut of the 5th nymph instar, 25 bands were detected. Only one band (No. 14) was highly detected.

In the newly emerged males, 15 bands were detected. Only three bands were detected at high concentration (Bands No. 21, 29 & 30).

α-naphthyl acetate was hydrolyzed by 22 bands in the non yellow young male locusts (before maturation). Only 2 bands were detected at high concentration (Bands No. 29 & 30).

TABLE (II)

Relative concentration of esterase bands detected in the mid gut of nymph and adults of *Sch. gregaria* by using α-naphthyl acetate and after inhibition of 2 enzyme inhibitors: eserine and paraoxon.

Band No.	% of different No. of esterases bands													
	Eserine							Paraoxan						
	Nymph	N_1	B_1	A_1	N_2	B_2	A_2	Nymph	N_1	B_1	A_1	N_2	B_2	A_2
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	4	4	-	-	11	-	-	-	-	-
4	-	-	-	-	-	-	-	3	-	-	-	-	-	-
5	-	-	-	-	2	-	-	5	-	-	-	-	-	-
6	7	6	14	10	9	13	8	4	-	-	-	-	-	-
7	4	4	9	5	3	-	-	-	-	-	-	3	-	-
8	4	4	9	-	3	10	19	-	-	-	-	-	-	-
9	4	-	7	4	4	8	-	-	-	-	-	-	-	-
10	-	-	4	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	3	-	-
12	-	-	-	-	-	-	10	-	-	-	-	3	-	-
13	-	-	-	-	-	-	-	9	-	-	-	8	-	-
14	-	-	-	-	8	-	-	20	-	4	-	13	5	4
15	-	2	3	7	5	-	-	11	-	-	-	6	4	3
16	-	3	3	5	6	4	-	10	-	-	-	-	-	-
17	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	6	-	4	-	7	4	-
19	-	-	3	4	5	4	-	-	-	-	-	-	-	-
20	3	4	4	4	-	6	-	4	-	-	-	4	6	-
21	-	-	4	3	6	6	7	-	12	4	-	4	6	5
22	5	4	6	4	12	5	8	3	-	4	-	-	6	7
23	6	4	15	-	5	6	-	-	-	-	-	-	-	-
24	9	-	-	-	-	-	-	-	-	7	-	3	-	6
25	15	19	-	-	7	7	12	-	-	6	-	4	6	5
26	-	-	-	-	5	-	-	-	-	-	-	-	-	5
27	10	13	-	29	-	9	10	4	15	9	21	8	11	7
28	8	10	11	12	4	3	10	3	17	20	26	11	8	12
29	8	10	-	-	3	3	4	5	18	15	24	8	16	20
30	17	17	8	13	9	12	12	13	27	27	29	15	28	22

N_1: newly emerged males B_1: males before yellow colour
A_1: males after yellow colour N_2: newly emerged females.
B_2: females before oviposition A_2: females after oviposition

In the yellow male adults (after maturation), 18 bands were hydrolyzed. Only two bands (No. 29 & 30) were highly detected.

On the other hand α-naphthyl acetate was hydrolyzed by 15 bands in the newly emerged females. Only 4 bands (No. 18, 20, 29 & 30) were highly detected.

Just before oviposition, α-napthyl acetate hydrolyzed 22 bands of the female locusts. Only one band (No. 30) was highly detected.

After oviposition, the female adults were hydrolyzed by α-naphthyl acetate and 14 bands were detected. Only six bands (No. 8,10,11,12, 29 & 30) were detected at high concentration.

Bands (No. 6, 20, 21, 28, 29 & 30) might be considered as the major esterase bands for the mid gut of *Sch. grigaria*, as they were commonly detected in all phases under investigation with different concentration. Most of these six major esterases were arylesterase. While the others were either cholinesterase or carboxyesterase according to the phase of insect Fig (1), Table (1).

TABLE (III)
Classification of non-specific esterases by inhibitors

Type of esterases	Inhibitors	
	Eserine	Paraoxon
Carboxyesterase	-	+
Cholinesterase	+	+
Arylesterase	-	-

(-): non-inhibition   (+): Inhibition

Kojima et al. (1970) demonstrated that the esterases exhibit a greater degree of polymorphism than enzymes involved in glucose metabolism or other metabolic path ways because they act on a class of molecules, many of which come directly from the external environment. Therefore, the substrates of esterases are qualitatively and quantitatively more variable than are the substrates of those enzymes involved in glucose metabolism.

Esterases exhibit wide divergences in the extent of their heterogeneity and characteristics of the multiple enzyme forms between even closely related species (Shaurab et al., 1999). This statement extends to our results where some esterase bands showed multiple enzymatic forms (Table 4). This occurrence may be correlated to the closely molecular weight of the bands or may be due to the band isozyme change of its function according to its phase.

TABLE (IV)

Response of non-specific esterases in the mid gut of nymph and adults of Sch. *gregaria* to specific inhibitors using α-naphthyl acetate as a substrate

Band no.	Nymph			Male N₁			Male B₁			Male A₁			Female N₂			Female B₂			Female A₂		
	Eserine	Paraoxon	Isozyme	Eserine	Paraoxon	Isozyme	Eserine	paraoxon	Isozyme	Eserine	paraoxon	Isozyme	Eserine	paraoxon	Isozyme	Eserine	paraoxon	Isozyme	Eserine	paraoxon	Isozyme
1	+	+	Choline																		
2	+	+	Choline																		
3	+	+	Choline		*								*			*	+	Carboxy	-	+	Carboxy
4		*											+	+	Choline	+	+	Choline	+	+	Choline
5	-	-	Aryl										-	+	Carboxy	+	+	Choline	-	+	Carboxy
6	-	-	Aryl	-	+	carboxy	-	+	Carboxy	-	+	Carboxy	-	+	Carboxy	-	+	Carboxy	+	+	Choline
7	-	-	Aryl				-	-	Carboxy	-	+	Carboxy	-	-	Aryl	+	+	Choline	+	+	Choline
8	*						*						*				+	Carboxy	+	+	Choline
9	-	-	Aryl	+	+	choline	-	+	Carboxy	*			-	+	Carboxy	-	+	Carboxy	-	-	Carboxy
10	+	+	Choline				-	+	carboxy				+	+	Choline	+	+	Choline			
11	+	+	Choline				+	+	Choline					*		+	+	Choline		*	
12	+	-	Choline				+	+	Choline	+	+	Choline		*		+	+	Choline		*	
13	-	-	Aryl				+	+	Choline	+	+	Choline		*							
14	-	-	Aryl	+	+	choline	+	+	Choline	+	+	Choline	*	*			*				
15	-	-	Aryl							-	+	Carboxy	*	*			*				
16	-	-	Aryl				-	+		-	+	Carboxy	-	+	Carboxy	*					
17	-	-	Choline	+	+	choline	+	+	Choline							+	+	Choline			

TABLE (IV) continued

Band no.	Nymph			Male									Female								
				N$_1$			B$_1$			A$_1$			N$_2$			B$_2$			A$_2$		
	Eserine	Paraoxon	Isozyme	Eserine	Paraoxon	Isozyme	Eserine	paraoxon	Isozyme	Eserine	paraoxon	Isozyme	Eserine	paraoxon	Isozyme	Eserine	paraoxon	Isozyme	Eserine	paraoxon	Isozyme
18		*		+	+	Choline	+	+	choline	+	+	choline	+	+	Choline		*				
19	+	+	Choline	+	+	Choline	-	+	Carboxy	-	+	Carboxy	*			-	+	Carboxy			
20	-	-	Aryl	-	+	carboxy	-	+	carboxy	-	+	Carboxy	+	+	Choline	-	-	Aryl	+	+	Choline
21	+	+	Choline	-	-	Aryles	-	-	Aryl	-	+	Carboxy	-	-	Aryk	-	-	Aryl	-	-	Aryl
22	-	-	Aryl	-	+	carboxy	-	-	Aryl	-	+	Carboxy				-	-	Aryl	-	-	Aryl
23	-	-	Aryl	*			-	+	carboxy	+	+	choline				-	+	Carboxy	+	+	Choline
24	*							*						*		+	+	Choline		*	
25	-	-	Aryl		+	carboxy	-	-	Aryl	+	+	choline	-	-	Aryl	-	-	Aryl	*	*	
26					+	Choline	+	+	choline				-	+	Carboxy					*	
27	-	-	Aryl		-	Aryk	-	-	Aryl	-	-	Aryl	*			-	-	Aryl	*	*	Aryl
28	-	-	Aryl		-	Aryl	-	-	Aryl	-	-	Aryl	-	-	Aryl	-	-	Aryl	-	-	Aryl
29	-	-	Aryl		-	Aryl	-	-	Aryl	-	-	Aryl	-	-	Aryl	-	-	Aryl	-	-	Aryl
30	-	-	Aryl		-	Aryl	-	-	Aryl	-	-	Aryl	-	-	aryl	-	-	Aryl	-	-	

Classification :

Type of esterases	Inhibitors	
	Eserine	Paraoxon
Carboxyesterase	-	+
Cholinesterase	+	+
Arylesterase	-	-

- An esterase band which was not detected by the substrate and activated by the inhibitor

N$_1$: newly emerged males	B$_1$: males before yellow colour	A$_1$: males after yellow colour
N$_2$: newly emerged females.	B$_2$: females before oviposition	A$_2$: females after oviposition

Inhibitor specificity

Using the classification in Table (3), we investigated the effects of inhibitors on esterases separated from the mid gut of *Sch. gregaria* at the present investigated phases Figs. (2 & 3).

Table (5) summarized the total number of esterase bands detected in the mid gut of *Sch. gregaria*. Three esterase enzymes (cholinesterase, carboxyesterase and arylesterase) were occurred.

Carboxyesterases are closely related to cholinesterases, since they hydrolyse awide range of aliphatic esters, including cholinesterase. They may be distinguished, however, by their resistance to 10^{-4} M eserine which completely inhibits cholinesterases.

In the present investigation, four bands of carboxyesterases were detected in the newly emerged males of the mid gut of *Sch. gregaria* which increased to eight bands before and after maturation. In spite of the absence of this esterase in the nymphal stage, the newly emerged females have detected five bands of carboxyesterase which increased just before oviposition to six bands and then decreased to three bands after oviposition.
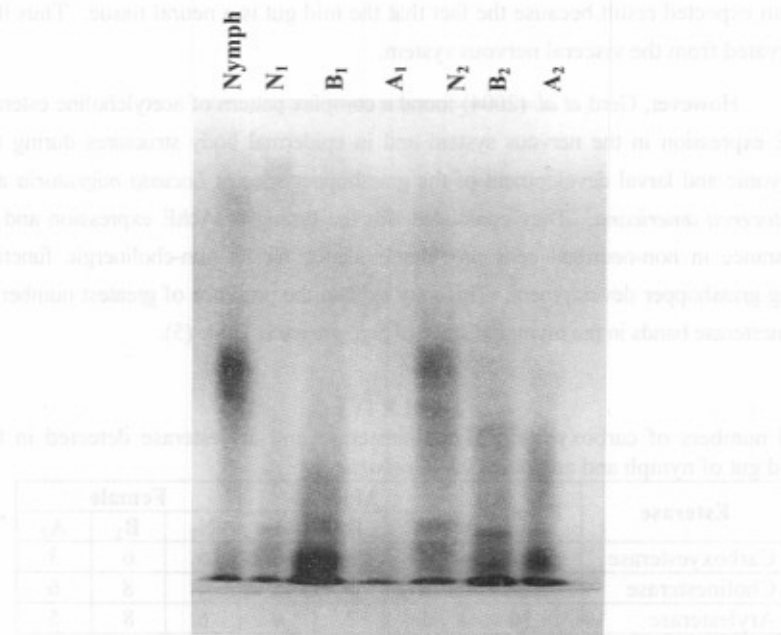

Fig. (3): Zymograph of esterase patterns in the mid gut of Sch. gregaria using α-naphthyl acetate as a substrate and paraoxon as an inhibitor. Explanation of the abbreviation as in table1.

From the previous results, it is concluded that carboxyesterase increased by increasing age in case of males while it was fluctuated in case of females *i.e.* it slightly increased before oviposition and then decreased after oviposition.

Megahed (1998) reported that carboxyesterase was increased by increasing age of female adults of *Culex pipiens*.

An elevated esterase-based mechanism is the major form of resistance in many pests (Graham and Janet 2000). They reported that widespread resistance to organophosphorus insecticides in *Nilaparvata lugens* is associated with elevation of carboxylesterase activity. The absence of carboxyesterase in nymphal stage of *Sch. gregaria* may be a suitable time to apply (use) organophosphorus insecticides.

The total number of cholinesterase bands detected in the mid gut of *Sch. gregaria* didn't increased by increasing age. Nine bands were detected in the nymph while it decreased to six bands in the newly emerged males then it increased to seven bands before maturation and returned to six bands after maturation. In case of newly emerged females, cholinesterase bands decreased to four bands. Then eight bands were detected just before maturation and decreased to six bands after maturation.

The occurrence of cholinesterase enzyme in the mid gut of *Sch. gregaria* was an expected result because the fact that the mid gut is a neural tissue. Thus it is innervated from the visceral nervous system.

However, Gerd *et al.* (2004) found a complex pattern of acetylcholine esterase AchE expression in the nervous system and in epidermal body structures during the embryonic and larval development of the grasshopper species *Locusta migratoria* and *Schistocerca americana*. They concluded that the timing of AchE expression and its appearance in non-neuronal cells provides evidence for its non-cholinergic function during grasshopper development. This may explain the presence of greatest number of cholinesterase bands in the mymphal stage of *Sch. gregaria* Table (5).

TABLE (V)

Band numbers of carboxyesterase, cholinesterase and arylesterase detected in the mid gut of nymph and adults of *Sch. Gregaria*

Esterase	Nymph	Male			Female		
		N_1	B_1	A_1	N_2	B_2	A_2
Carboxyesterase	Zero	4	8	8	5	6	3
Cholinesterase	9	6	7	6	4	8	6
Arylesterase	16	5	7	4	6	8	5

N_1: newly emerged males B_1 : males before yellow colour
A_1: males after yellow colour N_2 : newly emerged females.
B_2: females before oviposition A_2 : females after oviposition

Lie *et al.* (1984) reported that in the pupae of the corn borer, *Osterinia furnacolis*, there was difference in the zymograms detected between the different developmental stages and between the different sexes. This conclusion is in agreement with our results in the present investigation, as the isozymes detection was clearly fluctuated in the different developmental stages of the locust *Sch. gregaria*.

Also Krishnamurthy and Umakanth (1997) reported that esterases contributed to fecundity of the silk worm, Bombyx mori, as the activity of these enzymes decline in the females after oviposition. This conclusion agrees also with the present results, as the cholinesterase and arylesterase bands declined in the females of *Sch. gregaria* after oviposition.

One most interesting observation in the present work was the esterase bands which were activated by the enzyme inhibitor (Table 4). These esterase bands were capable of hydrolyzing α-naphthyl acetate only after addition of the eserine or paraoxone enzyme inhibitor. These esterase bands may be part of the detoxification mechanism of the insecticides used as inhibitors. A similar observation of esterase bands resistant to certain enzyme inhibitor was reported by Freyvoget *et al.* (1968), Edward *et al.* (1978) and Megahed (1998). This anomalous behaviour of esterase bands was explained by Mutero *et al.* (1994) in *Drosphila melanogaster* as mutations associated with the coding regions of the genes encoding these proteins (esterases).

SUMMARY

Mid gut esterase patterns, using polyacrylamid gel electrophoresis in grasshopper *Schistocerca gregaria* was studied in the 5th nymphal instar, male and female adults at three phases (newly emerged, before and after maturation). α-naphthyl acetate was used as substrate; meanwhile eserine and paraoxon were used as inhibitors.

Thirty esterase bands were capable of hydrolyzing α-naphthyl acetate.Three esterases (carboxyesterase, cholinesterase and arylesterase) were detected in the mid gut of *Sch. gregaria*.

Bands (No. 6, 20, 21, 28, 29 & 30) might be considered as the major esterase bands for the mid gut of *Sch. gregaria* as they were commonly detected in all phases under investigation. Most of these bands were arylesterase, while the others were either cholinesterase or carboxyesterase according to the phase of insect. The correlation between mid gut maturation and the occurrence of esterases was

discussed. Some esterase bands showed multiple enzymatic forms which may be due to the closely molecular weight of the bands or to the band isozyme change of its function according to its phase. Some esterase bands capable of hydrolyzing α-naphthyl acetate only after the addition of eserine and paraoxon enzyme inhibitor. These esterase bands may be part of the detoxification mechanism of the insecticides and function as inhibitors.

REFERENCES

AUGUSTINSSON, K.B. (1961): Multiple forms of esterases in vertebrate blood plasma *(Am. N.Y. Acad. Sci., 49:844-860)*.

BUSH, F.M.; J.R. PRICE and J.I. TOWNSEND (1970): Plasma esterases, their definitions and status as isozymes in the hous spasrow. *(Int. J. Biochem., 1:85-107)*.

EDWARD, J.H.; O.C. WILLIAM and L.H. JAMES (1978): Electrophoretic characterization of the nonspecific esterases of the Mosq. *Culex tarsalis*. Conventional and isoelectric focused acrylamide gels. *(Com. Biochem. Physiol., 6:291-295)*.

FREYVOGEL, T.A.; R.L. HUNTER and E.M. SMITH. (1968): Non-specific esterases of mosquitoes. *(J. Histochem. Cytochem., 16:765-790)*.

GRAHAM, J. and JANET, H. (2000): Molecular characterization of the amplified carboxylesterase gene associated with organophosphorus insecticide resistance in the brown planthopper, *Nilaparavata Lugens. (Insect molecular biology., 9 (6), 647-653)*.

GERD, B.; M. NAUJOCK and A. HAASE (2004): Cellular expression patterns of acetyle cholinesterase activity during grasshopper development. *(Cell tissue Res., 317:207-220)*.

IBRAHIM, S.I. (1971): Effect of different host plants on biocycle, fecundity and progeny of the desert locust. *Schistocerca gregaria* Forsk. *(Unpublished Ms. Sc. Thesis Cairo, Univ)*.

KOJIMA, J.; J. GILLESPIE and Y.N. TOBARI (1970): A profile of *Drosophila* species enzymes assayed by electrophoresis. I. Number of alleles, heterozygosites, and linkage disequilibrium in glucose-metabolizing system and some other enzymes. *(Biochemi. Genet., 4:627-637)*.

KRISHNAMURTHY, N.B. and R.S. UMAkANTH (1997): Contribution of esterase isozymes during mating in silkworm, *Bombyx mori* L. (Ind. J. Sericult., 36:11-16).

LI, Y.Y.; W.Q. ZHANG; X.Z. TANG. and H.X. LI (1984): The effect of Co^{60} gamma-ray irradiation on the esterase isozymes of pupae of the corn borer. *(Application of Atomic Energy Agriculture Yuanzing Nogye Yingyong, 3:44-48).*

MEGAHED, F.M. (1996): Effect of gamma radiation on the ultrastructure of the ovaries and protein and esterase patterns of female *Culex pipiens (Unpublished Ph. D. Thesis, Fac. Sci., Cairo Univ., Egypt, PP. 111).*

MEGAHED, F.M.; M.S. SALAMA, A.A. EL-MOURSY; H.M. SALEM; A.M. ABDEL-RAHMAN (1998): Effect of Gamma Irradiation on the Esterase patterns of female mosquito *Culex pipens. (Fourth Arab conference on the Peaceful uses of Atomic energy Tunis; 14-18/11/1998 AAFA).*

MUTERO, A.; M. PRALAVORIA, J.A. BRIDE AND D. FOURNIER (1994): Resistance associated point mutations in insecticide in sensitive acetylcholinesterase. *(Proc. Nati. Acad. Sci. USA, 91.:5922-5926).*

NOLT, D.J. (1974): Gregarization of locust. *(Biol. Rev. 49:1-14).*

SALAMA, M.S.; JR, L.P. SHOUEST and T.A. MILLER (1992): Effect of diet on the esterase patterns in the haemoloymph of the corn earworm and the tobacco budworm (Lepidoptera: Nocutuidae). *(J. Econ. Entomol., 45:1079-1087).*

SELL, D.K.; G.S. WHITT; R.L. METCLF and L.P.KANLEE (1974): Enzyme polymorphism in the corn earworm, *Heliothis zea* (Lepidoptera: Noctuidae) haemoloymph esterase polymorphism. *(Can. Entomol., 106:701-709).*

SHAURUB, E.H.; G.M. Ali and F.M.A.MEGAHED (1999): Esterase isozymes in the ovaries of gamma-irradiated cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). *(Bull. Ent. Soc. Egypt, Econ. Ser., 26: 109-122).*

SIMS, M. (1965): Methods for detection of enzymatic activity after electrophoresis on polyacrylamide gel in *Drospohila* species. *(Nature, 207:757-758).*

TURUNEN, S. and G.M. CHIPPENDALE (1977): Esterase and lipase activity in the mid gut of *Diatraea grandioeslla* digestive function and distribution. *(Insect Biochem., 7:67-71).*