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Biochemical studies of two forensically important insects in Egypt which had colonized rabbit carrions treated with organophosphorus compound

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ABSTRACT

The objective of this study is to elucidate the effect of Pirimiphos-methyl (Organophosphorus insecticide) on protein profile of insects that found in/above or around treated rabbit (*Oryctolagus cunicullus domesticus* L.) carrions. Additionally, an esterase analysis was performed to clarify the effect of this insecticide on necrophagous insects. Biochemical studies were carried out on collected adult *Dermestes maculatus* De-Geer and pupae of *Chrysomya albiceps* (Weidemann) for their forensic importance. Four protein fractions observed only in the treated beetles and one unique band for the control group. Treated *Chrysomya albiceps* pupae exhibited 14 specific bands whereas two bands were only exhibited by the control. Genetic distances calculated for treated and control insects were 0.38 and 0.42 for adult *Dermestes maculatus* and pupae of *Chrysomya albiceps*, respectively. Variations in protein bands may be interpreted that some pesticides enhance the transcription of certain sequences which are probably related to resistance and /or detoxification mechanisms.

Esterase pattern analysis using α-naphthyl acetate reflected 4 characteristic bands for treated beetles. However, using β-naphthyl acetate yielded 4 specific bands for the control group. For *Chrysomya albiceps pupae,* conduction of α- naphthyl acetate yielded one common band for both treated and control groups and one specific band for the control group. Whereas, using of β- naphthyl acetate substrate yielded two common bands shared by both groups and one specific band for the control group. Forensically important insects such as *Chrysomya albiceps* Weidemann and *Dermestes maculatus* De-Geer have an active esterase system.

Key words: *Dermestes maculates, Chrysomya albiceps,* esterase, Pirimiphos-methyl, organophosphate

INTRODUCTION

Drug-related deaths have been increased worldwide. In many parts of the world, however, the ease availability of Organophosphorus (OP) insecticides for agricultural purposes makes them the most important causes of poisoning by accidental exposure, suicide and sometimes, homicide, mostly in the developing countries (Güven *et al.*, 1997; Premaratna *et al.*, 2001; El-Karadawy *et al.*, 2006). Usually, victims are not discovered for many days to months and because of it, entomological techniques are useful in forensic analysis (Carvalho *et al.*, 2001). Deaths as a result of OP poisoning are usually detected by analysis of body fluids and tissues for the presence of the toxic agent. In the first few hours, a pathologist can provide a reasonable estimate of Post mortem interval (PMI) from the physical and histochemical consequences of death; which represent particular difficulties when performed on remains in an

advanced stage of decomposition (Gunatilake *et al.*, 1989). However, study of nechrophagous insects can be contributed to the qualitative identification of substances or abuse drugs. Recently, researches have shown that it is possible to detect chemical substances even in pupariae. (Carvalho *et al.*, 2001).

Nevertheless, electrophoresis is one of the best techniques for assessing the amount of variation within insects (Mohammed and Hafez, 2000). While study of individual proteins facilitates the investigation of the chemical nature and the physiological functions of each protein, measurements of the total proteins reveal the net result of protein biosynthesis and utilization. The changes of protein contents of the developmental stages of insects suggest their involvement in metabolism and probably reflect the balance between synthesis, storage, transport, degradation of structural and functional proteins during autogeny as well as a response to particular ecological and physiological conditions (Firling, 1977).

In addition, Polyacrylamide gel electrophoresis has been widely used to help in explanation of different biological processes that occur inside the living organisms (Sharaawi *et al.*, 2002). Specific esterase isozymes patterns were studied in several insects (Park *et al.*, 2002; Sharaawi *et al.*, 2002). Esterases are proteins that are defined by their ability to catalyze the hydrolysis of ester bonds within lipophilic compounds (El-Bermawy, 2004). Because most insecticides are esters of substituted phosphoric, carbamic, or cyclopropanecarboxylic acids, they are subjected to degradation by esterases (Devonshire, 1991). Therefore, esterases are the most significant enzymes for insecticide detoxification in insects. Organo-phosphates, carbamates, and pyrethroids contain carboxylester and phosphodiester bonds that are subjected to attack by esterase enzymes. These esterases can often be separated into isozymes with different substrate specificities. Insect esterases are very diverse and can include monomers, dimmers and multimers, which means that their relative molecular mass can cover a wide range (Mostafa *et al.*, 2003).

Pirimiphos-methyl is a cheap anticholinesterase OP pesticide that is widely used for agricultural purposes in the world and particularly in Africa to protect food against pests. Its acute and chronic toxicity had been well studied (Mantle *et al.*, 1997). We performed an early experiment as we investigated the succession and development of insects on remains of rabbits administered Pirimiphos-methyl via injection; the presence of dead flies, and beetles during the first 48 hours post-mortem of poisoned rabbits was interesting. *Chrysomya albiceps* (Weidemann), (Calliphoridae, Diptera) and *Dermestes maculatus* De-Geer (Dermestidae, Coleoptera) were considered of special forensic importance in Egypt (Tantawi *et al.*, 1996).

Insects can be used as alternative specimens for toxicological analysis when conventional post-mortem samples are not available (Bourel *et al.*, 2001). The main objective of this study is to elucidate the effect of OP on protein profile of two forensically important insects that found in/ above or around treated rabbit (with Pirimiphos-methyl) remains and to compare it with the other profile for control group. Additionally, an esterase analysis was performed to clarify the effect of this insecticide on these necrophagous insects.

MATERIALS AND METHODS

Insects specimens

Norish® pesticide (an-anticholin-esterase OP compound) was used in this experiment. Its constituents are as the following: Pirimiphos-methyl 50%, Sorbol

10%, odorless kerosene 40%). It was supplied in a liquid form from El-Fares Company, under registration # 757. This pesticide has a wide use in rural villages in Egypt for the control of walking pests (cockroaches, ants,….etc.). For agricultural use, it is always diluted: 2 ml: 1 liter distilled water. The active ingredient: pirimiphos-methyl has a concentration of 50%.

To elucidate the effect of OP on protein profile of insects that found in / above or around the rabbit remains, two male domestic rabbits (*Oryctolagus cunicullus domesticus* L.) weighting each approximately 1500 g were poison-killed by 10 ml pure dose of the pesticide shown above, directly injected to the apex of the heart. They are defined in this study as "treated" rabbits. These rabbits died within 5 min. after poison injection. Two other "control" rabbits were sacrificed by asphyxia. Within 10 min death, all rabbit carrions were immediately transferred into 4 separate wooden cages and placed 1 m apart in an uninhabited house under construction. The selected study area was visited at mid-day daily for the first two weeks and then once a week for the following four weeks. Approximately 25% of specimens were captured from each case. Alive insect specimens from both treated and control groups were collected and immediately killed by freezing (using domestic freezer at -20^0 C). Identifications of different insects were carried out at the Museum of Entomology Department, Faculty of Science, Ain Shams University, Cairo. Biochemical studies were performed on adult *Dermestes maculatus* (3-12 insects used) and pupae of *Chrysomya albiceps* (100-107 insects used) for their forensic importance. Beetle specimens processed were those fed on the carrions and sampled at 4^{th} and 8^{th} day post-mortem, whereas the dipterous pupae used for this biochemical study were those developed from the feeding maggots (5-6 days in age) and collected in pupal stage (1-2 days in age) at 8^{th} and 9^{th} day post-mortem.

Biochemical studies

Protein profile

Proteins were separated by SDS-PAGE according to the method of Davis (1964). Native-polyacrylamide Gel Electrophoresis: vertical bio-Rad gel electrophoresis apparatus was used in all proteins and isozymes run. Electrode and gel buffer were prepared according to Markert and Faulhaber (1965), while the polyacrylamide stock was prepared according to Bollag and Edelstein (1994).

Isozymes

Electrophoretic pattern of isozymes was studied in insect homogenate for both treated and untreated samples. Enzymes were extracted by grinding 0.5 g samples in one ml extraction buffer using a mortar and pestle. The extract was then transferred into clean Eppendorf tubes and centrifuged at 10,000 rpm for five minutes. Supernatants were transferred to new clean Eppendorf tubes and kept at -20^0 C until electrophoretic analysis.

α- and β – Esterases enzymes (α- and β – Est):

Separation of esterase patterns was achieved by polyacrylamide gel electrophoresis (PAGE) technique (Salama *et al*., 1992). After electrophoresis, the gel was soaked in 0.5 M borate buffer (pH: 4.1) for 90 min at 4^0 C. The gel then was rinsed rapidly into two changes of double distilled water. The gel was stained for esterolytic activity by incubation at 25^0 C in a solution of 100mg α- or β – naphthyl acetate (as a substrate) and 100 mg fast blue salt BN (as Diazo coupler) in 200 ml of 0.1 M phosphate buffer pH 6.5 (Sell *et al*., 1974). After incubation, the gel was destained in 7% acetic acid.

Gels were photographed, scanned and analysed using Gel Doc 2000 Bio-Rad system.

The similarity coefficient and genetic distance were calculated according to Nei and Li (1979).

S.I = 2Nxy / (Nx + Ny), G.d = 1- S.I

Where: S = similarity value, Nx and Ny are the number of bands in individuals x and y, Nxy is the number of shared bands. The value produced by this index ranges from zero, representing no bands sharing, to (1), representing complete identity while G. d is the genetic distance value.

RESULTS

SDS-Protein analysis: Protein fractions:

Electrophoretic analysis of proteins of control and treated *Dermestes maculatus* (Table 1) revealed 9 protein fractions. Four common bands (No. 1, 4, 8 and 9) were characteristic for the adult stage of this insect species; they were at 34.5, 20.7, 9.3, 6.0 kDa respectively. However, four additional protein fractions (No. 2, 3, 6, 7 at 28.8, 27.3, 15.3, 12.0 kDa) were observed in treated insects and were lacking in those fed upon the control corpses. Interestingly, one unique band (No. 5 at 18.9 kDa) was characteristic for the control insects. Similarity index for treated and control Dermestes sp was calculated as 0.62. Hence, the genetic distance reported was 0.38.

Table 1: Molecular weight (M.W), relative fragmentation (R.F), and amount (%) of protein profile of treated and control adults of *Dermestes maculatus* De Geer

Rows	Treated			Control		
	M.W (kDa)	R.F	%	M.W (kDa)	R.F	%
1*	34.5	0.64	0.39	34.5	0.64	40
2	28.8	0.71	9.8	-	-	-
3	27.3	0.72	8.78	-	-	-
4*	20.7	0.8	2.9	20.7	0.8	6.76
5	-	-	-	18.9	0.82	66.5
6	15.3	0.86	4.87	-	-	-
7	12	0.90	32.1	-	-	-
8*	9.3	0.93	34.8	9.3	0.93	30.07
9*	6	0.97	6.2	5.7	0.97	16.3

* Common bands

Protein profile of *Chrysomya albiceps* developing pupae in both control and treated groups were separated electrophoretically into 27 fractions (Fig. 1). Their molecular weights ranged from 7.2 to 83.9 kDa (Table 2).

Eleven bands (No. 1, 4, 8, 9, 12, 17, 22, 23, 24, 26, 27) were considered to be specific for *Chrysomya albiceps* pupal stages in both treated and control groups. However, the pupae from the treated group exhibited 14 unique bands (No. 2, 3, 5, 6, 7, 10, 11, 13, 14, 15, 16, 18, 19, 20) compared to two specific bands (No. 21, 25) in the control pupae. Treated and control similarity index (SI) was calculated as 0.58, their genetic distance was 0.42.

Table 2: Molecular weight (M.W), relative fragmentation (R.F), and amount (%) of protein profile of treated and control *Chrysomya albiceps* (Weidemann) developing pupae

Rows	Treated			Control		
	M.W (kDa)	R.F	%	M.W (kDa)	R.F	%
1*	83.9	0.08	1.06	83.6	0.083	13.91
2	79.9	0.12	3.68	-	-	-
3	69.7	0.24	5.40	-	-	-
4*	66.4	0.28	0.15	65.5	0.29	0.22
5	63.7	0.31	1.16	-	-	-
6	61.6	0.33	1.55	-	-	-
7	59.8	0.35	2.01	-	-	-
8*	58.6	0.37	2.13	57.1	0.38	18.69
9*	56.5	0.39	1.96	54.6	0.41	5.76
10	53.7	0.42	2.23	-	-	-
11	51.9	0.44	2.24	-	-	-
12*	48.6	0.48	3.65	48.3	0.48	8.26
13	44.1	0.53	3.85	-	-	-
14	42.3	0.55	4.60	-	-	-
15	39.3	0.59	5.17	-	-	-
16	37.5	0.61	6.12	-	-	-
17*	36.0	0.62	6.21	36.0	0.62	9.67
18	33.3	0.65	0.85	-	-	-
19	29.4	0.70	2.31	-	-	-
20	24.9	0.75	4.53	-	-	-
21	-	-	-	24.6	0.75	6.95
22*	20.7	0.80	5.46	20.7	0.80	11.52
23*	17.4	0.84	14.0	16.8	0.84	0.26
24*	16.2	0.85	14.02	15.0	0.86	12.93
25	-	-	-	12.9	0.89	1.73
26*	10.5	0.91	2.56	10.5	0.91	3.38
27*	7.2	0.95	3.58	7.2	0.95	6.63

* Common bands

Enzyme assay:

Eleven electrophoretic bands were yielded upon treatment of *Dermestes* samples with α- naphthyl acetate. Two bands were common for

both groups, indicating that they are characteristic for adult *Dermestes maculatus* De-Geer. The first common band (0.081) had RF densities of 0.41 and 0.89% for treated and control groups, respectively. The other common band (0.11) had higher densities of 12.78 and 67.36% for the same groups, respectively. However, the treated beetles had exhibited 4 different specific bands representing densities of 1.55, 2.93, 2.11, and 80.2%. On the other hand, the control group yielded other different 4 electrophoretic bands. These characteristic bands which were absent in the treated group represented densities of 4.32, 3.03, 0.63, 7.62, and 15.95%.

Table 3: Relative fragmentation (R.F) and density (%) of Esterase enzyme bands of *Dermestes maculatus* De-Geer detected by α –and β – naphthyl acetate as substrates

	α –naphthyl acetate				β –naphthyl acetate			
	Treated		Control		Treated		Control	
Rows	R.F	%	R.F	%	R.F	%	R.F	%
r1	0.081	0.41	0.081	0.89	0.052	14.27	0.055	9.11
r2	0.091	1.55	-	-	0.062	10.42	0.065	10.2
r3	0.11	12.78	0.11	67.36	0.1	12.78	0.1	7.45
r4	0.16	2.93	-	-	0.14	62.56	0.14	35.66
r5	0.18	2.11	-	-	-	-	0.4	5.15
r6	0.21	80.20	-	-	-	-	0.43	5.81
r7	-	-	0.45	4.32	-	-	0.47	4.04
r8	-	-	0.48	3.03	-	-	0.49	22.45
r9	-	-	0.5	0.63	-	-	-	-
r10	-	-	0.54	7.62	-	-	-	-
r11	-	-	0.57	15.95	-	-	-	-

Treatment with β-naphthyl acetate as a substrate yielded 4 common bands for both groups (0.052, 0.062, 0.1, 0.14), which arech ara cteristic to the adult stage of this species. Their densities were 14.27, 10.42, 12.78, 62.56 and 9.11, 10.20, 7.45, 35.66 for the treated and the control groups, respectively. Interestingly, the control group exhibited extra 4 bands which were absent in the treated groups. Their R.F densities were 5.11, 5.81, 4.04, and 22.45%.

The esterase patterns in *Chrysomya albiceps Weidemann* pupae after treatment with α- and β- naphthyl aceteate were illustrated in Figs. 2 and 3, respectively. Analysis of which was shown in Table 4.

Table 4: Relative fragmentation (R.F) and density (%) of Esterase enzyme bands of *Chrysomya albiceps* (Weidemann) pupae detected by α - and β- naphthyl acetate as substrates

Rows	α - naphthyl acetate				β- naphthyl acetate			
	Treated		Control		Treated		Control	
	R.F	%	R.F	%	R.F	%	R.F	%
r1	0.15	100	0.14	0.49	-	-	0.081	12.1
r2	-	-	0.35	99.5	0.13	12.4	0.13	0.30
r3	-	-	-	-	0.4	87.6	0.4	87.5

Conduction of α- naphthyl acetate as a substrate yielded one common band (0.15), which is characteristic to *Chrysomya albiceps Weidemann* pupae. Its density was 100% in the treated group and 0.5% in the control group. Additional band (0.35) with a density of 99.5% was produced by the control samples, and was lacking in the treated pupae. Two characteristic bands (0.13 and 0.4) were observed upon treatment with β-naphthyl acetate substrate. The first band density was 12.4 and 0.3 % for the treated and control groups respectively while the second common band exhibited density of 87.6 and 87.5% for the same groups, respectively. Like treatment with α-naphthyl acetate, one specific band was also observed in the control group (0.081). Its density was 12.1%. This band was absent in the treated group.

DISCUSSION

Four protein fractions were detected only in the treated beetles whereas they were absent in the control ones. However, one unique band was absent in the treated beetles profile. This figure was similar to the *Chrysomya* pupae (resulting from the feeding maggots) profile as well, since pupae from the treated group exhibited 14 unique bands whereas two bands exhibited by control ones were lacking in the treated pupae profile. Variations in protein bands, however, may be explained on the basis that some pesticides enhance the transcription of certain sequences which are probably

related to resistance and /or detoxification mechanisms (Salam *et al*., 1984). Data of this study suggest that the elevated level of DNA in the cell could transfer its message through RNA and protein synthesis. In other words, the appearance of extra bands due to the treatment with an insecticide indicates that resulting proteins are probably responsible for the detoxification of the insecticide. These bands are present in untreated individuals in a hidden form or are probably activated by the addition of insecticide (Mohammed and Hafez, 2000). Indeed, our previous experiment (Abd El-Bar and Sawaby, under publishing) revealed that this Pirimiphos-methyl was toxic to all insects that came to feed upon treated rabbit carrions in the first 48 hours post-mortem. Moreover, in the following days post-death, significantly lesser numbers of immature stages were developing on these treated rabbits, comparing to the other figure of control rabbits.

Interestingly, the genetic distance between the treated and control *Chrysomya* sp. pupae reached 0.42, reflecting the great effect of the OP on the developing progeny. Several reports indicated that some pesticides can produce important side effects including genetic damage to microorganisms, plants and animals (Mohammed and Hafez, 2000). The group of OP pesticides which is the most important group of pesticide is known to react with DNA, generally as alkylating agents and consequently, it is a potential mutagen and/or carcinogen. Early studies (e.g. Mohammed and Hafez, 2000) revealed that some organophosphorus and carbamate compounds proved to be mutagenic agents that may be represented either by the disappearance of the electrophoretic banding patterns in some individuals or the presence of extra bands in others. In other words, this means activation of other genes to produce these types of protein. Any unusual increase or decrease in the activities of the enzymes in the progeny of treated parents might be interpreted on the molecular level, to depression or mutation of the regulating genes responsible for biosynthesis, of polypeptide chains building these enzymes (El-Bermawy, 1994). However, the pupae tested were those maggots which could survive upon the treated rabbit remains and could develop to normal pupae. We therefore suggest that changes in the banding pattern in certain progenies may be attributed to three alternatives: i.e. selection, genetic drift, or mutational events (El- Bermawy, 2005).

Resistance to OP may arise due to inheritance of one or more of 3 main classes of enzymes which are ableto bind and metabolise insecticides (Conyers *et al*., 1998). The microsomal monooxygenases or mixed function oxidases (MFOs), glutathione *S*-transferases (GSTs), and hydrolases (more specifically esterases). Esterases represent a group of highly variable and multifunctional hydrolytic enzymes. In arthropods, these enzymes are involved in various physiological activities such as regulation of juvenile hormone levels, digestive processes, reproductive behavior, functioning of the nervous system and resistance to pesticides (Baffi *et al*., 2008).

Most OPI are irreversible inhibitors of acetylcholinesterase (AChE), a key enzyme that is characteristic of the neuromuscular junctions responsible for the hydrolysis of the neurotransmitter acetylcholine (ACh). ACh, a major neurotransmitter in both vertebrate and invertebrate nervous systems interacts with receptors on target cells and elicits a variety of post-synaptic responses. Insect AChE is the primary target of OPI, which by phosphorylating the serine hydroxyl group located in the active site of the AChE, inhibits its activity. As a consequence, ACh accumulation in the synapses and neuromuscular junctions leads to over

stimulation of cholinergic receptors that result in a general pattern of nerve poisoning, hyper excitability, tremors and paralysis and causes neurotoxic effects (Park *et al*., 2002, Jadhav and Rajini, 2009).

Our results suggest variation in esterase activity between treated and control groups for both tested insect species. Many more cases of esterase mediated OP resistance have been reported for example in mosquito, *Culex tarsalis* (Whyard *et al*., 1995). Increased enzyme activity however, can be either due to a more catalytically efficient enzyme able to hydrolyse insecticides, or to higher amounts of enzyme which protect the AChE by offering a large number of alternative sites of phosphorylation, and therefore reduce the amount of OP available to bind to AChE. (Conyers *et al*., 1998; Jian-Rong Gao, 2006).

Model substrates such as naphthyl esters are commonly used in preference to insecticidal esters, which are normally more difficult to use as assaying agents (Srinivas *et al*., 2004). However, the observation of reduction in the staining density of some original bands with simultaneous appearance of new EST bands, exhibited in the present work by treated beetles and blowfly pupae using different substrates, was noticed also by Perrotey *et al.* (2002), during blood digestion in the colonized population of sandflies *Phlebotomus duboscqi*,

Recently, forensic entomologists have introduced a procedure using insects as a silence witness interpreting information concerning death. The present data indicate that forensically important insects such as *Chrysomya albiceps* Weidemann and *Dermestes maculatus* De-Geer have an active esterase system. This conclusion highlights the importance of using different entomological techniques, such as protein profiles and esterase assays which are involved in physiological and endogenous metabolism functions, for identifying drugs in necrophagous insects that colonized drug-deaths suspecting corpses. Furthermore, in cases where the death is rather old, the only abundant materials are insect remains and particularly pupariae cases, which are often preserved for a long time. Other techniques such as radioimmunoassay method can be used, furthermore, following enzymatic hydrolysis to measure concentration of certain drug that found within nechrophagous insect (remain), (Bourel *et al*., 2001).

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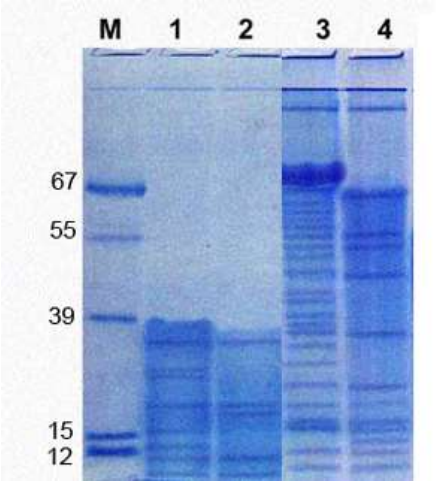

Fig.(1). Protein profile of treated and control *Dermestes maculates* De Geer adults and *Chrysomya albiceps* (Weidemann) developing pupae
M: Marker
Lane 1: Treated adults of *Dermestes maculatus*
Lane 2: Control adults of *Dermestes maculatus*
Lane 3: Treated pupae of *Chrysomya albiceps*
Lane 4: Control pupae of *Chrysomya albiceps*

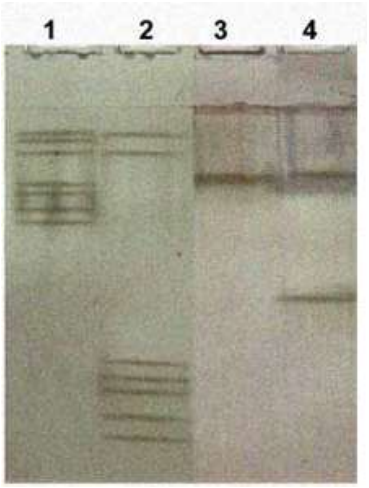

Fig. (2). Electrophoretic patterns of treated and control *Dermestes maculates* De Geer adults and *Chrysomya albiceps* (Weidemann) developing pupae using α-naphthyl as a substrate
Lane 1: Treated adults of *Dermestes maculatus*
Lane 2: Control adults of *Dermestes maculatus*
Lane 3: Treated pupae of *Chrysomya albiceps*
Lane 4: Control pupae of *Chrysomya albiceps*

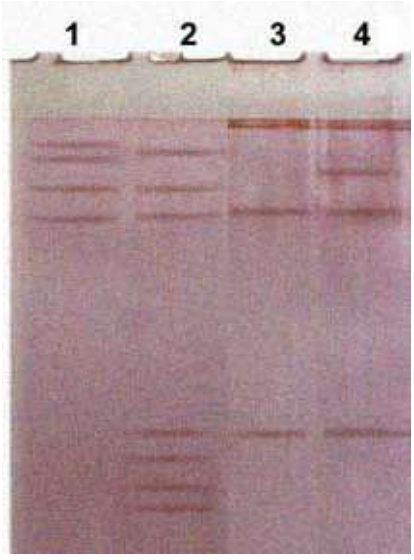

Fig. (3). Electrophoretic patterns of treated and control *Dermestes maculates* De Geer adults and *Chrysomya albiceps* (Weidemann) developing pupae using β-naphthyl as a substrate
Lane 1: Treated adults of *Dermestes maculatus*
Lane 2: Control adults of *Dermestes maculatus*
Lane3: Treated pupae of *Chrysomya albiceps*
Lane 4: Control pupae of *Chrysomya albiceps*

ARABIC SUMMARY

دراسات بيوكيميائية لحشرتين ذاتا أهمية في الطب الشرعي في مصر و اللاتى تغذيا على جيف أرانب عولجوا بمركب عضوي فوسفوري

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استهدف هذا البحث دراسة تأثير احدى مركبات المبيدات الفوسفورية (بيريميفوس ميثيل) على أنماط البروتينات و خمائر الاستيريز الخاصة بحشرات لها أهمية في الطب الشرعي و ذلك بعد تجميعها من(أو حول) جيف أرانب حقنت بهذا السم (مجموعة معاملة). أجريت الدراسة على الخنافس البالغة ديرمستس ماكيولاتس دي جير و عذارى ذبابة كريزوميا ألبيسبس (وايدمان). أظهرت الدراسة أربع شرائط لبروتينات خاصة فقط بديرمستس ماكيولاتس التي تغذت على جيف الأرانب المعاملة وواحدة فقط خاصة بديرمستس ماكيولاتس المتغذية على مجموعة الأرانب الضابطة. بالمثل، عذارى ذبابة كريزوميا ألبيسبس(المعاملة) أظهرت ١٤ شريط بروتين مميزة لها. أما المجموعة الضابطة فظهر فيها شريطين مميزين. كان التباين الوراثي ٠,٣٨ بين المجموعة المعاملة و الأخرى الضابطة في الخنافس البالغة ديرمستس ماكيولاتس، و سجل ب ٠,٤٢ بين المجموعتين في عذارى ذبابة كريزوميا ألبيسبس.كما فسرت الاختلافات في شرائط البروتينات على أساس أن بعض المبيدات قد تحفز التعبير عن بعض متتابعات الأحماض النووية مما له علاقة بظهور المقاومة في بعض الحشرات. وقد تم دراسة خمائر الاستيريز التى أظهرت٤ حزم مميزة في المجموعة المعاملة للخنافس البالغة ديرمستس ماكيولاتس و ذلك باستخدام ألفانفثيل أما استخدام بيتا نفثيل فقد كشف عن٤ حزم أخرى مختلفة مميزة للمجموعة الضابطة.وكذلك استخدام ألفانفثيل أظهر وجود شريط واحد مشترك ما بين المجموعة المعاملة و الأخرى الضابطة في عذارى ذبابة كريزوميا ألبيسبس و شريط واحد مميز للمجموعة الضابطة أما استخدام بيتا نفثيل فقد نتج عنه ظهور شريطان مشتركان للمجموعتين المعاملة و الضابطة في عذارى الذباب وشريط واحد مميز للمجموعة الضابطة . أثبت البحث وجود نظام انزيمي نشط في حشرات لها أهمية في الطب الشرعي.