BIOCHEMICAL VARIATION IN THE LIFE STAGES AND POPULATIONS OF *CHRYSOPERLA CARNEA* (STEPHENS) (NEUROPTERA :CHRYSOPIDAE)

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Abstract

Total protein and esterase activity were determined in eggs, larvae (1st, 2nd and 3rd instars) and cocoons of *Chrysoperla carnea* (Stephens) (Laboratory culture). There was a positive correlation between the total protein level and acetyl cholinesterase (AchE) activity. The gradual increase in (AchE) activity in larval stage of *Ch. carnea* are compatible with the larval age. The greatest increase in esterase activity was found in the third larval instar (9.3 mM/mg proteins).

Protein and esterase levels in the second larval instar (release stage) of *Ch. carnea* were determined to measure the biochemical variations in the collected populations of *Ch. carnea* from the fields using pesticides intensively (Menofia and Fayum) and from free pesticide fields (Abu Simbel and Baharia Oasis) comparing with Laboratory culture. Significant differences in change percentage of protein levels between the collected populations of *Ch. carnea* from the fields using pesticides and from free pesticides. The highest values of protein level (58.4 and 60.0 mg/ml) and esterase activity (7.1 and 7.2 mM/mg proteins) in the 2nd larval instar were obtained in the collected populations from Menofia and Fayum, respectively. The lowest values of protein level (53.2 and 56.5 mg/ml) and esterase activity (6.0 and 6.5 mM/mg proteins) were found in the collected populations from free pesticide fields, Abu Simbel and Baharia Oasis, respectively.

Electrophoresis data showed that the protein profile of the 2nd larval instar of *Ch. carnea* for the different populations were separated into 25 different bands with molecular weight ranged from 184437 to 12816 KDa.

Electrophoresis data of esterase patterns showed that the bands of the 2nd larval instar of Laboratory culture has only four bands , while the field populations, Abu Simbel, Baharia Oasis, Menofia and Fayum have 6, 9, 6 and 8 bands, respectively. The conformable estrease profile within the collected populations of *Ch. carnea* from the fields using pesticides intensively and collected populations from free pesticide fields was not detected.

The differentiation between the collected populations of *Ch. carnea* from fields using pesticides intensively (Menofia and Fayum) and collected populations from free pesticide fields (Abu Simbel and Baharia Oasis) was not possible with protein or esterase profiles.

INTRODUCTION

Vegetable crops growing in fields and greenhouses are heavily attacked with several insect pests and diseases. Severe attacks of these pests and diseases cause considerable loss and reduce the yield.

The intensive use of pesticides caused unexpected side effects. Judicious application of chemical products to minimize the risk of adverse effects is integral with the principles of integrated pest management (IPM). An ideal insecticide should be toxic to pests but not to predators and parasites (Ishaaya and Caside, 1981).

The main objective in IPM is increasing the role of biological control components under this system. The maintenance of biological components has proven difficult in practice where pesticide must be applied, and the beneficial species are often more sensitive than target species (Croft and Brown 1975). Classical biological control and inundation control programs require application high selective chemical control agents and also knowledge about their side effects on biological control agents to be combined within them.

Integrated pest management (IPM) was developed as a consequence of the incompatibility of pesticides with biological control. The common green lacewing, *Chrysoperla carnea* (Stephens) appears to be a good candidate for use in IPM programs Information on the toxicities of various pesticides using on vegetable crops in greenhouse to the *Ch. carnea* is therefore important in selection of compounds that will minimize mortality of this predator. While *Ch. carnea* has been shown to have greater tolerance to many pesticides, a blanket statement of lacewing tolerance to insecticide residues may not be appropriate.

First, insecticide tolerance varies geographically among *Ch. carnea* populations (Grafton-Cardwell & Hoy, 1985 and Zaki et al., 1999)

Second, there are differences between closely related Chrysopid genera and even within genera (Rumpf et al., 1997).

Numerous researchers have shown *Ch. carnea* has a relatively broad tolerance to many insecticides, particularly during the larval stage (Grafton-Cardwell & Hoy, 1985 and Mizell & Schiflhauer, 1990). Ishaaya and Casida (1981) came to the common green lacewing, *Ch. carnea* have a remarkable natural tolerance to pyrethroids. A portion of this tolerance is attributable to detoxification by pyrethroid esterase. Grafton-Cardwell and Hoy (1986),indicated that both enzyme systems (mixed function oxidases and hydrolyzing esterases) might be involved in detoxification of carbaryl by the resistance strain of *Ch. carnea* larvae. Patel et al., (1996) found that The activity of acetylcholinesterase (AchE) in resistant strain of *Ch. scelestes* was double that of

the susceptible strain. Rumpf et al., (1997) proved that the inhibition of acetylcholinesterase (AChE) in lacewings is a useful tool to study the impact of different organophosphates used in integrated pest management, in contrast, the change in whole body glutathione-s- transferase (GST) activity. Furthermore, the effect of some insecticides on acetylcholinesterase from *Coccinella septempunctata*, *Ch. carnea* and *Forficula auricularia* was tested by Bozsik et al., (2002). They found that the susceptibility of these beneficial insect AChE differed highly from each other.

The present work aimed to study the following points:

1-Determine the protein and esterase levels in the life stages of *Ch. Carnea* (Stephens). Data of this nature would be a necessary prerequisite for any future studies concerning the side effects of some insecticides on this predator and probably explain the susceptibility of different stages of *Ch. carnea* to some insecticides.

2- Biochemical changes in the second larval instar (release stage) of *Ch. carnea* in the populations of Fayum, Menofia, Baharia Oasis and Abu Simbel comparing with Laboratory culture through determining the protein & esterase levels and electrophoreses analysis of proteins and esterase enzymes. This contribution gives a complementary technique to distinguish between the different *Ch. carnea* populations in relation its susceptibility to pesticides. Moreover, this technique may be considered as a faster and more accurate in comparison with the conventional biological evaluation.

MATERIALS AND METHODS

Five populations of *Chrysoperla carnea* were used in this test. Two populations of *Ch. carnea* were collected from two locations free from pesticide application at March 2005. About 100 lacewing adults were collected from clover fields in El-Baharia Oasis and another 100 adults were collected from wheat fields in Abu Simbel. The other two populations of *Ch. carnea* were collected during July 2005 from cotton and pepper fields of Fayum and Menofia Governorates, which representative locations of intensive using of pesticides. The collected lacewings were reared in laboratory until there were sufficient numbers for testing. The 5th population of *Ch. carnea* that rearing since ten years in "*Chrysoperla* mass rearing unit" Faculty of Agriculture, Cairo University was used as Laboratory susceptible culture to candidate pesticides in the experiments comparing with the other four populations. T test (Sokal and Rohlf, 1973) was used for statistical analysis of the protein level and esterase activity in the second larval instar of *Ch. carnea* for the populations of Abu Simbel, Baharia Oasis, Menofia and Fayum comparing with the Laboratory culture.

Rearing of *Chrysoperla carnea*

The predatory insects were reared according to described method of El Arnaouty *et al.* (2007).

Sample preparation

Eggs, the three larval instars and cocoons of *Ch. carnea* (Laboratory culture) and the 2nd larval instar of Abu Simbel, Baharia Oasis, Menofia and Fayum populations, collected from wheat, clover, pepper and cotton, respectively, were used to determine the total proteins and esterase activity. Each sample (0.25 g) was homogenized with 1 ml phosphate buffer (pH: 7) in an Eppendorf homogenization. The homogenate was centrifuged at 10'000 rpm for 10 minutes at 10°C and resulting supernatants were held in ice until use.

Protein determination

Protein determination was conducted according to the method of Gornall (1949) by using Biuret reaction. The kit consists of two bottles of Biuret reagent, one bottle of comparison reagent (concentrate) and a vial of standard protein solution (60 g/I).

Reagent 1

-Potassium iodide 30 M mol / l -Potassium sodium tartrate 100 M mol / l

-Copper sulphate 10 M mol / l -Sodium hydroxide 3.8 mol / l

Reagent 2

The reagent's volume for protein determination was mentioned in the following Table:

	Blank	Standard	Sample
Reagent	1 ml	1 ml	1 ml
Distilled water	10 µl	---	---
Standard	---	10 µl	---
Sample	---	---	10 µl

After 10 minutes, the Optical Density of the samples and standard were read against of blank using spectrophotometer at 546 nm. The protein concentration was calculated according to the following equation:

$$\text{Protein concentration} = \frac{A_{sample}}{A_{standard}} \times C_{standard} = C \text{ sample}$$

A= Absorbance, C = Concentration

Esterase determination

Total esterases were determined according to the method of Gomori (1953).

Reagents

0.2 M Phosphate buffer (pH: 7).

Substrate solution: 1- Naphthyl acetate (0.25 m M) was prepared by dissolving 30 mM of 1- Naphthyl acetate in 1 ml acetone and 60 ml phosphate buffer, then adding Fast Blue B (0.3%).

Equal amount of protein (50 µg) of each sample was incubated with 1- Naphthyl acetate (0.25 m M) at 25 °C for 30 min. Fast blue B salt (0.3%) in 3.5% aqueous sodium dodecyl sulphate (0.5 ml) was added. The mixture was incubated for 15 min at 25 °C and the resulting color was measured at 605 nm spectrophotometrically by using JEN WAY, GENOVA spectrophotometer.

Gel electrophoresis analysis

-Acrylamide-Bisacrylamide (30:0.8 w/w) were dissolved in 100 ml of double distilled water

- Stacking gel buffer (Tris-HCl, 0.5 nmol/l pH 6.8): 6 g Tris-HCl was dissolved in 40 ml double distilled water and by using 1 N HCl adjust the pH to 6.8, then complete the volume with double distilled water to 100 ml.

- Resolving buffer (Tris-HCl 3 mol/l, pH 8.8): 36.3 g Tris-HCl was dissolved in 48 ml 1N HCl, adjust the pH value to 8.8, then complete the solution to 100 ml with double distilled water.

- Ammonium persulfate solution (1.5% W/V): 0.15 g Ammonium persulfate was dissolved in 10 ml double distilled water.

- SDS Solution (10% W/V): 10 g Sodium dodecylsulfat dissolved in 100 ml double distilled water.

- Running buffer (Stock solution: Tris base 0.25 mol/l, Glycin 1,92 mol.l, SDS 1%): 30.3 g Tris base, 144 g glycin and 10 g SDS were dissolved in double distilled water, then complete the volum to 1 l.

- Sample buffer: The sample buffer consists of stacking gel buffer, 10 % SDS, beta mercaptoethanol, glycerin and bromophenol blue at the ratio of (12 : 25 : 10 : 12 : 1), respectively.

1- Protein detection

According to the described method by Laemmli (1970) the slab SDS-Polyacrylamide gel (10%) was used to separate the proteins extracted from the second larval instar of *Ch. carnea* for Abu Simbel, Baharia Oasis, Menofia and Fayum populations. Equal amount of protein samples (40 µg) was mixed at ratio of 1:1 (v/v) with sample buffer contain 0.1% bromophenol blue as marker loaded in each well of the gel. The gel was run at constant 100V for 1 h. then at a constant 200V for 3 h. Proteins were fixed and stained over night using Commassie Blue until getting a clear background. The gel was immersed in the conserving solution for about 15 min. and let it drying in solifan at room temperature.

An image of the dried gel was done by using an Epson scanner GT 9500 then transferred into an image analyzer Phoretix 1D Quantifier to integrate the gel data.

2- Esterase detection

The described method by Devonshire (1975) was used to determine the esterase separated on native polyacrylamide gel. Discontinuous native polyacrylamide gel electrophoresis was performed in a 20 X 20 cm vertical unit, using a 7.5% acrylamide gel with a continuous Tris/glycine buffer system. Samples of each pooled replicates were mixed at ratio of 1:1 (v/v) with sample buffer contains 0.1% bromophenol blue as a marker. Individual wells were loaded with equal amounts of protein (150 μg) of each sample. Gels were run at a constant 100V for 1 h. then at 200V for 3 h. After the end of the run the gels were stained for esterase activity at 25°C for 90 min. The stain was prepared by adding 30mM 1-Naphthyl acetate in acetone (1 ml) to 0.2% (w/v) Fast blue B salt in 0.2M phosphate buffer, pH 6.0 (50 ml). The reaction was stopped by immersing the gel into 7% acetic acid. Then, it was transferred in conserving buffer for about 15 min. and let it drying in solifan at room temperature.

An image of the dried gel was carried out using an Epson scanner GT 9500 then transferred into an image analyzer Phoretix 1D Quantifier to integrate the gel data.

RESULTS AND DISCUSSION

1- Protein and esterase levels in the life stages of *Chrysoperla carnea*

The present work determines the total proteins and esterase activity in eggs, larvae (1^{st}, 2^{nd} and 3^{rd} instars) and cocoons of *Ch. carnea* (Laboratory culture).

The levels of protein and esterase in the different stages of *Ch. carnea* (Laboratory culture) are shown in Table (1). There are a positive correlation between the total proteins and esterase activity. It seems that the esterase activity affected by increasing the total proteins. The total proteins were 22.2, 37.0, 43.0, 53.5 and 41.0 mg/ml in eggs, 1^{st} , 2^{nd}, 3^{rd} larval instars and cocoons, while the corresponding esterase activity were 2.9, 4.0, 5.4, 9.3 and 5.5 mM/h./mg protein, respectively. It was found that if total protein concentration increases progressively, the esterase activity increases up to a maximum protein concentration (53.5 mg/ml).

On the basis of esterase activity, the descending order of the life stages of *Ch. carnea* was the 3^{rd} larval instar, cocoons, the 2^{nd} larval instar, the 1^{st} larval instar and then eggs. The gradually increase in esterase activity in larval stage of *Ch. carnea* are computable with the larval age. The greatest increase in esterase activity was found in the third larval instar (9.3 mM/h./mg protein), while the lowest level was showed in egg stage (2.9 mM/h./mg protein).

2- Protein and esterase levels in the different collected populations of *Ch. carnea*

The present work determined the protein level and esterase activity in the second larval instar of *Ch. carnea* for the populations of Abu Simbel, Baharia Oasis, Menofia and Fayum comparing with the Laboratory culture as shown in Table (2). It was conducted to measure the biochemical variations in the collected populations of *Ch. carnea* from the fields using pesticides intensively (Menofia and Fayum) and from free pesticide fields (Abu Simbel & Baharia Oasis) comparing with the Laboratory culture and their correlation with the susceptibility to the tested pesticides

The highest values of protein levels in the 2nd larval instar were obtained in the collected populations from the fields using pesticides intensively (Menofia and Fayum), which were 58.4 and 60.0 mg/ml with change percentage 40.1 and 43.9% comparing with the Laboratory culture, respectively. In contrary, the lowest values were in the collected populations from free pesticide fields (Abu Simbel & Baharia Oasis), which were 53.2 and 56.5 mg/ml with change percentage 27.6 and 35.5% comparing with the Laboratory culture, respectively. There are significant differences in change percentage of protein levels between the collected populations of *Ch. carnea* from the fields using pesticides intensively and collected populations from free pesticide fields. There are no significant differences in protein levels between the populations of Abu Simbel & Baharia Oasis. The same trend was also found in Menofia and Fayum populations.

Also, it was found that there is a positive correlation between the total proteins and esterase activity in the second larval instar of different populations. Accordingly, the highest protein levels in the collected populations of *Ch. carnea* from the fields using pesticides intensively (Menofia and Fayum) gave highly esterase activity in the second larval instar than those in the collected populations from free pesticide fields (Abu Simbel & Baharia Oasis). The esterase activity in the second larval instar of Menofia and Fayum was 7.1 and 7.2 mM/h./mg protein with change percentage 34.0 and 35.8% comparing with laboratory culture, while it was 6.0 and 6.5 mM/h./mg protein with change percentage 13.2 and 22.6% in Abu Simbel and Baharia Oasis populations, respectively. Also, it was found no significant differences in change percentage of esterase activity between the two collected populations from free pesticide fields (Abu Simbel and Baharia Oasis) and also between the two collected populations from the fields using pesticides intensively (Menofia and Fayum). In contrary, there are significant differences in change percentage of esterase activity between the collected populations of*Ch. carnea* from the fields using pesticides intensively and collected populations from free pesticide fields.

Table 1. Protein and esterase levels in the different stages of Chrysoperla carnea (Laboratory culture).

Stage	Total proteins (mg/ml)	Esterase activity (mM/mg protein)
Egg	22.2 ± 0.4	2.9 ± 0.10
Larva (1st instar)	37.0 ± 0.5	4.0 ± 0.09
Larva (2nd instar)	43.0 ± 0.8	5.4 0.05
Larva (3rd instar)	53.5 ± 0.0	9.3 ± 0.2
Cocoon	41.0 ± 0.5	5.5 ± 0.3

Table 2. Protein and esterase levels in the second instar larvae of the different populations of Chrysoperla carnea.

Populations	Total proteins		Esterase activity	
	(mg/ml)	Change (%)	(mM/mg protein)	Change (%)
Abu Sembil	53.2 ±1.80	27.6*	6.0 ± 0.08	13.2**
Baharia Oasis	56.5 ± 2.00	35.5*	6.5 ± 0.20	22.6**
Menofia	58.4 ± 0.87	40.1**	7.1 ± 0.08	34.0***
Fayum	60.0 ± 2.10	43.9**	7.2 ± 0.03	35.8***
Laboratory	41.7 ± 2.70	---	5.3 ± 0.10	---

* Significant at the level 5%.

** Highly significant at the level 1%.

*** Very highly significant at the level 0.1%.

3- Protein profile in the different populations of *Ch. carnea*

Electrophoresis data showed that the protein profile of the second larval instar of *Ch. carnea* for the different populations were separated into 25 different bands (Fig.1) with molecular weight ranged from 184437 to 12816 KDa (Table 3). The protein bands (13) with R_f values, 0.063, 0.117, 0.141, 0.184, 0.236, 0.288, 0.338, 0.378, 0.439, 0.488, 0.559, 0.587 and 0.860 representing molecular weights 184437, 155222, 144828, 127624, 109162, 92992, 80111, 70877, 59229, 50967, 41193, 37726 and 16401 KDa, respectively, were appeared in the Laboratory culture and the populations of Abu Simbel, Baharia Oasis,

Menofia and Fayum. The protein levels in the previously bands were differed in the field populations comparing with the Laboratory culture (reference) of *Ch. carnea*. The protein levels in bands with R_f values, 0.063, 0.141, 0.184, 0.236, 0.378, 0.559, 0.587 and 0.860 of the 2nd larval instar for Abu Simbel population were less than those in Laboratory culture. In contrary, the protein levels in bands with R_f values, 0.117, 0.288, 0.338, 0.439 and 0.488 were more than those in laboratory culture. In case of Baharia Oasis population, the protein levels were less than those in Laboratory culture except in the protein bands with R_f values, 0.141, 0.184, 0.288 and 0.559 were more than those in Laboratory culture. The most of protein levels in Menofia population were less than those in laboratory culture except in two protein bands have R_f values, 0.236 and 0.338 were slightly more than those in laboratory culture. The protein levels in bands with R_f values, 0.184, 0.378, 0,439, 0.488, 0.587 and 0.860 of the 2nd larval

instar for Fayum population were less than those in Laboratory culture. In contrary, the protein levels in bands with R_f values, 0.063, 0.117, 0.141, 0.236, 0.288, 0.338 and 0.559 were more than those in Laboratory culture. On basis of the protein levels in the different populations comparing with the Laboratory culture, the populations were ordered descending as Fayum, Abu Simbel, Baharia Oasis and Menofia. It was found that the protein bands with R_f values, 0.378, 0.587 and 0.860 in Laboratory culture were more than those in all field populations. The total bands of protein in the tested populations of laboratory, Abu Simbel, Baharia Oasis, Menofia and Fayum were 21, 20, 20, 22 and 21, respectively.

The protein bands, which distinguished by the R_f values, 0.212, 0.305, 0.616, 0.693 and 0.742 were disappeared in Abu Simbel population comparing with the Laboratory culture of *Ch. carnea*. The corresponding values of R_f for protein bands of Fayum population were 0.305, 0.616 and 0.805. One protein band was disappeared in Baharia Oasis and Menofia populations with R_f values, 0.253 and 0.909, respectively There are four new protein bands with R_f values, 0.074, 0.525, 0.833 and 0.941 and molecular weight, 177740, 45589, 17739 and 12816 KDa, respectively, were appeared in the electrophoresis pattern of Abu Simbel population comparing with the Laboratory culture. Fayum population has three new protein bands with the same R_f values (0.074, 0.525 and 0.941) of those obtained in Abu Simbel population. The same results were obtained in case of Menofia population but one new protein band only with R_f value 0.941 and molecular weight 12816 KDa was found. There is no new protein bands were appeared in the Baharia Oasis.

The differentiation between the collected populations of *Ch. carnea* from the fields using pesticides intensively (Menofia and Fayum) and collected populations from free pesticide fields (Abu Simbel & Baharia Oasis) was not possible with protein profile.

4- Esterase profile in the different populations of *Ch. carnea*

Data in Table (4) shows the R_f values and the percentage of esterase bands in the second larval instar of field populations, Abu-Simbel, Baharia Oasis, Menofia and Fayum comparing with the Laboratory culture. Electrophoresis data showed that the esterase bands of the 2nd larval instar of Laboratory culture (Fig.2) has only four bands with R_f values, 0.334, 0.354, 0.400 and 0.593, while the field populations, Abu Simbel, Baharia Oasis, Menofia and Fayum have 6, 9, 6 and 8, respectively. There is one esterase band with R_f value 0.334 was found in all field populations and also in Laboratory culture. The percentage content of this esterase band was decreased in all field populations than those in laboratory culture. The esterase band having R_f value 0.354 was increased to 112.74 and 168.76% in the populations of Abu-Simbel and Menofia comparing with the laboratory culture. Also, the esterase band with R_f value 0.593 was increased to 134.74% in Menofia population only.

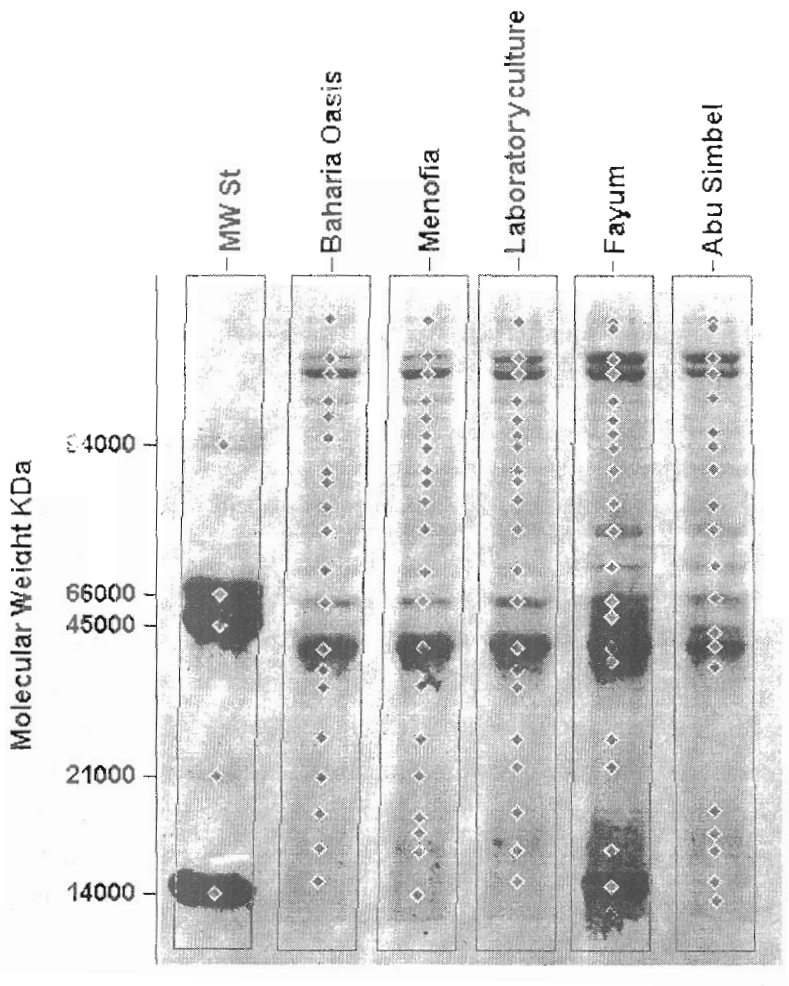

Fig. 1. Protein patterns of the second larval instar of *Ch. carnea* in the populations of Laboratory, Abu-Simbel, Baharia Oasis, Menofia and Fayum after separation on SDS-polyacrylamide gel (10%) electrophoresis.

Table 3. Molecular weight (MW), rate of flow (Rf) and the percentage of band content of electrophoretically separated proteins of the second larval instar of Ch. carnea for the field populations of Abu-Simbel, Baharia Oasis, Menofia and Fayum compared to Laboratory culture.

No.	MW	R_f	Laboratory culture		Abu Simbel		Baharia Oasis		Menofia		Fayum	
			Normal-ization	Band (%)	Normal-ization	Band (%)	Normal-ization	Band (%)	Normal-ization	Band (%)	Normal-ization	Band (%)
1	184437	0.063	100	1.99	91.61	2.17	96.92	2.32	96	2.05	116.13	2.21
2	177740	0.074	---	---	N	1.49	---	---	---	---	N	1.11
3	155222	0.117	100	4.04	124.33	5.98	81.2	3.95	71.87	3.11	142.52	5.49
4	144828	0.141	100	4.79	94.02	5.36	101.9	5.87	84.79	4.35	136.16	6.22
5	127624	0.184	100	2.89	76.35	2.62	101.17	3.52	97.43	3.01	99.75	2.75
6	117872	0.212	100	1.98	---	---	139.13	3.31	120.1	2.54	168.79	3.18
7	109162	0.236	100	2.89	86.62	2.98	97.03	3.38	102.06	3.16	103.89	2.86
8	103414	0.253	100	2.19	91.53	2.39	---	---	83.95	1.97	114.25	2.39
9	92992	0.288	100	3.09	142.32	5.24	115.72	4.31	77.18	2.56	169.63	5.01
10	88555	0.305	100	1.43	---	---	103.35	1.79	113.43	1.74	---	---
11	80111	0.338	100	4.88	100.24	5.82	99.07	5.82	102.21	5.34	105.35	4.9
12	70877	0.378	100	7.16	81.11	6.92	88.72	7.65	89.43	6.86	82.92	5.67
13	59229	0.439	100	3.83	109.58	5.0	76.72	3.54	85.68	3.52	78.8	2.88
14	50967	0.488	100	5.54	107.67	7.1	87.77	5.85	83.59	4.96	89.94	4.75
15	45589	0.525	---	---	N	4.3	---	---	---	---	N	5.37
16	41193	0.559	100	10.31	76.63	9.41	102.61	12.75	98.16	10.84	106.16	10.45
17	37726	0.587	100	4.58	87.15	4.76	84.82	4.68	83.02	4.08	98.72	4.32
18	34515	0.616	100	5.24	---	---	57.97	3.66	51.8	2.91	---	---
19	27240	0.693	100	3.92	---	---	110.88	5.23	68.05	2.85	166.53	6.22
20	23433	0.742	100	3.93	---	---	110.33	5.22	89.1	3.75	119.97	4.5
21	19386	0.805	100	5.45	63.57	4.13	49.45	3.25	80.6	4.71	---	---
22	17739	0.833	---	---	N	6.91	---	---	N	3.14	---	---
23	16401	0.860	100	11.85	39.73	5.61	46.23	6.6	62.99	7.99	46.75	5.29
24	14161	0.909	100	8.03	69.7	6.66	75.34	7.29	---	---	159.02	12.18
25	12816	0.941	---	---	N	5.16	---	---	N	14.57	N	2.25
Total bands			21		20		20		22		21	

N = New band ---= Not detecte

Three esterase bands conformable with laboratory culture bands were appeared in Abu-Simbel population with R_f values, 0.334, 0.354 and 0.400, in Baharia Oasis population (R_f values, 0.334, 0.400 and 0.593) and in Menofia population (R_f values, 0.334, 0.354 and 0.593). In case of Fayum population, there are only two esterase bands with R_f values, 0.334 and 0.593 were conformed with laboratory culture.

There are three new esterase bands were appeared in the electrophoresis pattern of Abu-Simbel population having R_f values, 0.052, 0.407 and 0.435 and also in Menofia population with R_f values, 0.052, 0.380 and 0.407. It was also found 6 new esterase bands in Baharia Oasis (R_f values, 0.262, 0.295, 0.380, 0.435, 0.522 and 549) and Fayum (R_f values, 0.052, 0.380, 0.407, 0.435, 549 and 0.639) populations. The electrophoresis pattern of Menofia and Fayum populations (collected from intensively using pesticide fields) were conformed in three new esterase bands having R_f values, 0.052, 0.380 and 0.407 but one and two of them were found in Baharia Oasis and Abu Simbel populations (collected from free pesticide fields), respectively. On the other hand, one new esterase band with R_f value 0.435 was conformed in the both populations of Abu-Simbel and Baharia Oasis but the same band was found in Fayum population. One new esterase band having the R_f value 0.639 was only appeared in the electrophoresis pattern of Fayum. It can be concluded that the differentiation was not possible between the collected populations of *Ch. carnea* from the fields using pesticide intensively (Menofia and Fayum) and collected populations from free pesticide fields (Abu Simbel & Baharia Oasis) with estreases profile. On the other hand, the conformable estreases profile within the collected populations of *Ch. carnea* from the fields using pesticides intensively and collected populations from free pesticide fields was not detected.

The determination of protein levels and esterase activity in the different stages of *Ch. carnea* (Laboratory culture) indicated that the esterase activity is affected by increasing the total proteins. Also, the gradual increase in esterase activity in the larval stage of *Ch. carnea* are compatible with the larval instar. These results agree with those of Call *et al.*, (1977) who found that the third instar of the corn root worm, *Diabrotica virgifera* (Lecont) had considerably higher cholinesterase specific activity levels than the second larval instar. Ishaaya and Casida (1981) reported that the larger larvae of lacewing, *Ch. carnea* have more pyrethroid esterases than those in the smaller larvae. The same finding was obtained by Kandil *et al.* (1983) who stated that the 23-day old larvae of confused flour beetle, *Tribolium confusum* (DUV.) had higher cholinesterase specific activity than those of 15-day larvae. To the contrary, Abdallah *et al.* (1973) found that the larval stage of cotton leaf worm, *Spodoptera littoralis* (Boisd.), was characterized by a very high initial cholinesterase activity in 2-day old larvae, followed by a steady decrease until the termination of the larval period.

Table 4. Rate of flow (R_f) and the percentage of band content of electrophoretically separated esterases of the second larval instar of *Ch. carnea* for the field populations of Abu-Simbel, Baharia Oasis, Menofia and Fayum compared to Laboratory culture.

No.	R_f	Laboratory culture		Abu Simbel		Baharia Oasis		Menofia		Fayum	
		Normal-ization	Band (%)	Normal-ization	Band (%)	Normal-ization	Band (%)	Normal-ization	Band (%)	Normal-ization	Band (%)
1	0.052	---	---	N	19.4	---	---	N	9.17	N	13.36
2	0.262	---	---	---	---	N	10.39	---	---	---	---
3	0.295	---	---	---	---	N	6.44	---	---	---	---
4	0.334	100	28.02	59.92	18.88	46.78	8.33	70.18	14.45	97.7	18.18
5	0.354	100	13.93	112.74	17.65	---	---	168.76	17.27	---	---
6	0.380	---	---	---	---	N	8.11	N	7.63	N	10.57
7	0.400	100	28.42	40.87	13.06	99.69	18.01	---	---	---	---
8	0.407	---	---	N	18.08	---	---	N	22.14	N	12.8
9	0.435	---	---	N	12.93	N	9.23	---	---	N	10.02
10	0.522	---	---	---	---	N	9.02	---	---	---	---
11	0.549	---	---	---	---	N	14.53	---	---	N	10.69
12	0.593	100	29.63	---	---	84.57	15.93	134.74	34.29	88.19	17.35
13	0.639	---	---	---	---	---	---	---	---	N	7.03
Total bands		4		6		9		6		8	

N = New band ---= Not detected

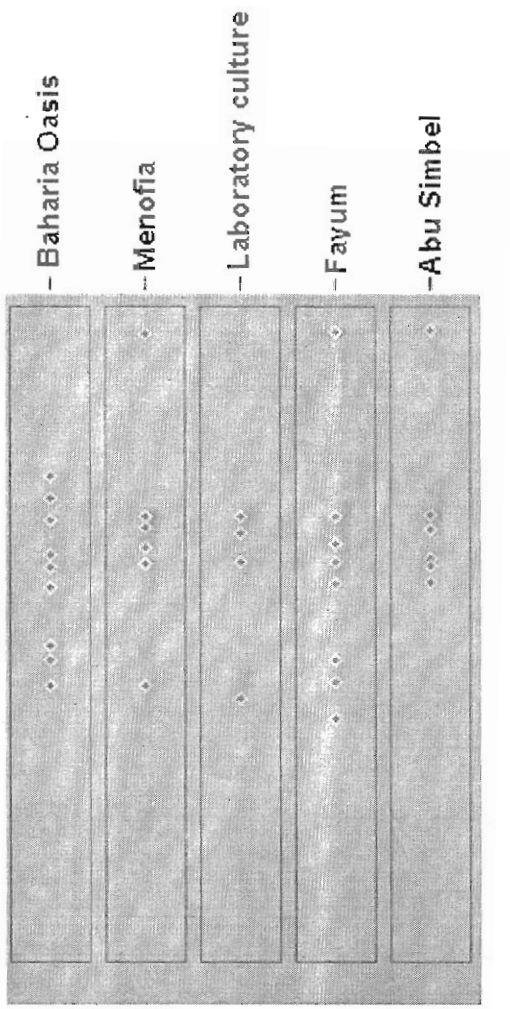

Fig. 2. Esterase patterns of the second larval instar of *Ch. carnea* in the populations of Laboratory, Abu-Simbel, Baharia Oasis, Menofia and Fayum after separation olyacrylamide gel (7.5%) electrophoresis.

Badawy *et al.*, (2006) confirmed that 3-day old eggs were more tolerant to the tested pesticides than the second larval instar of *Ch. carnea*. Also, Grafton-Cardwell and Hoy (1985) stated that the eggs and pupae are the most resistant stages and larvae are more tolerant to pesticides than adults of *Ch. carnea*. To the contrary, the data in Table (1) presented that the esterase activity in egg stage (2.9 mM/mg protein) was less than those in the second larval instar (5.4 mM /mg protein). So, the esterase activity may be, of no impact on eggs tolerance to pesticides application.

This is the first documentation about variance of pesticide tolerance in different populations of *Ch. carnea* in Egypt. The results in the present study confirmed that the collected *Ch. carnea* populations from fields using pesticides intensively (Fayum and Menofia) were more tolerant or resistant to pesticides than those collected from free pesticide fields (Baharia Oasis and Abu Simbel) and of course laboratory culture. Accordingly, the biochemical variations in these field populations were studied through determining the protein & esterase levels and electrophoreses analysis of proteins and esterase enzymes. It was found that the esterase activity in the second larval instar of populations collected from Menofia and Fayum was more than those in the collected populations from free pesticide fields (Abu Simbel & Baharia Oasis). This remarkable difference in esterase activity between the two groups may lead to tolerance or resistance of the collected *Ch. carnea* populations from fields using pesticides intensively more than the collected populations from free pesticide fields. Also this finding suggests that there are gradually developing tolerance to the pesticides in Fayum and Menofia populations due to a high esterase activity. These results agree with Plapp and Bull (1978), who noticed that the toxicity data are interpreted as indicating that lacewing larvae may have high activity of esterase acting on pyrethroids and other insecticides. Also, Ishaaya and Casida (1981) reported that the *Ch. carnea* larvae have an unusually active estereases that contributes to their natural tolerance.

Consequently, the susceptibility and tolerance of populations to pesticides could not only be differentiated on the basis of biological evaluation, but also by their esterase activity using spectrophotometer method.

Although the populations collected from the fields using pesticides intensively have protein levels and esterase activity more than those in the collected populations from free pesticide fields but the differentiation between the two groups was not possible by the electrophoretic patterns of proteins and esterases. The conformable profiles of proteins and esterases within the collected populations of *Ch. carnea* from the fields using pesticides intensively and collected populations from free pesticide fields was not detected.

High esterase activity is important but probably not the only factor in tolerance of *Ch. carnea* larvae. Although not examined specifically, other factors such as mixed-function oxidase activity, slow penetration, and a relatively insensitive nerve target could theoretically combine with the esterases in making up the complement of *Ch. carnea* defenses against insecticides.

Finally, this contribution gives a complementary technique to distinguish between the different *Ch. carnea* populations in relation its susceptibility to pesticides. Moreover, this technique may be considered as a faster and more accurate in comparison with the conventional biological evaluation.

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الاختلافات البيوكيميائية في أطوار الحياة وعشائر مفترس أسد المن

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تم تقدير مستوى البروتين الكلى ونشاط الأستريز في البيض، الأعمار اليرقية الثلاثة والشرانق المحتوية على العذارى لمفترس أسد المن. و يمكن الرجوع إلي نتائج هذه الدراسة في الدراسات المعنية بالتأثير الجانبي للمبيدات الحشرية على المفترس أسد المن والتي يمكن بها تفسير حساسية الأطوار المختلفة للمفترس لبعض المبيدات الحشرية.

أظهرت النتائج وجود علاقة طردية بين مستوى البروتين الكلى ونشاط الأستريز. كما أوضحت نتائج نشاط الأستريز أنه بزيادة العمر اليرقى لمفترس أسد المن فإن نشاط الأستريز يزداد تدريجياً. ووجد أن أعلى زيادة في نشاط الأستريز في العمر اليرقى الثالث (٩,٣ مل مول / ملجم بروتين).

تم تقدير مستوى كل من البروتين والأستريز ليرقات العمر الثاني لمفترس أسد المن وذلك لقياس التغيرات البيوكيميائية في العشائر المجموعة من مناطق يستخدم فيها المبيدات بصورة مكثفة (المنوفية – الفيوم) وأخرى محظور فيها استخدام المبيدات (أبو سمبل – الواحات البحرية) ومقارنتهم بالحشرات المرباة مختبرياً. وأظهرت نسب التغير لمستويات البروتين اختلافات معنوية بين العشائر التي جمعت من المناطق التي تستخدم المبيدات وتلك التي جمعت من مناطق محظور فيها استخدام المبيدات. وكانت اعلي مستويات للبروتين ٥٨,٤ و ٦٠ ملجم/ملل وبلغ اعلي نشاط للأستريز ٧,١ و ٧,٢ مل مول/ ملجم بروتين في يرقات العمر الثاني والتي تم جمعها من المنوفية والفيوم على التوالي. وكانت أقل معدلات للبروتين والأستريز قد ظهرت في تلك العشائر التي جمعت من المناطق التي يحظر فيها استخدام المبيدات(أبو سمبل و الواحات البحرية)، فقد قدرت نسبة البروتين (٥٣,٢ و ٥٦,٥ ملجم / ملل على التوالي) والأستريز(٦ و ٦,٥ ملل مول/ ملجم بروتين على التوالي). وتشير النتائج السابقة إلى إمكانية استخدام هذه التقنية للتمييز بين عشائر المفترس المختلفة تبعاً لحساسيتها تجاه المبيدات الكيميائية. كما تمتاز هذه التقنية بالسرعة والدقة مقارنة بطرق التقييم الحيوية التقليدية الأخرى.

أظهرت نتائج التفريد الكهربائي للبروتين ليرقات العمر الثاني لمفترس أسد المن في مختلف العشائر ظهور ٢٥ حزمة مختلفة ذات أوزان جزيئية تراوحت بين ١٨٤٤٣٧ إلى ١٢٨١٦.

أظهرت نتائج التفريد الكهربائي للأستريز ليرقات العمر الثاني لمفترس أسد المن المرباة معملياً أربعة حزم ، فى حين كان عدد الحزم ٦ ، ٩، ٦ و ٨ حزمة في يرقات العشائر الحقلية المجمعة من أبو سمبل، الواحات البحرية، المنوفية والفيوم على التوالي.

وتشير الدراسة انه من غير الممكن الاعتماد على نتائج التفريد الكهربائي للبروتين والأستريز في التفرقة بين عشائر مفترس أسد المن التي جمعت من مناطق تستخدم المبيدات بكثرة وأخرى محظور فيها استخدام المبيدات.