J. Agric. Sci. Mansoura Univ., 34 (10): 10203 - 10216, 2009

EFFECT OF SOME NATURAL AND SYNTHETIC COMPOUNDS ON THE PROTEIN PATTERN OF *Muscina stabulans* (FALLEN) PUPAE.

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ABSTRACT

The present investigation deals with the effect of Insect growth regulator (Flufenoxuron), volatile oil extract of *Imperata cylinderica* and fixed oil extract of *Glycine soja* on the protein pattern of *Muscina stabulans* pupae resulting from treated third larval instar. The mean total protein content was disturbed by the LC_{50} of the tested compounds. These results were verified electrophoretically as the tested compounds caused disturbance in the protein fractions through the appearance and disappearance of some protein bands compared with that of control.

INTRODUCTION

Several studies in different parts of the world show that non-biting flies (like our fly under study, *Muscina stabulans*) carry different stages of helminth and protozoan parasites. For example, Sulaiman *et al.* (1988), isolated eggs of *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms from fly species of *Chrysomya, Sarcophaga* and *Musca*, which were collected from refuse dump and peridomestic sites in Malaysia. Non-biting flies are often associated with domestic dwellings, human and animal excreta and other wastes, especially during the dry season when they breed prolifically and cause a constant annoyance for humans. The free interchange that flies have with such sites ensures that they are laden with disease causing organisms on their mouthparts, body hairs and sticky pads of their feet, as well as in their stomachs, faeces and vomitus (Graczyk *et al.*, 2005). The use of environmentally friendly and biodegradable natural insecticides of plant origin has received renewed attention as agents for disease vector control such as microbial sprays, and insect growth regulators admist other control measures such as beneficial insects and all, necessitate an integration of supervised control (Ascher *et al.*, 1995; Senthil *et al.*, 2004, 2005). Owing to the medical importance of *Muscina stabulans*, the present work was undertaken to investigate the effects of some plant extracts and insect growth regulators against this fly.

MATERIALS AND METHODS

1. Insect culture:

Muscina stabulans adults were collected from Dakahlia provience. They were reared in the laboratory for five successive generations before being used in experiments, according to the method described by El-Shazly *et al.* (1996).

2-Tested compounds:

i- Volatile oil extract of *Imperata cylinderica*

Steam distillation technique was used for extraction of this oil according to the methods of Anderson *et al.* (1980).

ii- Fixed oil extract of *Glycine soja*

Extraction of the fixed oil was performed from crushed and previously steam distilled plant parts. Where the dry powdered plants were macerated in petroleum-ether (40-60) according to Harborne (1984) and El-Sayed *et al.* (1989).

iii- IGR (Flufenoxuron).

2. Biochemical studies:

Third instar larvae were dipped for five seconds in (LC_{50S}) petroleum ether (40-60) solution of *Imperata cylinderica* volatile oil, *Glycine soja* fixed oil and flufenoxuron at a concentration of 0.139, 0.19 and 0.0052 percentage respectively as reported by Khalaf and Hussein (1997). The total proteins and the protein fraction SDS-Page were determined in the fresh whole body homogenate.

A. Determination of total proteins:

The total proteins were determined according to Lowery *et al.* (1951).

B. Separation of protein bands by electrophoresis:

Haemolymph proteins of the pupae came from control and treated 3rd instar larvae of *Muscina stabulans* were electrophoretically separated using SDS-page according to (Davis, 1964).

RESULTS AND DISCUSSION

Treatment of third instar larvae of *Muscina stabulans* with (LC50) of *Imperata cylinderica* volatile oil, *Glycine soja* fixed oil and flufenoxuron induced a disturbance in the total protein content of the resulting pupae.

A. Determination of total proteins:

The insect proteins are numerous; among them are storage protein, vitellogenin, lipophrin and a large amount of other major proteins with unknown fractions Kyung and Kim (1990).

Data in table (1) and figs. (1, 2&3) illustrate that the LC_{50s} of IGR (Flufenoxuron) caused significant reduction in mean total protein content at two and four days old pupae, while it gave no significant effect at six days old pupae in comparison with that of control. LC_{50} of fixed oil of *Glycine soja* significantly decreased the determined criterion only at four days old pupae compared with control groups.

Table (1): Effect of the tested compounds on the total proteins of *M. stabulans* pupae treated as 3rd larval instar (mg/g fresh body weight):

Treatment	Age of pupae per days		
	2	4	6
IGR's (Flufenoxuron)	15.86±0.012 *	23.15±0.019 *	25.1±0.016
Volatile oil of *Imperata cylinderica*	24.94±0.021 *	26.68±0.013 *	25.91±0.021
Fixed oil of Glycine soja	19.98±0.017	28.8±0.015 *	27.22±0.023
Control	20.13±0.016	36.36±0.018	23.7±0.016

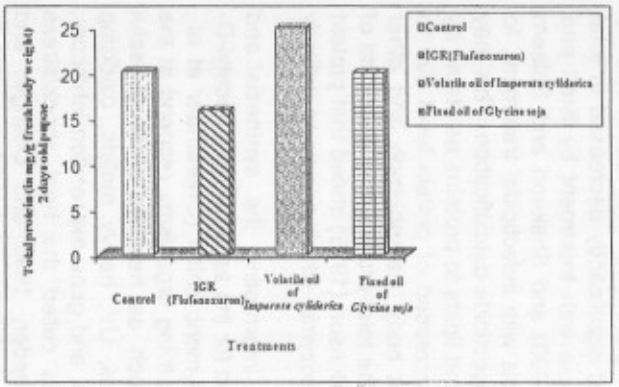


Fig. (1): **Effect of the tested compounds on total protein content of *M.stabulans* pupae (2 days old).**

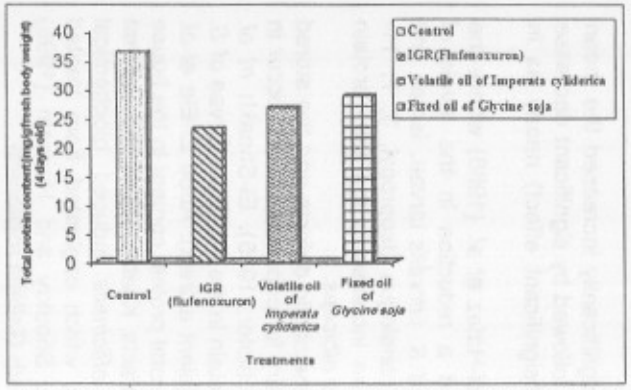


Fig. (2): **Effect of the tested compounds on total protein content of *M.stabulans* pupae (4days old).**

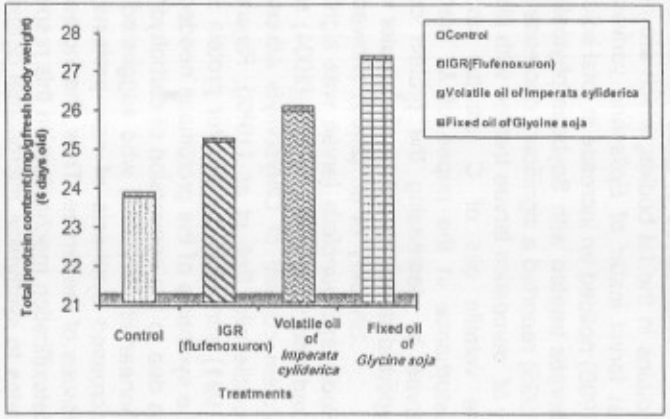


Fig. (3): **Effect of the tested compounds on total protein content of *M.stabulans* pupae (6 days old).**

Volatile oil of *Imperata cylinderica* significantly increased the mean total protein content at two days old pupae, followed by significant decrease at four days old pupae then recovered (insignificant effect) nearly as in control at six days old pupae.

Similar results were arrived by Abdel-Hafez *et al.* (1988) where the IGR's diflubenzuron and triflumuron caused a reduction in the levels of proteins and free amino acids of the treated *S. littoralis* larvae. Ismail and Fouad (1985) found that juvenile hormone analogue (Isopropyl, 3, 7, 11-trimethyl, 2, 4, Dodencadienoate) induced an increase in the total protein content all over the pupal period of *Chrysomia albiceps*.

High doses of azadirachtin caused metabolic defects and the stored proteins in the fat bodies, which are necessary for pupation, did not occur in last larval instar of *Epilachna varivestis* (Schloter, 1985). El-Sheakh *et al.* (1990) noticed an increase in total soluble protein in the 4th instar larvae of *S. littoratis* treated with Soybean phytoalexins (plant extract). Abou El-Ela *et al.* (1995) reported a significant decrease in the total protein content in the house fly *M. demostica* larvae treated with plant extracts. Khalaf (1998) showed that the volatile oils of *C. citratus* and *R. officinalis* induced biochemical disturbance in the pupae of *M. stabulans*, which originated from treated larvae by decreasing the protein content. Shoukry and Hussein (1998) reported similar results on the greater wax moth *Galleria mellonella*.

Shoukry *et al.* (2003) showed that haemolymph proteins content of *Plodia interpunctella* larvae was significantly increased in all treatments by fixed and volatile oils. Sabry (2004) reported significantly decreased in total protein content of *Chrysomyia albiceps* larvae with treatment by fixed and volatile oils. Fell *et al.* (1982), Rajender (1990) and Shakoori and Saleem (1991) attributed the greater protein synthesis with insecticidal treatment, to the synthesis of the proteinase needed for insecticide detoxification. This may be due to the conversion of carbohydrates and lipids to proteins as stated by Kinnear *et al.* (1971) who suggested that increased of protein level due to increased synthesis of new proteins by fat body, haemolymph and other tissues of the larvae. Thus, the increase in the total protein may be a kind of detoxification mechanism. In this respect, Wilkinson (1976) stated that protein helps to synthesize microsomal detoxifying enzyme, which assists to detoxify the toxicants that entered into the body.

Chitin synthesis inhibitors act by interrupting the synthesis and transport of specific proteins that are required for the assembly of N-acetyl-D-glucosamine (Glc-NAC) monomers into polymeric chitin (Oberlander et al., 1998). It is also a well-established fact that living organisms respond at the cellular level to unfavorable conditions such as heat or other stressful situations including exposure to xenobiotics, UV, heavy metals, oxidizing agents, mutagens, carcinogens, insecticides, and gene expression inhibitors, by expression of specific sets of proteins called the heat shock/stress proteins (Linquist, 1986; Nover, 1991; Feeder, 1996 and Delinger and Yocum, 1998). Recent studies indicate that stress proteins play a role in toxicity since they are induced as a result of damages caused to the cell by the toxicant (Hightower, 1991 and Sanders, 1993).

B. Separation of protein bands by electrophoresis:

Haemolymph proteins of the pupae came from control and treated 3rd instar larvae of *Muscina stabulans* were electrophoretically separated using SDS-page. The separating gel was 10% acrylamide.

Plates (I&II) show the electrophoretic patterns (electrophoregrams) obtained for haemolymph proteins from the control pupae with LC_{50} of *Imperata cylinderica*, *Glycine soja* and the IGR (Flufenoxuron) respectively. At the same time protein M.Wt. standard were separated to be used as a reference for the molecular weights of the separated protein bands.

1-) Two days old pupae:

A total number of forty six different protein bands with molecular weights ranging from 3-91.8 k.dalton, were distinguished. Sixteen protein fractions were separated from haemolymph proteins of the control pupae (fig. 4). Eighteen, seventeen and fourteen bands were separated from the *Imperata cylinderica* volatile oil, *Glycine soja* fixed oil and the IGR (Flufenoxuron) (figs. 5, 6 and 7), respectively. Among these five fractions (no. 9, 10, 20, 22 and 38) were always permanent or dominant in both treated and untreated pupae with M.Wts. equal, 71.9, 67.4, 57.9 and 3.1 K.Da, respectively. However their relative percentages and intensities fluctuated, they are stage specific proteins. The other protein fractions (41 fractions) are often related to the type of applicable materials and therefore they are considered as treatment dependent.

2-) Four days old pupae:

A total number of thirty six different protein bands with molecular weights ranging from 3.16 – 72.17 k.dalton, were distinguished. Thirteen protein fractions were separated from haemolymph proteins of the control pupae (fig. 8).Ten; thirteen and twelve bands were separated from the *Imperata cylinderica* volatile oil, *Glycine soja* fixed oil and the IGR (Flufenoxuron) (figs. 9, 10 and 11), respectively. Among these three fractions (no.4,5 and 17) were always permanent or dominant in both treated and untreated pupae with M.Wts. equal, 67.4, 66.95 and 57.9 K.Da, respectively. However their relative percentages and intensities fluctuated, they are stage specific proteins. The other protein fractions (33 fractions) are often related to the type of applicable materials and therefore they are considered as treatment dependent.

3-) Six days old pupae:

A total number of forty four different protein bands with molecular weights ranging from 1.7– 91.58 k.dalton, were distinguished. Fourteen protein fractions were separated from haemolymph proteins of the control pupae (fig. 12). Eleven, sixteen and thirteen bands were separated from the *Imperata cylinderica* volatile oil, *Glycine soja* fixed oil and the IGR (Flufenoxuron) (figs. 13, 14 and 15), respectively. Among these three fractions (no.6, 8 and 19) were always permanent or dominant in both treated and untreated pupae with M.Wts. equal, 67.4, 66.94 and 57.9 K.Da, respectively.

Fig. (3):

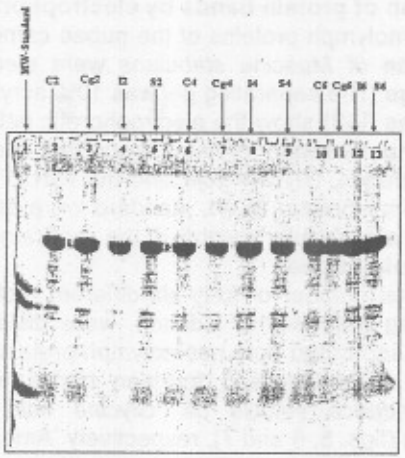


Plate (I): Electrophoregraph of haemolymph protein of different pupal stages of Muscina stabulans (SDS-polyacrylamide gel electrophoresis).

C2: control pupae (2 days old).

Cg2: treated pupae (2 days old) with *I .cylinderica*

I2: treated pupae (2 days old) with IGR'S (Flufenoxuron).

S2: treated pupae (2 days old) with *Glycine soja*.

C4: control pupae (4 days old).

I4: treated pupae (4 days old) with IGR'S (Flufenoxuron).

S4: treated pupae (4 days old) with *Glycine soja*

C6: control pupae (6 days old).

Cg6: treated pupae (6 days old) with *I. cylinderica*.

I6: treated pupae (6 days old) with IGR'S (Flufenoxuron).

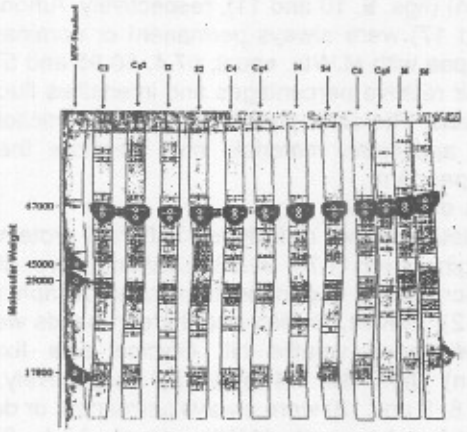
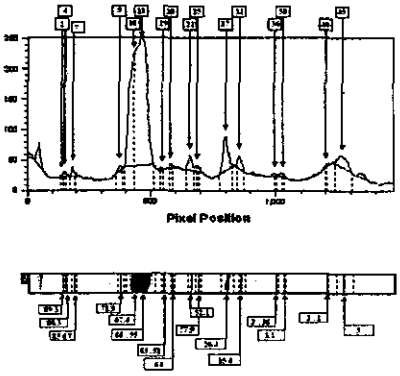


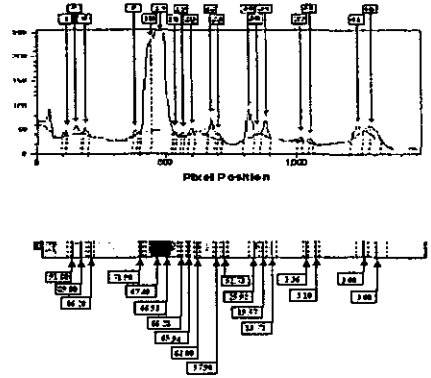
Plate (II): Computerized diagram of plate (I)



Fraction	Relative percent	M.wt (K.Da)
2	0.00	89.30
4	0.25	88.30
7	0.71	85.67
9*	0.76	71.90
10*	19.30	67.40
13	61.87	66.95
19	0.24	65.51
20*	0.44	64.00
22*	2.28	57.90
25	0.33	52.10
27	7.01	26.40
31	2.08	15.40
36	0.13	3.36
38*	0.11	3.10
40	0.10	3.10
45	4.42	3.00

* Permanent fraction

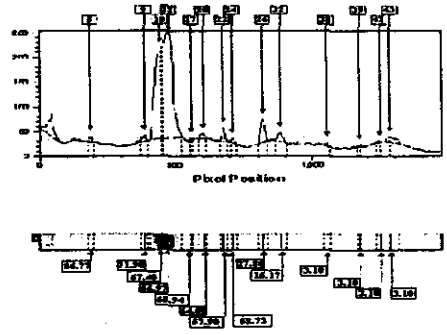
Fig. (4): Typical electrophoregrams, densitometer scans, and relative percent of protein fractions of untreated 2 days old pupa of *Muscina stabulans*.



Fraction	Relative percent	M.Wt. (K.Da)	Fraction	Relative percent	M.Wt. (K.Da)
1	0.47	1	22*	2.52	57.90
3	2.60	3	23	0.81	52.73
6	0.79	6	28	5.65	25.92
9*	0.59	9*	30	0.98	19.57
10*	18.86	10*	34	2.76	13.78
14	57.32	14	36	0.53	3.36
16	0.19	16	38*	0.46	3.10
19	0.35	19	41	2.31	3.00
20*	0.74	20*	46	2.35	3.00

* Permanent fraction

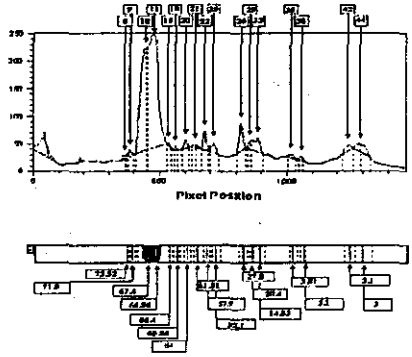
Fig. (5): Typical electrophoregrams, densitometer scans, and relative percent of protein fractions of *I. cylinderica* 2 days old pupa of *Muscina stabulans*.



fraction	Relative percent	M.Wt. (K.Da)	fraction	Relative percent	M.Wt. (K.Da)
5	0.97	86.77	32	2.06	15.17
9*	1.04	71.90	38*	0.94	3.10
10*	27.81	67.40	39	0.37	3.10
12	55.99	66.97	42	0.24	3.10
17	0.11	65.94	43	1.47	3.10
20*	1.08	64.00	32	2.06	15.17
22*	2.20	57.90			
24	0.63	52.73			
26	5.08	27.80			

* Permanent fraction

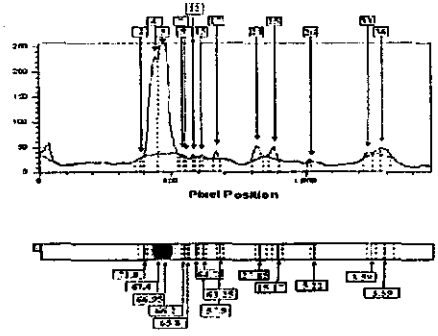
Fig. (6): Typical electrophoregrams, densitometer scans, and relative percent of protein fractions of IGR 2 days old pupa of *Muscina stabulans*.



Fraction	Relative percent	M.Wt. (K.Da)	Fraction	Relative percent	M.Wt. (K.Da)
8	0.11	73.52	25	1.85	52.10
9*	0.89	71.90	26	4.87	27.80
10*	26.16	67.40	29	0.55	20.40
11	50.85	66.96	33	4.59	14.83
15	0.26	66.40	35	0.75	3.81
18	0.28	65.86	38*	0.62	3.10
20*	1.26	64.00	42	1.66	3.10
21	0.41	61.51	44	2.27	3.00
22*	2.62	57.90			

* Permanent fraction

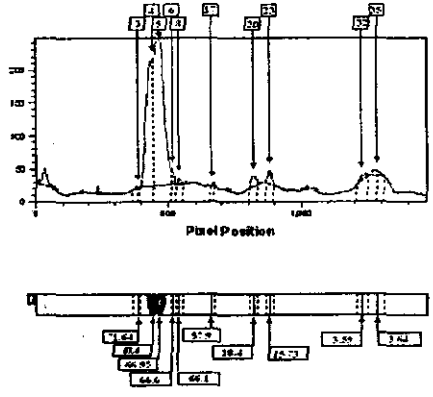
Fig. (7): Typical electrophoregrams, densitometer scans, and relative percent of protein fractions of *G.soja* 2 days old pupa of *Muscina stabulans*.



Fraction	Relative percent	M.wt (K.Da)
2	0.22	71.80
4*	35.03	67.40
5*	52.77	66.95
7	0.19	66.20
9	0.15	65.80
12	0.15	64.78
15	0.33	63.25
17*	1.00	57.90
21	3.70	27.85
25	2.76	15.17
28	0.48	3.80
33	0.95	3.59
36	2.26	3.59

* Permanent fraction

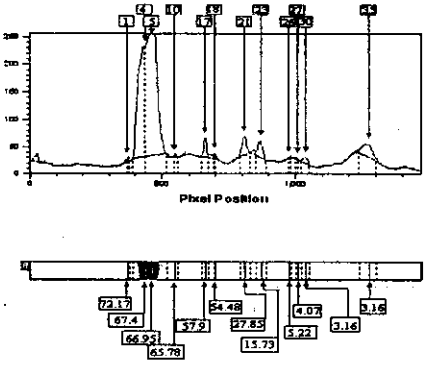
Fig. (8): Typical electrophoregrams, densitometer scans, and relative percent of protein fractions of untreated 4 days old pupa of *Muscina stabulans*.



Fraction	Relative percent	M.wt (K.Da)
3	0.26	71.64
4*	31.08	67.50
5*	59.70	66.95
6	1.37	66.60
8	0.77	66.10
17*	0.27	57.90
20	2.00	28.40
23	2.47	15.73
32	1.27	3.59
35	0.82	3.64

* Permanent fraction

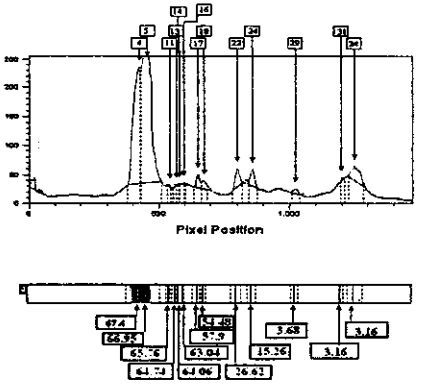
Fig. (9): Typical electrophoregrams, densitometer scans, and relative percent of protein fractions of *I. cylindrica* 4 days old pupa of *Muscina stabulans*.



Fraction	Relative percent	M.wt (K.Da)
1	0.31	72.17
4*	26.47	67.40
5*	57.36	66.95
10	0.31	65.78
17*	2.36	57.90
18	0.18	54.48
21	3.31	27.85
23	3.10	15.73
26	0.24	5.22
27	0.13	4.07
30	1.09	3.16
35	0.31	3.64

* Permanent fraction

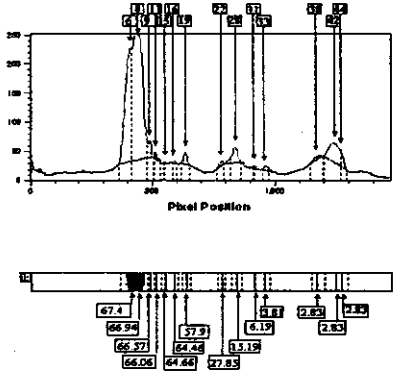
Fig. (10): Typical electrophoregrams, densitometer scans, and relative percent of protein fractions of IGR 4 days old pupa of *Muscina stabulans*.



Fraction	Relative percent	M.wt (K.Da)
4*	31.82	67.40
5*	51.90	66.95
11	0.27	65.76
13	0.17	64.74
14	0.01	64.06
16	0.12	63.04
17*	1.97	57.90
18	1.36	54.48
22	3.21	26.62
24	2.98	15.26
29	0.87	3.68
31	0.23	3.16
34	5.08	3.16

* Permanent fraction

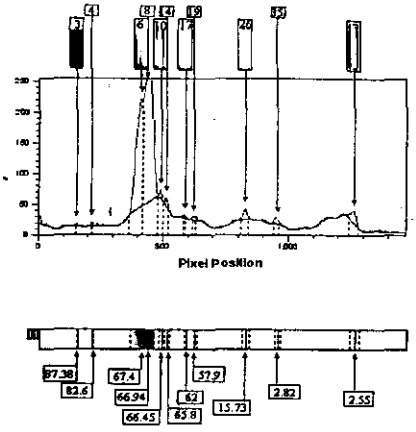
Fig. (11): Typical electrophoregrams, densitometer scans, and relative percent of protein fractions of *G.soja* 4 days old pupa of *Muscina stabulans*.



Fraction	Relative percent	M.Wt. (K.Da)	Fraction	Relative percent	M.Wt. (K.Da)
6*	28.13	67.40	31	0.14	6.19
8*	52.22	66.95	33	0.75	3.81
9	1.84	66.57	38	0.83	2.83
11	0.76	66.06	42	7.48	2.83
15	0.06	64.66	44	2.05	2.83
16	0.40	64.46			
19*	1.64	57.90			
22	0.61	27.85			
28	3.09	15.19			

* Permanent fraction

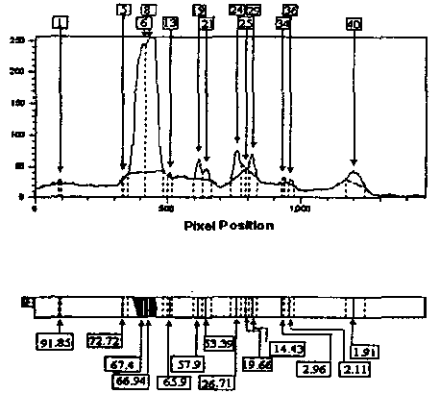
Fig. (12): Typical electrophoregrams, densitometer scans, and relative percent of protein fractions of untreated 6 days old pupa of *Muscina stabulans*.



Fraction	Relative percent	M.wt (K.Da)
3	0.12	87.38
4	0.23	82.60
6*	32.50	67.37
8*	58.34	66.94
10	0.87	66.45
14	1.01	65.80
17	0.35	62.00
19*	0.33	57.90
26	1.98	15.73
35	1.11	2.82
43	3.15	2.55

* Permanent fraction

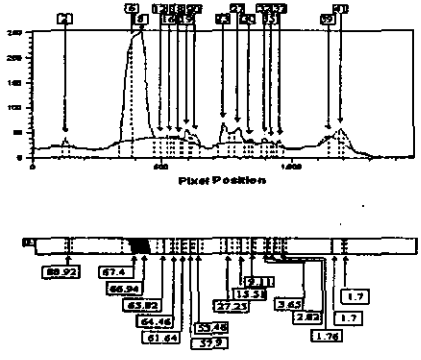
Fig. (13): Typical electrophoregrams, densitometer scans, and relative percent of protein fractions of *I. cylinderica* 6 days old pupa of *Muscina stabulans*.



Fraction	Relative percent	M.wt (K.Da)
1	0.20	91.58
5	0.13	72.72
6*	35.77	67.40
8*	46.78	66.94
13	00.30	65.90
19*	2.90	57.90
21	1.70	53.39
24	4.42	26.71
25	0.68	19.66
29	2.46	14.43
34	0.28	2.96
36	0.50	2.11
40	3.87	1.91

* Permanent fraction

Fig. (14): Typical electrophoregrams, densitometer scans, and relative percent of protein fractions of IGR 4 days old pupa of *Muscina stabulans*.



Fraction	Relative percent	M.Wt. (K.Da)	Fraction	Relative percent	M.Wt. (K.Da)
2	1.04	88.92	27	3.64	15.51
6*	26.56	67.40	30	0.37	9.11
8*	55.31	66.94	32	0.73	3.65
12	0.04	65.82	35	0.08	2.82
16	0.39	64.46	37	0.88	1.76
18	0.32	61.64	39	0.15	1.70
19*	1.93	57.90	41	2.18	1.70
20	1.94	53.46			
23	4.53	27.25			

* Permanent fraction

Fig. (15): Typical electrophoregrams, densitometer scans, and relative percent of protein fractions of *G.soja* 6 days old pupa of *Muscina stabulans*.

However their relative percentages and intensities fluctuated, they are stage specific proteins. The other protein fractions (41 fractions) are often related to the type of applicable materials and therefore they are considered as treatment dependent.

From the above mentioned data we can deduce that, treatment with plant fixed oil, volatile oil and IGR make disturbance in the protein fractions of treated pupae. The appearance of new bands may be due to liberation of free radicals which affect directly the nitrogenous compounds this in turn lead to break down of the peptide linkage causing fragmentation of protein molecules. Also, the formation of extra-molecular size of one or more molecules is expected as the suggestion of Megahed (1996), and Gehad and Shaurub (1997). Another possible source of haemolymph free protein is the haemocytes which play a part in the metabolic process as growth and moulting (Wigglesworth, 1959).

Generally insects exihibit both cellular (haemocytes) and non cellular (humoral) defense mechanisms (Gotz and Boman, 1985; Brehelin, 1986 and Dunn, 1986). Recent studies on insect haemolymph indicated the presence of a variety of immune protein or molecules formed in response to the oil treatments. Such molecules act as agglutinins, lysis, precipitin, opsonin and microbicides (Fries, 1984 and Ratcliffe, 1985).In conclusion, the protein bands of treated samples were completely different from those of control, so it may be a difference in biological and biochemical activities as recorded by Mostafa *et al.* (1995). El-Beramawy and Abdel Fattah (2000) recorded that, it is worthy to note that the protein type has specific biological role according to this role the DNA secretes enzymes which act as catalyst to produce specific type of protein, this protein is responsible for specific biological process, due to the difference in protein bands between treated samples and control, the biological process may be different too. During stress conditions, animals need more energy to detoxify the toxicants to overcome stress (Tiwari and Singh 2003). At the chronic period of stress, the proteins were synthesized as a source of energy. If the carbohydrates are limited, the next alternative source of energy is protein to meet the increased energy demand. Perhaps, total protein level can also be low during some developmental stages of *Muscina stabulans* because of the reason mentioned above. On the other hand, the toxic effect of the tested compounds may have severe implications for the permeability of membranes. This leads to the release of some proteases, enzymes that degrade protein molecules, this in agreement with the findings of Agar *et al.* 2005.

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تأثير بعض المركبات الطبيعية والمخلقة على المحتوى البروتينى لعذارى ذبابة مسينا ستابيولانس (فالن)

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بدراسة تأثير التركيز نصف المميت لإثنين من الزيوت النباتية المستخلصة من نباتى الحلفا بر وفول الصويا و نوع واحد من منظمات النمو الحشرى (الفلوفينوكسورون) على عذارى ذبابة مسينا ستابيولانس وذلك بعمر الطور اليرقى الثالث لمدة خمس ثوانى فى هذا التركيز ودراسة التغيرات الناتجة فى المحتوى البروتينى تم التوصل إلى النتائج التالية:

١. ادت المعاملة إلى ترجح محتوى البروتين للعذارى الناتجة من المعالجة ما بين الزيادة والنقصان وكان منظم النمو الحشرى أكثرهم فاعلية يليه الزيت المستخلص من نبات الحلفا بر ثم الزيت المستخلص من نبات فول الصويا.

٢. أدت المعاملة إلى إختفاء بعض الأجزاء البروتينية وظهور اخرى جديدة عند تحليل الدم بطريقة الهجرة الكهربية.

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