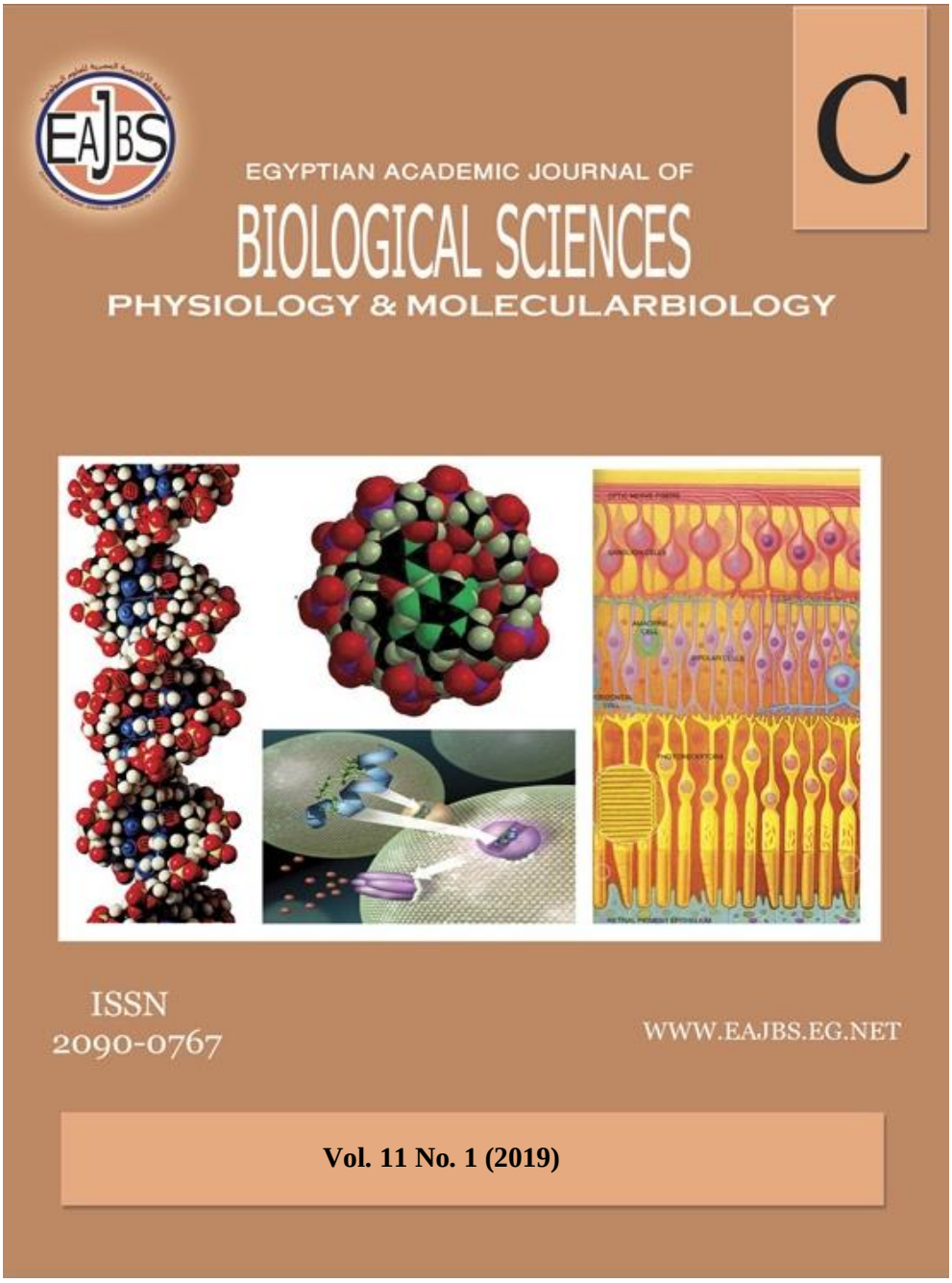

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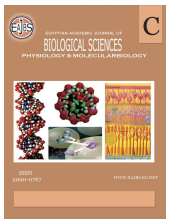

Electrophoretic Protein and Amino Acid Analysis of *Lucilia sericata, Chrysomya albiceps (Diptera: Calliphoridae) And Musca domestica (Diptera: Muscidae)* Larval Excretion/Secretion

Moneer S. Amer, Kotb M. Hammad , Ahmed Z.I. Shehata, Ahmed I.A. Hasballah and Mahmoud M.M Zidan
Department of Zoology, Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt
E.Mail : ahmedzeinhom00@gmail.com

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ABSTRACT

Lucilia sericata, *Chrysomya albiceps* and *Musca domestica* larvae have been successfully used routinely for the clinical treatment of difficult necrotic and infected wounds. Degradation by proteinases contained in larval excretory/secretory (ES) products is thought to contribute to wound debridement by removal of dead tissue. However, proteinase activity may also affect host tissue remodeling processes (Chambers *et al*., 2003), for hundreds of years, the beneficial effects of maggots on wound healing have been documented. Insects have to defend themselves from infection by a wide variety of potential bacteria and fungi. In addition to some of flies have beneficial effects in medicine especially, *L. sericata* which used in Maggot Debridement Therapy (MDT). Maggot therapy success is partly due to the ingestion and killing of living microorganisms but antibacterial activity of excretion/secretion (ES) that included the salivary gland secretions and faecal products of *L. sericata* has been demonstrated in vitro.

INTRODUCTION

The proteins of (ES) may be affected by antimicrobial activity and beneficial effects of maggots. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is a reliable method for determining the molecular weight (MW) of an unknown protein. **Van der plas *et al.*, (2008)** showed that the molecular weight of protein involved in beneficial effects of maggots is believed to be contained in their excretion/secretion, antimicrobial peptides found in insects playing an important role in innate immune systems and host defense mechanisms. They have attracted much attention as a novel class of antibiotics, in particular for antibiotic-resistant pathogens, because of their action mechanism of non- selective interaction with cell surface membranes of microbes **(**Hancock and Rozek, 2002; Zasloff 2002; Boman 2003 and Bulet *et al*., 2004). The insect peptides/ polypeptides that have a broad spectrum of activity against both Gram- positive and Gram- negative bacteria and against fungi (Hoffmann 1995; Hoffmann *et al*., 1996). The amino acid-like compounds present in (ES) of *L. sericata* larvae in order

to determine the compounds present and their potential role in the wound healing process. The classical methods for the separation of amino acids by column chromatography.

MATERIALS AND METHODS

Tested Flies:

Lucilia sericata Meigen, *Crysomya albiceps* Wiedemann (Diptera: Calliphoridae) and *Musca domestica* Linnaeus (Diptera: Muscidae).

1. Collection and Laboratory Maintenance of Tested Flies:

1.1. The Green Bottle Fly, *Lucilia sericata* Meigen:

Larvae of *L. sericata* were collected from Tonamel-Village, Aga Center, Al- Dakahlya Governorate, Egypt using bait trap. collected larvae transferred to Medical Entomology Insectary, Animal House, Zoology Department, Faculty of Science, Al-Azhar University (Cairo) and maintained for several generations under controlled conditions of temperature (27±2ºC) and relative humidity (70±5%) and photoperiods (12h light: 12h dark). Adults reared in mesh cages 30×30×30cm with three sides of the wire, larvae were feed on an artificial diet (liver), the emerged flies were feed on a dry diet (milk powder) and sucrose solution (cotton pads soaked in 10% sucrose solution) (Queiroz and Milward-de-Azevedo, 1991).

1.2 *Crysomya albiceps* Wiedemann:

Crysomya albiceps larvae were collected beside Animal House of Al-Azhar University (Cairo) and maintained for several generations in Medical Entomology Insectary under controlled conditions of temperature (27±1ºC), relative humidity (60±10%) and photoperiods (12h light: 12h dark). A standard rearing procedure described for *L. sericata* was adopted to provide larvae needed for experiments.

1.3. The House Fly, *Musca domestica* Linnaeus:

larvae of the housefly *M. domestica* were collected around Animal House, Al-Azhar University (Cairo) by hand trap and maintained for several generations under controlled conditions of temperature (27±2ºC) and relative humidity (70±5%) and photoperiods (12h light: 12h dark). The emerged flies were fed on a dry diet (milk powder) and sucrose solution (cotton pads soaked in 10 % sucrose solution). Eggs could be collected from paper strips or from cotton pads of feeding, where the females deposited them. Larvae were reared on an artificial diet (wheat bran, milk, powder yeast; 200:100:5gm) per 200 ml distilled water according to the method described by **Busvine, (1962)**.

Collection Larval Native Excretions/Secretions (nES):

Excretions/secretion (ES) of each *L. sericata, C. albiceps* and *M. domestica* maggots were collected by washing 3rd instar larvae (about 5000 larvae) with 70% ethanol and sterile ultrapure water (ddH_2O) then incubated overnight (10hrs) at 30°C **(Kerridge *et al.*, 2005),** after which ES was collected and centrifuged at 20,000 rpm for 15 min. to remove large particles. The ES from each insect species was then stored frozen at -20°C until required.

Detection of Different Proteins in Larval Native ES and Body Extracts by Gel Electrophoresis (SDS-PAGE):

Separation of protein was carried out by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE).

Using the methods described by Laemmli, (1970), Hames and Rick-wood, (1981) and Stegeman *et al*., (1988), protein bands were separated.

- Stock solutions for gel preparation:
- Stock solution1:

- Acrylamide (30%):

Thirty grams acrylamide and 0.8 GRAM N, N-methylenebisacrylamide (BDH) were dissolved in 100 ml. distilled water. The solution was filtered stored in a dark bottle at 4 °C till used

- Stock solution2:
- Stacking buffer:

Tris-HCl buffer solution (pH 6.6) was prepared by dissolving 9.0 gramsTrishydroxymethylaminometha ne in 100 ml dist. water. The pH was adjusted using 1N HCl and the solution stored at 4°C.

- Stock solution3:
- Separating buffer:

Tris-HCl solution (pH 8.8) was prepared by dissolving 27.3 grams Trishydroxy methyl amino methane in 100 ml dist. water and about 20 ml 2N HCl were added until the optimum pH, then the solution stored at 4 °C.

- Stock solution4:
- Running buffer:

Fifteen grams Tris-base, 72.0 grams glycine and 5.0 grams sodium dodecyl sulfate (SDS) were dissolved in 5000.0 ml dist. water, adjusted to pH 8.3 with 1N HCl, and then stored at 4°C.

- Stock solution 5:
- Sample buffer:

Tris-borate solution pH 8.2 was prepared by dissolving 3.25 gramstris- base and 3.66 grams boric acid in 100 ml dist. water, the pH was adjusted with 1N HCl. The solution was filtered and stored at 4°C.

- Stock solution 6:
- 10 % SDS solution:

This solution was made by dissolving 10.0 grams sodium Dodecyl sulfate in a small amount of water with gentle stirring and then dist. water was added to 100 ml.

- **Procedures:**

A set of 2 glass plates of 4 mm thickness was used for gel 1 mm thickness with a total 14 x 16 cm area; 11 cm separating plus 3 cm stacking gel. Owl vertical slab apparatus was used in protein separation. The separating and stacking gels were prepared according to the following table:

Solution	Separating gel	Stacking gel
Acrylamide (30%)	12ml	3ml
Tris-HCl buffer (pH 8.8)	6ml	---
Tris-HCl buffer (pH 6.6)	---	3ml
Distilled water	10ml	12ml
Glycerol	4ml	---
SDS (10%)	300µl	150µl
TEMED	30µl	15µl
Ammonium persulphate (10%) Freshly prepared.	150µl	75µl
Total volume	**32.48ml**	**18.24ml**

Eight cm. of separating gel was poured between the glass plates immediately after adding ammonium persulphate (APS). After polymerization of the separating gel, stacking gel was poured into the glass plate's space, and then the comb was inserted to form sample wells.

- **Sample preparation:**

Samples were prepared according to Stegmann *et al.,* (1988). Serum was diluted with distilled water (1:3 v/v, respectively), i.e.

150µl-distilled water was added to 50µl serum. Then, 50µl SDS and 25µl β-mercaptoethanol were added to the diluted sample and put in a boiling

water bath for 5 min. The sample was cooled at room temperature, added 5µl of bromophenol blue stain "tracking dye" and 10µl from each were applied to the gel wells according to the method of Laemmli, (1970) and Hames and Rick-Wood, (1981) with some modifications.

- **Running condition:**

The run was carried out with a constant voltage of 200 volts at the stacking gel and 30 m.A. at the separating gel. When the tracking dye becomes one cm. before the end of the gel, the current switched off. The usual run time was approximately 120 min.

- **Staining:**

The separated proteins were stained with Coomassie brilliant blue R-250, which had been prepared as follow:

Coomassie brilliant blue R-25 (0.1gm)
Distilled water (400ml)
Acetic acid (70ml)
Methanol (200ml)
Trichloracetic acid (60ml)

The gels were soaked in excess of staining solution till the appearance of bands.

- **Destaining:**

After gel staining, the gel was transferred to a distaining solution to remove the excess stain, until the clearance of the background.

- **Destaining Solution:**

Methanol (150ml)
Acetic acid (50ml)
Distilled water (300ml)

The slab gel of proteins was photographed. Analysis percentages of the peaks of each line were carried out using Hoofer Scanning densitometer with corresponding software Gel-scan version 5. Sigma protein marker wide range (M.W. 6,500-205,000) was used to determine the approximate molecular weight of protein fractions.

- **Analysis of gel lanes:**

Analysis of gel lanes was carried out using gel documentation and analysis system consisting of dark room, trans illuminator, Integrating CCD video camera and image analysis software (AAB software©).

1. Determination of amino acids in larval (nES) and body extracts:

Method of preparation of protein hydrolysate: First of all, samples should be fat free, using ether, then the sample containing exactly 50 milligrams protein, was mixed with 5 ml hy drochloric acid, 5.7 normal (constant boiling) hydrochloric acid and the mixture was prepared in Digestion tube then closed under vacuum according to the method of Block *et al*., (1958).

The content of the tube digested for 24 hours at 110 degrees Celsius. After cooling, a sample was filtered and the contents were washed with distilled water, the filtrate was completed to 50 ml in a volumetric flask. Five ml of the filtrate were evaporated at room temperature till a dry powder was obtained, the bioassays were kindly carried out at Agriculture studies & consultant center (ASCC), Faculty of Agriculture, Al – Azhar University, Cairo, Egypt.

Preparation of the Sample for Injection:

The dry film powder was dissolved in 5 ml loading buffer (0.2 N sodium citrate buffer Ph 2.2), filtered through 0.22 micrometers membrane. Twenty microliters contained the amino acids that resulted from digestion of 20 microgram sample were used.

Instrument:

Alpha plus amino acid analyzer, high-performance system (LKB).

Column: column used was high-pressure stainless steel column, with peltier heating-cooling system, the resin was ultrospek 8 cation exchange

sodium from resin, particle size 8 Um, length 200 mm, diameter 4.6 mm.

Buffers: Three citrate buffers 0.2 N with the following Ph 3.2, 4.25, 6.45 were used for fractionation of the amino acids in a specified program that lasted for 51 minutes. 0.4 N sodium hydroxide solution was used for regeneration of the column after every run.

The flow rate for buffer was 35 ml per minute, the flow rate for ninhydrin was 25 ml per minute, reaction coil temperature was kept at 135 degree Celsius. Pressure 15 bar for a buffer, 15 bar for ninhydrin.

Area of each peak was recognized using Hewlett Packard 3390 integrator, a concentration of each amino acid (gram per 16 gram nitrogen) was calculated.

RESULTS

Determination of electrophoretic proteins of maggot's (ES), *L. sericata, C. albiceps and M. domestica*.

The electrophoretically separated proteins of body extracts of *L. sericata, C. albiceps and M. domestica* larvae and the densitometric analysis are shown in fig. (37). Also, the numbers, percentages and calculated molecular weight of different protein bands are given in table (1).

Referring to the marker in table (1) and figure (1) and densitometric scan of protein electrophoregrams sodium dodecyle sulphat-polyacrylamide gel electrophoresis (SDS–PAG) in figure (2), the obtained results displayed that the number of protein bands of marker was 7 bands with molecular weights varied from 20.35 up to >150 kDa.

The protein bands which separated by SDS-PAGE electrophoresis from maggots (ES) of *M. domestica* were 10 bands with molecular weights varied from 57.09 to 143.33 kDa and *C. albiceps* were 12 bands with molecular weights varied from 21.05 to 143.33 kDa. while the number of protein bands which separated from *L. sericata* were 15 bands with molecular weights varied from 20.35 up to >150 kDa.

Table (1): The calculated molecular weights of different protein bands and percentages (ES) of 3rd larval instar of *L. sericata*, *C. albiceps* and *M. domestica*.

Peak No.	M wt. kDa	Marker %	*C. albiceps* %	*L. sericata* %	*M. domestica* %
1	>150	-	-	7.8	-
2		-	-	2.9	-
3	150	8.4	-	-	-
4	143.33	-	1.2	-	1.5
5	141.67	-	-	0.6	0.6
6	126.67	-	-	0.1	-
7	120	0.7	2.6	-	2.8
8	102.67	-	5.1	-	5.4
9	100	32	-	-	-
10	98.29	-	-	7.6	-
11	94.29	-	6.1	-	-
12	92	-	-	16.2	16.2
13	86.86	-	14.8	-	13.9
14	85.71	-	-	10	-
15	80	8.8	-	-	-
16	73.85	-	2.5	-	2.0
17	72.31	-	-	1.3	-
18	68.85	-	6.9	-	6.7
19	67.31	-	-	6.4	-
20	60	33.3	-	-	-
21	59.09	-	5.4	-	5.1
22	57.95	-	0.8	-	0.9
23	57.5	-	-	2.4	-
24	55.91	-	-	0.3	-
25	52.27	-	1.4	-	-
26	51.82	-	-	0.0	-
27	43.41	-	-	0.8	-
28	40	6.1	-	-	-
29	33.33	-	-	36.9	-
30	32.98	-	46.9	-	-
31	21.05	-	6.3	-	-
32	20.35	-	-	6.7	-
33	20	10.7	-	-	-

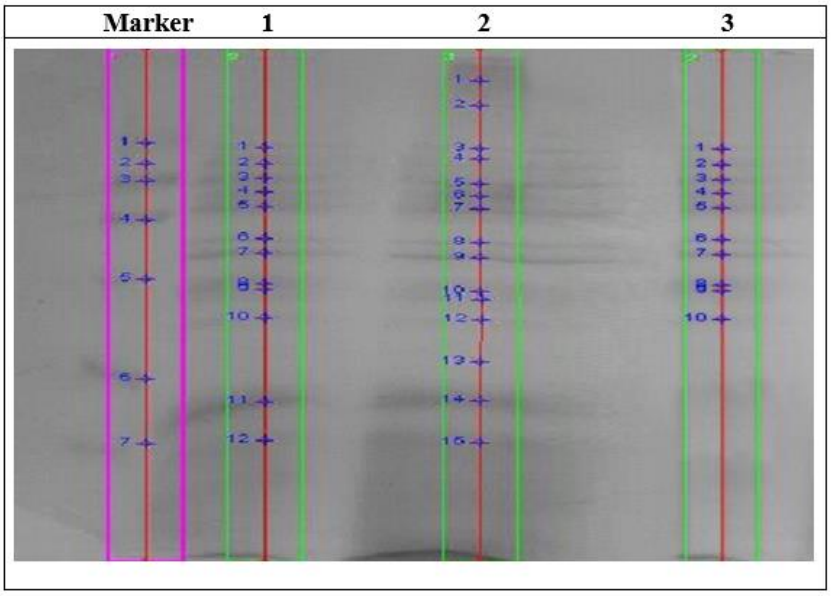

Fig. (1): Electrophoregrams **SDS**–**PAG** of marker (ES) 3rd larval instar of *C. albiceps* (1), *L. sericata* (2) and *M. domestica*. (3)

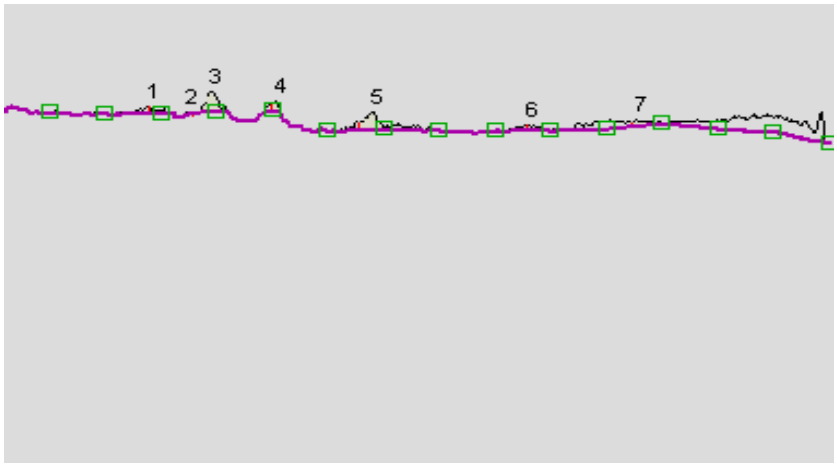

Fig. (2): Densitometric scan of protein electrophoregrams **SDS**–**PAG** of marker

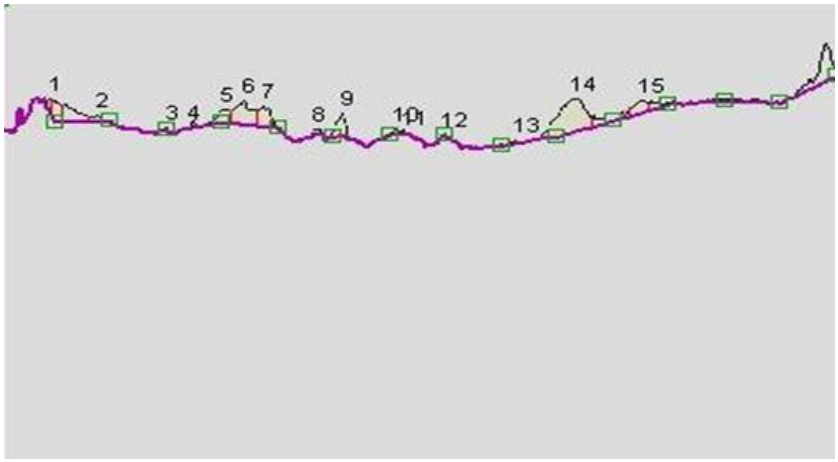

Fig. (3): Densitometric scan of protein electrophoregrams **SDS**–**PAG** of (ES) 3rd larval instar of *L. sericata*.

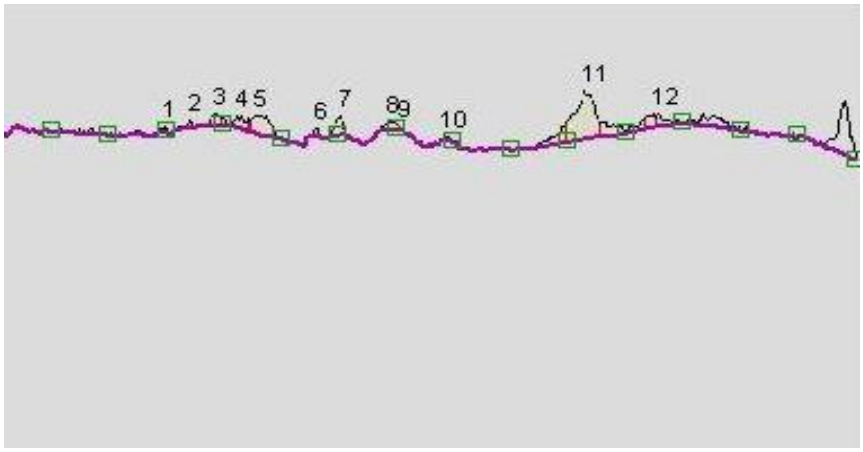

Fig. (4): Densitometric scan of protein electrophoregrams **SDS–PAG** of (ES) of 3rd larval instar of *C. albiceps.*

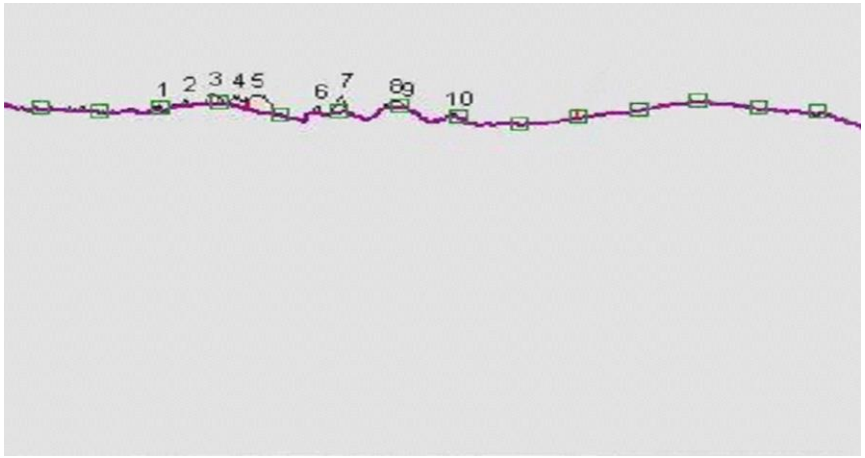

Fig. (5): Densitometric scan of protein electrophoregrams **SDS**–**PAG** of (ES) 3rd instar larvae of *M. domestica.*

By comparing, we found the following:

1- The number of protein bands was different from *L. sericata*, figure (3), *C. albiceps* figure (4) and *M. domestica*, figure (5)
2- The lowest number of protein bands (10) was recorded for (ES) of *M. domestica* larvae.
3- Some bands were appeared or disappeared according to variance between the tested species.
4- The number of protein bands with high molecular weights (<150 kDa) was recorded for (ES) of *L. sericata* larvae.

According to the bands which appeared in the marker and disappeared of (ES) larvae, possible clarification by referring to the table (1), as there are two bands appeared in (*L. sericata*) with molecular weights (<150 kDa.) and concentration (7.8% & 2.9%), while disappeared in the marker, the 1st band of the marker with MW (150 kDa.) and concentration (8.4%) has not been matched with MWs of all (ES) larvae and the next band appeared in *M. domestica* and *C. albiceps* with MW (143.33 kDa.) with concentrations (1.2%) and (1.5%). It is also noticeable that there are two bands appeared in *L. sericata, M. domestica* with MW (141.67) kDa. With concentration (0.6%), consequently.

The next band in marker appeared in MW (126.67 kDa.) with (0.1%) for *L. sericata* and run across of *C. albiceps* and *M. domestica* with concentrations (2.6 & 2.8%),

respectively appeared in marker with MW (120 kDa.), the follow down with MW (102.67 kDa.) and the concentrations (5.1 & 5.4%) appeared in *C. albiceps* and *M. domestica* respectively but disappeared in marker, and the molecular weight (100 kDa.) appeared in marker with concentration (32%) while disappeared in all of (ES), the 5th band of *L. sericata* appeared with concentration (7.6%) and MW (98.29 kDa.)

The 4th band of *C. albiceps* which appeared with MW (94.29 kDa.) and concentration (6.1%), followed by *L. sericata* and *M. domestica* bands with MW (92 kDa.) with both concentration (16.2%) but disappeared in marker, followed by *C. albiceps* and *M. domestica* bands with MW (86.86 kDa.) and concentrations (14.8 & 13.9%), respectively, but disappeared in marker, followed by solo band for *L. sericata* with MW (85.71 kDa.) and concentration (10%) but disappeared in marker. The 4th band of marker appeared in MW (80 kDa.) with concentration (8.8%) while disappeared in all of species, the band with concentrations (2.5 & 2%) with MW (73.85 kDa.) appeared in *C. albiceps* and *M. domestica* respectively, but disappeared in marker, the molecular weight (72.31 kDa.) with concentration (1.3%) just appeared in *L. sericata* where disappeared in marker, followed by MW (68.85 kDa.) with concentrations (6.9 & 6.7%) appeared in *C. albiceps* and *M. domestica* respectively, but disappeared in marker.

The protein band which appeared in MW (67.31 kDa.) in *L. sericata* with concentration 6.4% and disappeared in marker, followed by MW (60 kDa.) just appeared in marker with concentration (33.3%).

The 8th and 9th bands of both *C. albiceps* and *M. domestica* appeared in MWs (59.09 & 57.95 kDa.) with concentrations (5.4 & 0.8%) for *C. albiceps* and for *M. domestica* (5.1 & 0.9%), respectively, but disappeared in marker. The 10th and 11th bands of *L. sericata* appeared in MWs (57.5 & 55.91 kDa.) with concentrations (2.4 & 0.3%), respectively, but disappeared in marker, the molecular weight of (52.27 kDa.) just appeared in *C. albiceps* and disappeared in marker with concentration (1.4%), we find the concentration (0.0%) just appeared in both *L. sericata*, *M. domestica* and disappeared in marker.

The concentration (0.8%) just appeared in *L. sericata* and disappeared in marker with MW (43.41 kDa.), it is possible to called the previous molecular weight with (Ovalbumin), is the main protein found in egg white, making up 60-65% of the total protein. Ovalbumin displays sequence and three-dimensional homology to the serpin superfamily, but unlike most serpins. The function of ovalbumin is unknown, although it is presumed to be a storage protein. The 6th band of marker showed in MW (40 kDa.) with concentration (6.1%), but there are no bands showed in all tested species.

The concentration (36.9%) just showed in *L. sericata* with MW (33.33 kDa.), followed by (46.9%) showed in *C. albiceps* with MW (32.98 kDa.), by next band in the same species showed with MW (21.05 kDa.) in concentration (6.3%), it is possible to call the previous molecular weights with (Carbonic anhydrase), several forms of carbonic anhydrase occur in nature, the primary function of carbonic anhydrase is to interconvert carbon dioxide and bicarbonate to maintain acid-base balance in blood and other tissues, and to help transport carbon dioxide out of tissues.

Finally, the last band in *L. sericata* obtained with MW (20.35 kDa.) with (6.7%), may be named the protein with the previous molecular weight with (Trypsin), as it is found

in the digestive system of many vertebrates, where it hydrolyses proteins. Trypsin is formed in the small intestine. It is used for numerous biotechnological processes.

Determination of Amino Acids of maggot (ES) for *L. sericata, C. albiceps and M. domestica*:

Data are given in table (2) showed the determination and concentration of amino acids detected for maggots (ES) of *L. sericata, C. albicep* and *M. domestica*, and obtained 14 amino acids and observed the Methionine is the lowest percent amino acid in *L. sericata, C. albicep* and *M. domestica* (0.93, 1.08 & 1.03 µg/ml), with concentrations (1.03, 0.94 & 0.61 %), frequently, in the other hand the highest percent recorded to Glutamic amino acid in *L. sericata, C. albicep* and *M. domestica* (26.18, 29.78 & 27.43 µg/ml), with concentrations (28.86, 26.05 & 25.84 %), frequently. Determination of amino acid derivatives for maggots (ES) showed 4 groups as Aspartate group that included (Aspartic acid, Methionine and Threonine), Pyruvate group (Serine and Histidine), Glutamate group (Glutamic acid, Proline and Arginine), and Aromatic group (Tyrosine and Phenylalanine).

Table (2): Determination and concentration of amino acids detected for maggots (ES) of *L. sericata, C. albicep* and *M. domestica*.

Amino Acids	*L. sericata*	%	*C. albiceps*	%	*M. domestica*	%
Threonine	2.68	2.95	3.12	2.73	2.42	1.85
Valine	2.47	2.72	4.98	4.36	3.67	2.67
Methionine	0.93	1.03	1.08	0.94	1.03	0.61
Isoleucine	2.7	2.98	3.82	3.34	2.73	2.39
Leucine	4.15	4.57	5.94	5.2	4.82	4.2
Aspartic	5.22	5.75	8.36	7.31	7.66	7.11
Glutamic	26.18	28.86	29.78	26.05	27.43	25.84
Serine	2.09	2.3	3.18	2.78	2.45	2.1
Histidine	5.39	5.94	6.82	5.96	5.37	5.63
Arginine	5.19	5.72	6.48	5.67	5.71	5.22
Alanine	5.3	5.84	6.52	5.7	6.13	5.73
Tyrosine	3.92	4.32	5.28	4.62	4.92	4.21
Phenylalanine	1.19	1.31	2.77	2.42	1.97	1.52
Proline	11.18	12.32	9.23	8.07	10.23	9.09
Total A.A	78.59		97.36		86.54	

1. **Classification of Amino Acids of maggot's (ES) into Essential and non-Essential:**

The classification of amino acids into essential and non-essential amino that the body cannot manufacture and therefore it must be obtained from its food sources of insect protein, the essential amino acids that have been investigated from the (ES) of tested species was 7 amino acids (phenylalanine, Valine, Theronine, Methionine, Isoleucine, Lysine and Histidine), Non-essential amino acids are amino acids that the body can manufacture itself and can be obtained from plant source, non-essential amino acids that have been investigated from (ES) of tested species was 7 amino acids (Alanine, Arginine, Asparagine, Aspartic acid, Glutamic acid, Proline, Serine and Tryosine), showed in table (3).

Table (3): Classification of Amino Acids into Essential and non-Essential for maggots (ES) of *L sericata, C. albiceps and M. domestica*.

E.A.A	*L. sericata*	*C. albiceps*	*M. domestica*	n-E.A.A	*L. sericata*	*C. albiceps*	*M. domestica*
Threonine	2.68	3.12	2.42	Aspartic	5.22	8.36	7.66
Valine	2.47	4.98	3.67	Glutamic	26.18	29.78	27.43
Methionine	0.93	1.08	1.03	Serine	2.09	3.18	2.45
Isoleucine	2.7	3.82	2.73	Arginine	5.19	6.48	5.71
Leucine	4.15	5.94	4.82	Alanine	5.3	6.52	6.13
Histidine	5.39	6.82	5.37	Tyrosine	3.92	5.28	4.92
Phenylalanine	1.19	2.77	1.97	Proline	11.18	9.23	10.23
Total	19.51	28.53	22.01	Total	59.08	68.83	64.53

DISCUSSION

1. Electrophoretic proteins of maggot's excretion/secretion.

Insect respond to the bacterial and fungal challenge or injury by rapid production of potent bactericidal and fungicidal peptides/polypeptides that have a broad spectrum of activity against both Gram-positive and Gram-negative bacteria and against fungi **(**Hoffmann, 1995; Hoffman *et al*., 1996).

The results of this study are comparable to those obtained by Tsuji *et al*., (1998), where they reported that (26 kDa.) protease showed antibacterial activity against several bacteria, but in the present study the antibacterial activity was (20 kDa.).

It was interesting to note that the larval extracts with protein bands of low-molecular weights recorded the highest activities against bacterial and fungal strains tested. In agreement with these results, Leem *et al*. (1999) suggested that these low-molecular weight proteins might play a role in the defense system of insects.

The analysis of native (ES) products from 3rd instar larvae of *L. sericata*, was in agreement with these results, Mariena *et al.*, (2007), where they showed that the molecules involved in beneficial effects of maggots are believed to be contained in their excretion/secretion.

As sown from the results of the present study, the proteins in secretory/excretory products with low molecular weights 20 kDa. are responsible for the antibacterial activity of the larval (ES). These results are consistent with those obtained by Chambers *et al.* (2003) and Taha *et al*. (2010), where they found the same protein band with molecular weights 20 kDa for secretory/excretory products from *L. sericata* larvae, respectively.

Several antibacterial proteins have been isolated and characterized from various insects, in the present study the antibacterial protein with a molecular weight of 20 kDa, this conclusion was similar to Abraham *et al*., (1995) which purified antibacterial protein with a molecular weight of 16 kDa in the silkworm, *Bombyx mori*, Lung *et al*., (2001) detected a 28 kDa protein derived from accessory glands of male *Drosophila melanogaster* which showed antibacterial activity.

The analysis of native (ES) products from 3rd instar larvae of *L. sericata*, was in agreement with these results, Van der plas *et al.*, (2008), where they showed that the molecules involved in beneficial effects of maggots are believed to be contained in their excretion/secretion.

Fu *et al*., (2009), evaluated purification and molecular identification of an antifungal peptide from Musca domestica hemolymph and they found that, the antibacterial and antifungal peptides found in *M. domestica* with large number are

indispensable components of its immune defense mechanism, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), they are showed its molecular weight was 17 kDa. Mass spectrometry showed the precise molecular weight of MAF-1 was 17203.384 Da. Its isoelectric point was acidic, this conclusion is similar to the present study.

2. Amino Acids Analysis of Maggots Excretion/Secretion.

The present study provides evidence, that the free amino acids were affected in (ES) and consequently affected the protein synthesis.

The results obtained in this study recorded 17 different amino acids in (ES), These results may be compared with that of Bischof (1996) who identified 16 amino acids in the haemolymph of control *L. dispar* larvae showing that histidine occurred at the highest concentration followed by alanine and cystine, the free amino acids of insect haemolymph are usually high variable in different species, successive instare and stages of a given species and also affected by the nature of diet (Jeuniaux, 1971).

Okada and Natori (1985) showed that the proteins induced in the haemolymph of larvae of *Sarcophaga peregrina* (flesh fly), were found to consist 39 of amino acid residues and to differ in only 2-3 amino acid residues. Matsuyama and Natori (1988) purified the other protein, named sapecin, is a new protein consisting of 40 amino acid residues.

Hara and Yamakawa (1995a) isolated three structurally novel antibacterial peptides from the haemolymph of the silkworm, *Bombyx mori*, these peptides were 32 amino acids. Hara and Yamakawa (1995b) showed that a novel antibacterial peptide from the haemolymph of the silkworm, *B. mori*. The novel peptide consisted of 42 amino acids.

Hemmi *et al.*, (2002) showed that a novel antibacterial peptide, moricin, isolated from the silkworm, *Bombyx mori*, consists of 42 amino acids, all the above conclusions were in consistency with the present study, in agreement with these results.

Bexfield *et al.*, (2010) reported that the amino acid-like compounds present in (ES) of *L. sericata* larvae may have a role in wound healing, by stimulating angiogenesis, in agreement with the present study.

The results obtain in this study recorded 14 different amino acids in (ES), these results may be compared with that of Miyanoshita *et al.*, (1996) who isolated a new family member of insect defensin an antibacterial peptide, from larvae of a beetle, *Allomyrina dichotoma*. The peptide consisted of 43 amino acids and 6 cystein residues were conserved in the same position as that of other insect defensins. The new defensin was found to be inducible by bacterial injection.

The results of Ueda *et al.*, (2005) are in agreement with the present study, where they demonstrated, purified a novel antibacterial peptide from the hemolymph of the coleopteran insect *Acalolepta luxuriosa*, of the family Cerambyocidae, and named it luxuriosin. This peptide showed growth-inhibitory activity against *Micrococcus luteus* and germination- and/or growth-inhibitory activity against the conidia from rice blast fungus, *Magnaporthe grisea*. But it differs in the amino acid sequence determined and identified luxuriosin as a peptide of 88 amino acids with a theoretical molecular weight of 10368.34 kDa and in the present study has been determined and identified to 14 amino acids with a theoretical molecular weight of 261.49 kDa for *L. sericata, C. albiceps* and *M. domestica*.

Conclusion:

Electrophoretic protein analysis varied from the different tested species and showed that the antibacterial agents present in (ES) of maggots with molecular weights ranging from (20-

150) kDa. and the highest antibacterial activity was to low molecular weight (20 kDa.)

Amino acid-like compounds present in (ES) of *L. sericata, C. albiceps* and *M. domestica* larvae in order to determine the compounds present and their potential role in the wound healing process. Amino acid analysis varied different tested species and classified to 14 amino acids in (ES).

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