Effect of a chitin synthesis inhibitor and a waste product on embryogenesis of Musca domestica

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Abstract: The change in the amounts of DNA, RNA and total protein content (quantitative and qualitative) during embryogenesis of normal and treated *Musca domestica* eggs (with Lufenuron and waste product) were investigated. DNA content increased gradually reaching its maximum level (2.2µg/ 10 mg DNA) at 7 hrs postoviposition (late embryogenesis). However ,RNA content and total protein decreased gradually reaching their minimum levels (5.3µg/10mgRNA, 1.17mgprotein/10mg egg) at 7 hrs postoviposition (late embryogenesis).Treatment of *Musca domestica* eggs with a chitin synthesis inhibitor (Lufenuron) and a waste product from rice straw resulted in a significant decrease DNA and RNA content throughout embryogenesis (0-7hr post oviposition).The treatment also resulted in a significant decrease in total protein of waste product treated eggs compared with the control except at 0hr old eggs (early embryogenesis). Treatment with Lufenuron resulted in a significant increase of total protein in treated eggs compared with the control at 0hr old eggs and 7hrs old eggs (late embryogenesis).However the protein content decreased ($p>0.05$) in Lufenuron treated eggs to a level similar to that of control at 1, 3, 5 hrs postoviposition(cleavage, gastrulation and organogenesis). The protein profile of normal and treated eggs at different time intervals (0 , 1, 3 , 5 and 7 hrs) during embryogenesis was evaluated to explain their mode of action. A total of 6 -13 protein bands with molecular weight of 5 -120 kilodaltons (KDa) were separated by electrophoresis during normal embryogenesis of *Musca domestica* . Treatment of *Musca domestica* eggs with Lufenuron resulted in separation of 9 – 11 protein bands with molecular weight of 25 -150 KDa. Treatment of *Musca domestica* eggs with waste product resulted in separation of 13 -16 protein bands with molecular weight of 10-200 KDa. The appearance and disappearance of certain protein fractions by application of these compounds may explain their ovicidal activity and disorders occurred during embryogenesis.

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Introduction

The housefly, *Musca domestica* is one of the medically important insects worldwide. Their adults are not only pestiferous insect in human environment (**Zumpt, 1965; Greenberg, 1971**), but also medical carriers and reservoir hosts of several pathogens, i.e., bacteria, viruses, protozoon cysts, and helminth eggs which can cause disease in humans (**Monzon *et al.*, 1991, Fotedar *et al.*, 1992; Sukontason *et al.*,2007**). The larvae of this fly can also be myiasis – producing agents in humans and animals, thus leading to economic loss, particularly in agronomic livestock (**Zhu and lin, 1999; Jiang, 2002; Sehgal *et al.*, 2002**).

Chitin synthesis inhibitors proved to be active in controlling a variety of insects especially those of orders *Lepidoptera*, *Diptera* and *Coleoptera* (**Grosscurt, 1978**). Numerous studies have demonstrated that benzoylphenyl urea compounds act to inhibit chitin synthesis in a variety of insects (**Verloop and Ferrell, 1977; Marks *et al.*, 1982)**. The exact step in chitin biosynthesis that is inhibited is unclear, but may include the polymerization stage in chitin biosynthesis(**Hajjar and Casida, 1978**) or a step in chitin precursor transport **(Nakagawa and Matsumura, 1994)**. The need for environmental safe, degradable and target specific insecticides has directed the search toward the plant kingdom, where many plant chemical have larvicidal, pupicidal and adulticidal activities. Most of these chemicals being repellants, ovipositional deterrents and antifeedant against both agricultural pests and medically important insect species (**Miller and chamberlain**,**1989; Sharma *et al.*, 1993** ; **Su and Mulla**,**1998 ; Dillio *et al.*,1999 ; Bakr *et al.*, 2010)**.

Such intoxication effects were found correlated with some biochemical changes in the tested species particularly in a decrease or increase of the total and certain protein fraction pattern , which may lead to certain functional and physiological interactions (**Mohamed and Hafez, 2000**)

Effects of IGRs on the protein content of *M. domestica* were studied by **Ishaaya and Casida (1974)** with diflubenzuron; **Hamdy (1988)** with Chlorfluazuron and teflubenzuron. Effects of several aziridinyl compounds on protein patterns of haemolymph, eggs and ovaries of the house flies were studied by **Gadallah *et al.* (1972)**.

The present study was conducted to study the effect of Lc50 dosage of a chitin synthesis inhibitor (Lufenuron) and a waste product of *Oryza sativa* on total protein (quantitative and qualitative) , RNA and DNA contents of housefly, *Musca domestica* during embryogenesis(0-7hr postoviposition).

2. Materials and Methods

Insect rearing:

The housefly, *Musca domestica L.* was obtained from Institute Of Medical Entomolgy,Ministry of Health, Dokki,Giza, Egypt. Both sexes were reared in wire cages with wooden frames (30 x 30 x 30 cm) at 27 ± 1 ºC, 60 – 70% RH, and constant light as described by **Rockstein (1957) and Busvine (1962).**

Tested compounds

- A chitin Synthesis inhibitor **Lufenuron** 10% EC) of chemical formula :
  N.[2, 5-dichloro-4-(1, 1, 2, 3, 3, 3-hexafluoroproxy) - phenyl/amino] -2, 6-difluorobenzamide.
- Waste product from rice straw (*Oryza sativa*)

Bran of oryza sativa was extracted by different solvents (acetone and ethanol), each extracted , **Bakr *et al.* (2006).**

Solvent was evaporated till dryness.

- **.Egg treatment**

Newly laid eggs (0-15 min) postoviposition were immersed by dipping technique in 1000 ml
water containing one of the four different doses 0.1, 0.5, 1 and 2 ppm for **Lufenuron** inhibitor and in 1000 ml water containing one of the four different doses (1, 10, 100 and 200 ppm) for the water product / 30 eggs. Thirty eggs were used for each concentration and repeated 3 times (30x 3). Eggs were dipped for 1 minute in each solution.

Each of the selected compounds were applied to newly oviposited (0 – 15 min) housefly eggs by dipping technique for 1 min at concentration of Lc 50. Different time intervals (0, 1, 3, 5 &7 hrs postovipostion) during embryogenesis were submitted to biochemical studies.

Biochemical studies:

Total protein content.

Ten mg of egg samples were homogenized with 0.1 ml of 50% of sucrose in 0.1 M tris- HCL buffer PH 7.3. The homogenate was diluted to 2 ml with the same buffer and then centrifuged at 4000 r.p.m. for 20 min. The soluble protein content of the supernatant was determined by the method of **Lowery *et al.* (1951)** using bovine albumim as standard .The values indicated in the tables represent average of three determination. Protein concentration was determined by UV absorbance at 595nm.

Electrophoretic analysis:

Eggs were homogenized with liquid nitrogen and buffer solution; (00.125MTris, 2% SDS, 10%glycerol, 5% 2-mercaptothanol, 0.001% bromophenol blue) centrifuged at 4000 r.p.m. for 20 min. at 4°C. The supernatants were withdrown carefully using automatic pipettes and transferred to a new Eppendorf tube and kept frozen at - 70°C till used.

Proteins were separated by polyacrylamide gel electrophoresis according to the method of **Smith (1976**); using slab gel that consists of 4% polyacrylamide stacking gel and an acrylamide gel (10%). High and low molecular weight standards were used for the determination of protein profiles of all fractionated samples. Electophoresis was done at 27 ± 2ºC using 20 mA/plate for about 4hrs. The gels were then stained with coomassie blue for protein. The gels were photographed and scanned using a gel-pro analyzer (version 3.1) for the protein analysis of tested samples.

DNA level and RNA content

DNA and RNA were extracted using DNeasy mini kit and RNeasy mini kit **(Qiagen, Germany)** according to the manufacturers'instruction **(Pinto *et al.*, 2003)**. DNA and RNA concentration were determined by UV absorbance at 260nm.

Statistical analysis:

Mean and standard error was analyzed by SPSS ver .16 while analysis was carried out using STATISTICA ver 6. The level of significance was expressed as highly significant ($p \leq 0.001$), significant ($p \leq 0.05$) and non – significant ($p > 0.05$).

3. Results

Total protein content

In normal eggs, protein content increased during the first hr (2.8 mg protein/ 10mg egg) ($P<0.05$)(cleavage) and then decreased ($P<0.05$) gradually reaching a minimum level at 7hrs postoviposition (1.17mg Protein /10 mg egg) (Table 1). In eggs treated with Lufenuron the protein content was higher than that of the control(4.3mg protein / 10mg egg) ($P<0.05$) in newly deposited eggs (0 – 15 min) postovipostion and at 7hrs postovipostion (3.1 µg Protein \mg egg). However the protein content decreased ($P \geq 0.05$) in Lufenuron treated eggs to a level similar to that of control at 1 ,3, 5hrs postovipostion . In eggs treated with waste product, the protein content decreased gradually (1-7hr) reaching a minimum level at 7hr postovipostion

(1.53µg Protein \mg egg). There was a significant ($P<0.05$) decrease in protein content between waste product treated eggs and the control at all the examined periods during embryogenesis(0-7hr) except at 0 hr postoviposition.

Electrophoretic analysis of protein

Changes in total protein profile of treated *Musca domestica* eggs with Lufenuron and waste product as compared with control group are represented in tables (4-8) and figures (1-3).

- **At 0 hr postoviposition.**

Six bands, No. 3, 7, 8, 13, 14, 16 (Mwt ranging from 120- 52 KDa) disappeared in the treated eggs with both compounds. Bands No. 1, 4, 6, 10, 15, 18, 19, 22, 24 (Mwt ranging from 142.7- 29 KDa) were detected only in treated eggs with Lufenuron whereas they disappeared in control and eggs treated with waste product. Bands No. 2, 5, 9, 11, 12, 17, 20, 21, 23, 25, 27, 28, 29, (Mwt ranging from, 126.6 - 10 KDa) were detected only in eggs treated with waste product (Table 4).

Densitometry scanning of electropherogram of SDS revealed that band No. 13 (Mwt67 kDa) showed the highest concentration (15.7%) in control group as shown in table (4), while band No. 14 (Mwt 51 KDa) had the highest concentration in Lufenuron treated eggs (17.5%). In case of waste product treatment band No. 19 (Mwt 41.7 KDa) had the highest concentration (15.5%) (Table 4, Figs. 1-3).

- **At 1 hr postoviposition.**

9 Bands No. 4, 6, 8, 10, 13, 15, 16, 19 and 30 (Mwt ranging from 109-5 KDa) were detected only in control group. Whereas bands No. 7 & 21 (Mwt 92 & 34) were common between control and Lufenuron treated eggs. Bands No. 26 & 28, (Mwt 23 & 17, KDa) were common between control and waste product treatments. Bands No. 18 & 23 (Mwt 42 & 27 KDa, respectively) were common between Lufenuron and waste product treatments. Bands No. 1, 3, 9, 11, 12, 20, 22, 27 & 29 (Mwt 166.6, 112, 82, 70, 63.8, 32, 29, 19 & 9 KDa, respectively) were detected only in waste product treated eggs. While bands No. 2, 5, 14, 17, 25 (Mwt ranging from 119-24 KDa) were detected only in Lufenuron treated eggs (Table 5).

Densitometry scanning of electropherogram of SDS Figs (1 – 3) revealed that band No. 15 (Mwt 59.2 KDa) showed the highest concentration in control group (12.3 %) as shown in Table (5). While band No. 14 (Mwt 52.2 KDa) had the highest concentration in Lufenuron treated group (16.5 %). In waste product treated group band No. 18 (Mwt 42 KDa) had the highest concentration (15.5 %).

- **At 3 hr postoviposition.**

Bands No. 5, 6, 9, 13, 15, 17, 21 & 34 (Mwt ranging from 108- 4 KDa) were detected only in control group. While band No. 28 (Mwt 24.4 KDa) were common between control and Lufenuron treated groups. Bands No. 10, 25 (Mwt 81, 28.4 KDa, respectively) were common between waste product and Lufenuron treated group.

Band No. 11, 31 (Mwt 74, 17 KDa respectively) were common between control and waste product treated group. bands No. 1, 3, 8, 12, 19, 23,24, 26, 27, 29, 30, 32 (Mwt ranging from 166.6-15 KDa) were detected in only waste product treated groups. bands No. 2, 4, 7, 14, 16, 18, 20, 22 (Mwt ranging from 131.3-33.6 KDa) were detected only in Lufenuron (Table 6) .

Densitometry scanning of electropherogram of SDS (Figs. 1-3) revealed that band No. 13 (Mwt 60 KDa) had the highest concentration (12.3%) in control group, while band No. 14 (Mwt 52 KDa) had the highest concentration (14.4 %) in Lufenuron treated group as shown in table (6). On the other hand, band No. 19 (Mwt 41.7) had the highest concentration (15.6 %) on waste product treated group.

At 5 hrs postoviposition

Bands No. 2, 4, 6, 7, 12, 14, 16, 20, 23, 29, 33 (Mwt ranging from 122- 4 KDa) were present only in control group. In Lufenuron treated group bands No. 3, 24 (Mwt 121, 33 KDa respectively) were common with waste product treated group, While band No. 31 (Mwt 18 KDa) was common between control and waste product group. In waste product group bands No. 1, 9, 11, 13, 18, 22, 25, 26, 27, 30, 32 (Mwt ranging from 211.1-15 KDa) were detected only in this group. Bands No. 5, 8, 10, 15, 17, 19, 21, 28 (Mwt ranging from 108- 33 KDa) were detected only in Lufenuron treated group (Table 7) .

Densitometry scanning of electropherogram of SDS (Figs 1-3) revealed that band No. 14 (Mwt 58 KDa) had highest concentration (10.3%) in control group. Band No. 15 (Mwt 52 KDa) had highest concentration (14.4%) in Lufenuron treated group.while band No.18 (Mwt 43 KDa) had highest concentration (14.2%) in waste product treated group as shown in table (7).

At 7 hrs postoviposition

Bands No. 3, 5, 7, 8, 10, 13, 15, 20, 30 (Mwt ranging from 118-5 KDa, respectively) were detected only in control group. While band No. 17, (Mwt 43 KDa) were common between Lufenuron treated eggs and control group. In Lufenuron treated group bands No. 9, 19, 22 (Mwt 83, 35, 29 KDa respectively) were common with waste product treated group. Band No. 24, 27 (Mwt 26, 21 KDa) were common between control and waste product treated eggs. Bands No. 1, 6, 11, 12, 16, 26, 28, 29 (Mwt ranging from 130-16 KDa) were only detected in waste product treated group. Bands No. 2, 4, 14, 18, 25 (Mwt ranging from

124- 24 KDa) were detected only in Lufenuron treated group(Table 8)

Densitometry scanning of electropherogram of SDS (Figs 1-3) revealed that band No. 13 (Mwt 63.8 KDa) had highest concentration (13.3%) in control group. Band No. 14 (Mwt 51 KDa) had highest concentration (17.5%) in Lufenuron treatment. Band No. 16 (Mwt 45 KDa) had highest concentration (14.8%) in waste product group as shown in table (8).

DNA Level and RNA Content:

In normal eggs, the DNA content recorded a gradual increase ($P<0.05$) during cleavage, gastrulation and organogenesis, until hatching at 7hr postoviposition (2.2µg DNA /10mg egg). However, there was a significant decrease ($P<0.05$) between both treated eggs with Lufenuron and waste product, and that of the control throughout embryogenesis (0-7hrs postovipostion) (Table 2).

On the other hand, RNA content decreased ($P<0.05$) gradually during normal embryogenesis reaching a minimum level at 7hrs postoviposition (5.34µg RNA/10mg egg) .There was also a significant decrease ($P<0.05$) between both treated eggs with Lufenuron and waste product), in comparison with the control throughout embryogenesis (0-7hrs postovipostion) (Table 3).

Comparing between DNA and RNA content in both Lufenuron and waste product treated eggs, there was a significant decrease between waste product and Lufenuron treated eggs (except at 3 and 7 hrs postoviposition). The same significant decrease in protein content was recorded (except at 3 hrs postoviposition) throughout embryogenesis.

Table (1) Total protein content in normal and treated *Musca domestica* eggs.

Incubation Hours	mg Protein /10mg egg (Mean±S.E)		
	Control	Lufenuron	Waste product
0	2.7± 0.05	4.3 ± 0.8	3 ± 0.17
1	2.8± 0.05	2.5 ± 0.05	1.8 ± 0.005
3	2.5± 0.05	2.4 ± 0.05	1.74 ± 0.005
5	2.3 ± 0.05	2.5 ± 0.05	1.68 ± 0.005
7	1.17± 0.005	3.1 ± 0.05	1.53± 0.005

Table (2) DNA level in normal and treated *Musca domestica* eggs.

Incubation Hours	µg DNA /10mg egg (Mean±S.E)		
	Control	Lufenuron	Waste product
0	1.24 ±0.005	1.5 ±0.005	1.1 ±0.005
1	1.28 ± 0.005	1.1 ± 0.005	1.1 ±0.005
3	2.1±0.005	1.2 ±0.008	1.43± 0.05
5	2.12 ± 0.005	1.3 ±0.005	1.5 ± 0.05
7	2.2 ± 0.005	2 ± 0.005	1.57 ±0.05

Table (3) RNA content in normal and treated *Musca domestica* eggs

Incubation Hours	µg RNA /10mg egg (Mean±S.E)		
	Control	Lufenuron	Waste product
0	7.25 ± 0.005	5.4 ± 0.05	4.1 ±0.05
1	6.18 ± 0.005	4.7 ± 0.05	4.2 ± 0.05
3	5.79± 0.005	3.9 ± 0.05	3.75± 0.08
5	5.43 ±0.005	4± 0.03	3.87 ±0.005
7	5.34± 0.005	4.4 ± 0.05	3.9 ± 0.05

Table 4: Molecular weight and concentration of different SDS protein bands detected at 0 hr *Musca domestica* eggs treated with Lufenuron and waste product.

Band number	**Molecular weight (KDa)**	**Control (%)**	**Lufenuron (%)**	**Waste product (%)**
1	142.7	-	1.55	-
2	126.6	-	-	1.69
3	120	3.59	-	-
4	119.6	-	3.44	-
5	107	-	-	1.34
6	106	-	2.03	-
7	102	3.84	-	-
8	94.3	4.32	-	-
9	97.6	-	-	1.42
10	84.1	-	5.53	-
11	80	-	-	2.56
12	68	-	-	3.65
13	67	15.7	-	-
14	57	4.73	-	-
15	51	-	17.1	-
16	52	3.99	-	-
17	49	-	-	15.5
18	45	-	3.25	-
19	41	-	6.72	-
20	38	-	-	2.77
21	36	-	-	5.37
22	35	-	4.06	-
23	30	-	-	7.08
24	29	-	5.47	-
25	26	-	-	3.5
26	25	-	8.53	-
27	22	-	-	3.09
28	18	-	-	7.46
29	10	-	-	5.4
Total number of bands		6	10	13

Table 5: Molecular weight and concentration of different SDS protein bands detected at 1 hr *Musca domestica* eggs post treatment with Lufenuron and waste product

Band number	**Molecular weight (KDa)**	**Control (%)**	**Lufenuron (%)**	**Waste product (%)**
1	166.6	-	-	1.85
2	119	-	2.98	-
3	112	-	-	2.53
4	109.8	1.91	-	-
5	105.2	-	2.31	-
6	100.3	4.15	-	-
7	92	4.48	4.72	-
8	84	2.84	-	-
9	82	-	-	3.72
10	74	3.98	-	-
11	70	-	-	4.79
12	63.8	-	-	6.02

13	50.3	3.93	-	-
14	52.5	-	16.5	-
15	59.2	12.3	-	-
16	46	1.96	-	-
17	45	-	3.15	-
18	42	-	6.54	15.5
19	41	4.43	-	-
20	32	-	-	4.9
21	34	3.82	3.8	-
22	29	-	-	5.99
23	27	-	4.07	3.65
24	26	-	-	-
25	24	-	8.34	-
26	23	9.25	-	5.61
27	19	-	-	3.82
28	17	4.03	-	6.81
29	9	-	-	2.8
30	5	5.56	-	
Total number of bands		13	9	13

Table 6: Molecular weight and concentration of different SDS protein bands detected at 3 hr *Musca domestica* eggs post treatment with Lufenuron and Waste product

Band number	Molecular weight (KDa)	Control (%)	Lufenuron (%)	Waste product (%)
1	166.6	-	-	1.68
2	131.3	-	2.31	-
3	121.1	-	-	1.71
4	112.1	-	1.81	-
5	108	1.65	-	-
6	99	4.26	-	-
7	97	-	3.05	-
8	92	-	-	1.74
9	90	4.1	-	-
10	81	-	3.94	2.63
11	74	4.1	-	4.57
12	62	-	-	4.89
13	60	12.3	-	-
14	52	-	14.4	-
15	51	4.66	-	-
16	44.4	-	3.3	-
17	43.8	5.01	-	-
18	42.1	-	1.76	-
19	41.7	-	-	15.6
20	38.2	-	2.93	-
21	35.3	3.13	-	-
22	33.6	-	3.08	-
23	32.8	-	-	5.91
24	29.8	-	-	2.28
25	28.4	-	6.39	4.08
26	26.1	-	-	4.05
27	23.6	-	-	2.98
28	24.4	6.54	10.2	-
29	19	-	-	5.12
30	18	-	-	7.39
31	17	4.61	-	6.9
32	15	-	-	2.29
33	8	-	-	-
34	4	5.15	-	-
Total numbers of bands		11	11	16

Table 7: Molecular weight and concentration of different SDS protein bands detected at 5 hr *Musca domestica* eggs post treatment with Lufenuron and Waste product.

Band number	Molecular weight (KDa)	Control (%)	Lufenuron (%)	Waste product (%)
1	211.1	-	-	1.74
2	122	1.66	-	-
3	121	-	1.93	2.16
4	109	1.81	-	-
5	108	-	3.04	-
6	97	3.86	-	-
7	89	4.18	-	-
8	86	-	4.39	-
9	84	-	-	2.97
10	77	-	3.61	-
11	74	-	-	5.15
12	70	4.14	-	-
13	63	-	-	5.66
14	58	10.3	-	-
15	52	-	14.4	-
16	49	5.17	-	-
17	45	-	2.37	-
18	43	-	-	14.2
19	42	-	3.11	-
20	41	5.16	-	-
21	38	-	4.24	-
22	36	-	-	4.17
23	34	5.55	-	-
24	33	-	2.48	4.84
25	30	-	-	2.69
26	27	-	-	5.59
27	25	-	-	4.2
28	24	-	5.67	-
29	23	7.67	-	-
30	21	-	-	4.63
31	18	4.67	-	6.61
32	15	-	-	4.25
33	4	6.8	-	-
Total numbers of bands		12	10	14

4. Discussion

Ecdysteriods, juvenile hormone and chemosterilant disturb the synthesis of protein and nucleic acids in many insect species **Painter and Kilgore (1967)**; **Kamel *et al.*, 1982; Gadallah *et al.* (1989).** In the present study, it appeared that chitin synthesis inhibitor and waste product of rice straw also induce disturbance of protein and nucleic acid synthesis in treated eggs. It is not clear, whether this chemical has a direct or indirect effect on protein and nucleic acid synthesis in the developing eggs. The change in total protein concentration between control and treated eggs of *Musca domestica* may be attributed to the toxic action of the tested compounds which change the expression process of protein. The protein type has a specific biological role, due to this role the DNA secretes enzymes that act as catalysts to produce specific type of protein, this protein is responsible for a specific biological process, **Bakr *et al.* (2010).**

Table 8: Molecular weight and concentration of different SDS protein bands detected at 7 hr *Musca domestica* eggs post treatment with Lufenuron and Waste product .

Band number	Molecular weight (KDa)	Control (%)	Lufenuron (%)	Waste product (%)
1	130	-	-	1.89
2	124	-	3.5	-
3	118	2.25	-	-
4	110	-	4.18	-
5	109	2.1	-	-
6	102	-	-	2.07
7	99	4.13	-	-
8	91	3.67	-	-
9	83	-	4.68	2.45
10	80	4.11	-	
11	75	-	-	2.22
12	66	-	-	5.68
13	63.8	13.3	-	-
14	51	-	17.5	-
15	50	4.52	-	-
16	45	-	-	14.8
17	43	4.78	3.6	-
18	40	-	4.35	-
19	35	-	3.94	4.74
20	33	6.53	-	-
21	32	-	-	-
22	29	-	5.07	4.3
23	28	-	-	-
24	26	5.91	-	9.06
25	24	-	9.03	-
26	22	-	-	4.27
27	21	5.05	-	5.16
28	18	-	-	6.25
29	16	-	-	4.63
30	5	6.82	-	-
Total numbers of bands		12	6	13

In the present study,the amount of total protein content of normal eggs decreased during the period of embryogenesis(0 hr- 7 hr) . In eggs treated with Lufenuron, the protein content was higher than that of the control eggs ($P<0.05$) in newly oviposited eggs (0 – 15 min) postovipostion and in 7 hr postovipostion .However the protein content decreased ($P\geq0.05$) in Lufenuron treated eggs to a level similar to that of the control at 1, 3, 5hr postovipostion . In eggs treated with waste product the protein content decreased gradually(1-7 hr) reaching its minimum level at 7 hrs postovipostion. There was a significant decrease ($P<0.05$) between waste product treated eggs and control (except in 0 hr postovipostion).

Similar results were reported in *Argas arboreus* **(Gadallah *et al.*, 1989)** where the total protein decreased gradually during embryogenesis. However , in *Hyalomma dromedarii* **(Kamel *et al.*, 1982)** recorded that total protein content of eggs remained unchanged and ; in ladybird beetles **Slogget and Lorenz(2008)**found that protein content declines weakly over development. Some of this protein are probably storage compounds that are degraded for use in the synthesis of enzymes and structural protein. However ,part of the degradation products may be used as a source of energy or used for synthesis of nonprotein components of the developing larva.

In the present study ,treatment of newly oviposited eggs (0 – 15 min postovipostion) of *Musca domestica* with Lufenuron and a waste product extract produced some differences in protein patterns(mobility and number of bands) in treated eggs compared with control group. Also , the molecular weight of protein bands is higher in the treated eggs with both compounds than the control; however the maximum weight(211.1 KDa) recorded for that treated with waste product. **Kamel *et al.* (1982), Gadalla *et al.*(1972 and 1989)** observed considerable changes in protein patterns from egg homogenates during embryogenesis of *Hyalomma dromedarrii* , *Argas arboreus* and housefly *Musca domestica* treated with 20-hydroxyecdysone juvenile hormone and a chemosterilant respectively.

In *Rhodnius prolixus,* **Kelly and Huebner (1987)** suggested that embryonic disturbance caused by treatment with fenoxycarb may be due to alterations in normal molecular events accompaning development. Three proteins bands normally absent, were expressed in fenoxycarb-treated embryos.

Bakr *et al.*(2010) beleived that proteins help to synthesize microsomal detoxifying enzymes. The change in arrangement of protein bands control group and treated eggs of *Musca domestica* may be attributed to the toxic action of the tested compounds which change the expression process of protein and may led to larval mortality and the appearance of latent effects on other developmental stages.

In the present study, the RNA content decreased ($P<0.05$) gradually reaching its minimum level at 7hr postoviposition in normal eggs. There was a significant decrease ($P<0.05$) between both treated (with Lufenuron and waste product) and control eggs throughout embryogenesis (0-7 hr postovipostion).

Chemosterilization of *Musca domestica*(**Gadallah *et al.*,1970**) during Oogenesis and embryogenesis alters DNA metabolism in both ovaries and eggs. Sexual sterilization may be due to alkylation of some portion of the DNA moiety in the ovary, which is in turn reflected in the inability of the eggs to synthesize DNA .

On the other hand, DNA content showed an increase during embryonic development (0-7 hr postovipostion).The same results was observed by (**Painter and Kilgore,1967 and 1973)** in house fly eggs from 1hr until time of hatching.

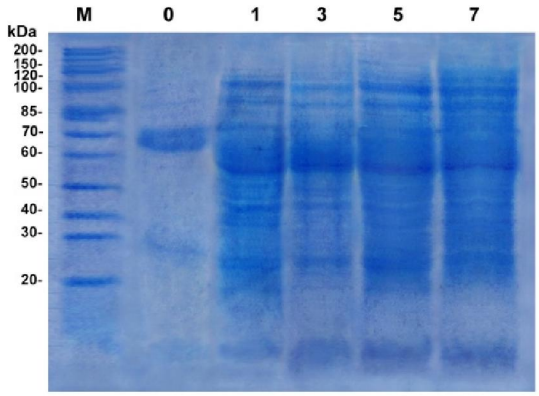

Fig (1): Protein banding pattern using SDS-PAGE technique of normal *Musca domestica* eggs at different time intervals
whereM: marker; 0:Newly oviposited eggs; 1: One hour old eggs; 3: Three hour old eggs; 5: Five hour old eggs; 7: Seven hour old eggs.

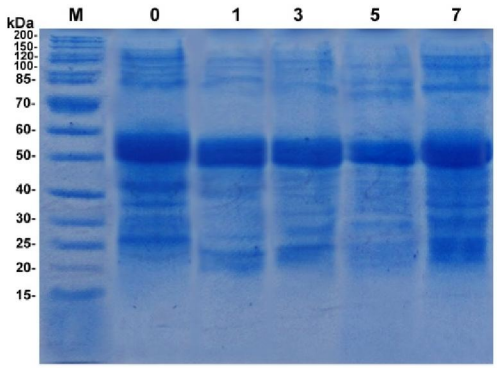

Fig (2): Protein banding pattern using SDS-PAGE technique of treated *Musca domestica* eggs with
Lufenuron at different time intervals
whereM: marker; 0:Newly oviposited eggs;
1: One hour old eggs 3:Three hour old eggs;
5: Five hour old eggs; 7: Seven hour old eggs

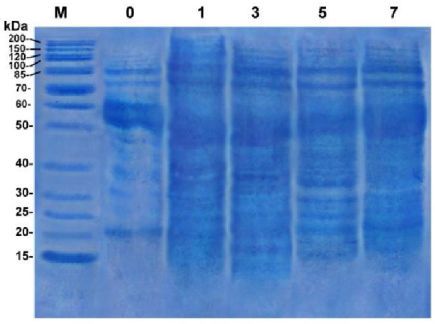

Fig (3): Protein banding pattern using SDS-PAGE technique of treated *Musca domestica* eggs with waste product at different time intervals
whereM: marker; 0:Newly oviposited eggs; 1:One hour old eggs; 3:Three hour old eggs; 5: Five hour old eggs; 7: Seven hour old eggs

Harris and forrest (1971) found comparable changes in DNA and RNA activity in the course of embryonic development of normal eggs of *Drosophila and Opeltus.* However, in *Tribolium confusum* the concentration of DNA and RNA showed a sharp ,steady increase at the beginning of embryogenesis, reached its maximum level, and finally dropped rapidly **(Kamel *et al.,* 1982)**. In *Argas arboreus* **,**similar results was observed by **Srinivasan and Kesavan(1979)** however, the minimum level was at 4 hr old eggs, this may indicate that RNA was consumed during the process of segmentation and differentiation of organs during this period. Whereas in *Hyalomma dromedarii* **(Kamel *et al.,* 1982)** the change in the amount of DNA and RNA exhibited a lag period of nine and six days, then a rapid increase occurred until hatching. **Harris and Forrest (1967)** studied DNA synthesis in the developing embryos of *oncopeltus* and showed that DNA increase rapidly up to gastrulation.

During embryogenesis marked changes in the total macromolecules observed because of cell division and differentiation of tissues. Changes in nucleic acids levels reflect the synthetic activities of the developing embryo and the degree of embryonic dependence on material of maternal origin. Housefly has a higher RNA content as the oocyte is supplied with RNA by nurse cell **(Srinivasan and Kesavan, 1979).**

The increase in DNA may be explained as related with nuclear divisions which are characteristically rapidly in housefly eggs as in *Drosophila melanogaster* and a complete mitotic cycle takes around 8 min (**West *et al.,* 1968**) . As in many other organisms, the insect egg contains a large amount of cytoplasmic DNA and this storage DNA is believed to supply precursors through the action of nucleases for such rapid nuclear multiplications **(Muhammed *et al.,* 1967).**

It is worth mentioning that the protein type has a specific biological role, due to this role the DNA secretes enzymes that act as catalysts to produce specific type of protein, this protein is responsible for specific biological processes. The alteration of protein bands in treated sample consequently affecting the biological process during embryogenesis. Protein fractions that fluctuated in number during embryogenesis might be due to the

protein breakdown and/or incorporation into other protein or both.

Results of this study may provide good evidence of possible use of chitin synthesis inhibitors and waste products in management of control programs of house fly during the stages of embryogenesis.

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