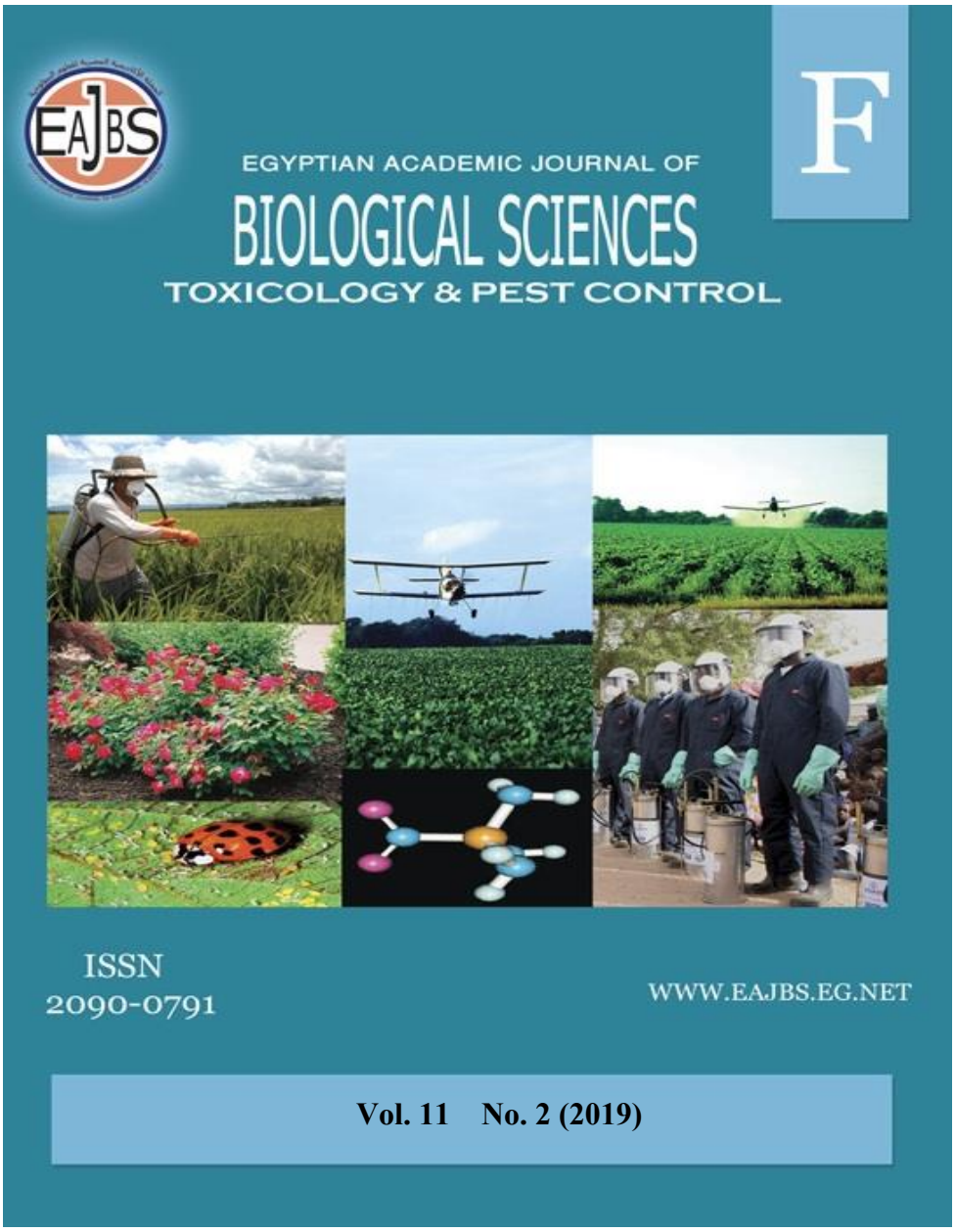

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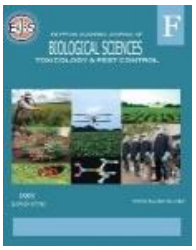

Toxicological and Biochemical Effects of Lufenuron and Rice Bran on Desert Locust, *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae)

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ABSTRACT

Lufenuron (chitin synthesis inhibitors) and *Oryza sativa* bran extract (waste product) exhibited insecticidal activity on females of *Schistocerca gregaria* when they were treated as one day old of the 5th nymphal instar with different concentrations of tested compounds. Some biological aspects, failure of ecdysis to adults, adult mortality, prolongation in the age of last nymphs and developmental events were investigated after treatment.

Quantitative and qualitative determination of protein and DNA contents were investigated in normal and affected eggs which resulted from females *Schistocerca gregaria* treated as one day old 5th nymphal instar with LC_{50} of Lufenuron and *Oryza sativa* bran extract during embryogenesis. The total protein content of normal eggs at 0-day old, was 81.07 mg protein/gm eggs, after one day of oviposition the total protein increase significantly ($p<0.05$). Then the protein content, during the early incubation period, to the 4th day post oviposition (pop) significantly ($p<0.05$) declined sharply to 38.74mg protein / gm eggs. While in the 7th, 11th &12th it was decreased significantly ($p<0.05$) during the late organogenesis stage. The treatment resulted a significant decrease in the total protein of rice bran extract affected eggs compared with the control at (1, 11& 12) days old eggs, and a significant increase at 0 and 4 days pop compared with control. Treatment with Lufenuron resulted a significant decrease of total protein in (0, 1 & 12) days old eggs compared with the control but there were no significant increase at 4, 7 and 11 old eggs. A total of (10 -12) protein bands were separated by electrophoresis during normal embryogenesis of *Schistocerca gregaria*. Fraction protein of affected eggs with LC_{50} of Lufenuron and rice bran extract resulted a separation of (10 – 14) and (11- 14) protein bands with molecular weights from 12 to 325 KDa. Data recorded significant differences between DNA content of normal and the affected *Schistocerca gregaria* eggs throughout embryogenesis (0-12 days) post-oviposition.

INTRODUCTION

Desert locust, *Schistocerca gregaria* (Forskal), is a serious polyphagous pest of crops. This species in its gregarious phase can cause up to 100% of crop loss (Simpson *et al.,* 1999), it is a threat to agricultural production in Africa and Western Asia for

thousands of years.

Current locust control system is mainly based on organophosphorus insecticides (Ghoneim *et al*., 2014). However, the widespread use of these chemicals resulted in inducing resistance by insect pests beside contamination of human food, mammalian toxicity, reducing beneficial non-target biota and environmental pollution (Garriga and Caballero, 2011).

IGRs are a class of new chemicals that interfere with maturation and reproduction in insects. They are not directly toxic, but act selectively on the development, metamorphosis or reproduction of the target insect species (Brar *et al.,* 2015).

Chitin synthesis inhibitors (CSIs) are usually classified in IGRs interfering with chitin biosynthesis in insects and thus prevent moulting, or produce an imperfect cuticle. These compounds have a good lethal effect on the desert locust (Azam and Seegh, 1993; Coppen and Jepson, 1996a; Bakr *et al.*, 2008).

Some plant extracts have thus become "active substances" of the so-called botanical insecticides (Regnault-Roger *et al*., 2012; Pavela, 2015; Zyaan *et al*., 2017), they have shown insecticidal properties on the development, growth, adult emergence, fecundity, fertility and embryogenesis of number of insect species including *Schistocerca gregaria* (Barbosa *et al*., 2011, França *et al*., 2012)

The aim of the present study is to evaluate the effect of the selected compound ,a chitin synthesis inhibitor (Lufenuron) and waste product of rice bran (*Oryza sativa*) on some biological aspects of *Schistocerca gregaria* female and biochemical effects on protein and nucleic acid (DNA) synthesis of *Schistocerca gregaria* egg, during embryonic development

MATERIALS AND METHODS

Experimental Insects:

The experimental nymphs were segregated from the gregarious stock colony of *Schistocerca gregaria* (maintained for several years at the Locust Research Department, Plant Protection Research Institute, Agricultural Research Center, Dokki, and Giza) at the beginning of the first nymphal instar and held up in cages (30x30x30 cm). The cages were wooden farmed equipped with zinc bottom covered by a thin layer of sand, glass-covered sides and a wire-gauze top provided with a little door. All cages were incubated at (32±2ºC) and (30-50% RH). Unconsumed food, dead locusts and feces were removed daily.

The whole cage was thoroughly washed and effectively sterilized with an antiseptic agent every (4-6 weeks) or whenever it becomes empty or at the end of any experiment.

Compounds Used:

One of Chitin Synthesis Inhibitors (CSIs), Lufenuron (EC.10%) and plant waste product extract, *Oryza sativa* bran extracted by petroleum ether according to the method described by (Bakr *et al.*, 2006).

Bioassays Studies:

Treatment of Experimental Insects:

One-day old females of the 5th nymphal instars of *S. gregaria* were treated by feeding technique with serial appropriate concentrations of Lufenuron (10%) and rice bran extract (*Oryza sativa*).

Leaves of *M. sativa* were immersed in 25, 50, 100 and 200 ppm and (5, 10, 15 and 20) ppm for lufeneroun and rice bran extract respectively. Leaves were dipped for two minutes in each concentration. They were air dried before being offered to the nymphs for feeding on it. Twenty nymphs were used for each concentration and the experiment

was repeated 3 times (20*3).

After feeding for 24 hours on the treated leaves, the alive nymphs were transferred to untreated leaves and left to feed for 24 hours. Mortality counts or malformed individuals were recorded. The LC_{50} was determined after 15 days of treatment.

Eggs from normal and adult females resulted from treated 5th nymphal instars with LC_{50}, were processed for biochemical studies.

Biochemical Studies:

Estimation of Total protein:

Total proteins of the eggs were estimated according to the method described by Bradford (1976). This method is based on the observation that Coomassi Brilliant Blue G-250 exists in two different color forms, red and blue. The red color is converted to the blue color upon binding to the protein; this binding causes a shift in maximum absorption of the dye from 465 to 595 nm. The intensity of the color was measured at 595 nm.

Electrophoretic Analysis of Proteins:

Eggs were homogenized with liquid nitrogen and a buffer solution (00.125 M Tris, 2% SDS, 10 ml glycerol, 5% mercaptoethanol, 0.001% bromophenol blue) centrifuged at 4000 r.p.m. for 20 min at 4°C. The supernatants were withdrawn 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).

Determination of DNA Level:

The eggs of *S. gregaria* were subjected to DNA extraction using the extraction genomic DNA kit.

Statistical Analysis:

The obtained data were subjected to analysis of variance using **Costat statistical software**

RESULTS

Biological Effect of Lufenuron(CSI) and *Oryza sativa* Bran Extract (Plant Waste Product) on the Development of *Schistocerca gregaria* Females:

1-Lethal and Malformation Effects of Lufenuron on *S. gregaria*:

Results in Table (1) showed the effects of lufenuron on the 5th nymphal instars of *S. gregaria* during one day old by feeding technique.

Data clear that the percentages of the 5th nymphal instars failed to ecdysis to adults increased significantly to be 0.0, 16.67, 18.33 and 26.67% after one day old treatment with 25, 50, 100 and 200 ppm of lufenuron, respectively compared with control (0.0%) (Fig. 3), whereas, the percentages of adult mortality significant increased to be 0.0, 16.67, 31.67 and 51.67%, respectively compared with control (0.0%). While, the percentages of malformed adults reached 76.67, 66.67, 50.00 and 21.67% after treated with 25, 50, 100 and 200 ppm of lufenuron concentrations, respectively compared with control (0.0%), on the other hand the percentages of non-malformed adults were 23.30 % for concentration 25 ppm and 0.0% for other concentrations compared with the control (100%) whereas no adult was alive.

Table (1): Effect of Lufenuron on some biological aspects of the desert locust, *Schistocerca gregaria* treated as 1-day old of the 5th nymphal instar females.

Conc. (ppm)	Nymphal instar			Adult stage					
	Failure to ecdysis to adults Mean ± SE	%	Duration of of the 5th nymphal instar/day Mean ± SE	Adult mortality Mean ± SE	%	Malformed adult Mean ± SE	%	Non malformed adult Mean ± SE	%
Control	0.0±0.0 c	0.0	8.0±0.5c	0.0±0.0 d	0.0	0.0±0.0 e	0.0	20.0±0.0 a	100
25	0.0±0.0 c	0.0	8.50±0.125c	0.0±0.0 d	0.0	15.33±0.2a	76.67	4.67±0.272b	23.3
50	3.33±0.272b	16.67	9.18±0.119b	3.33±0.272c	16.67	13.33±0.2b	66.67	0.0±0.0 c	0.0
100	3.67±0.272b	18.33	9.61±0.125b	6.33±0.272b	31.67	10.0±0.47c	50.00	0.0±0.0 c	0.0
200	5.33±0.272a	26.67	10.50±0.191a	10.33± 0.272a	51.67	4.33±0.27d	21.67	0.0±0.0 c	0.0
LSD 0.05	0.814		0.493	0.814		1.151		0.470	

LSD: Least Significant Deference
Means followed with the same small letter within the same column were not significantly difference $p < 0.05$.

2-Lethal and Malformation Effects of *Oryza sativa* Bran Extract on *S. gregaria* Females:

Data in Table 2 showed that the effects of rice bran extract on the 5th nymphal instars of *S. gregaria* of one day old after feeding technique that The percentages of nymphal instars failed to ecdysis to adults were 0.0, 8.33, 13.33 and 16.67% after treatment with rice bran extract concentrations (5, 10, 15 and 20) ×10³ ppm, respectively comparing with the control (0.0%) (Table 2). While, the percentages of adult mortality were 16.67, 28.33, 37.67 and 46.67%, respectively comparing with the control (0.0%).

Also, the percentages of malformed adults were 18.33, 36.67, 48.33 and 36.67% after treated with rice bran extract concentrations (5, 10, 15 and 20)×10³ ppm, respectively comparing with the control (0.0%). On the other hand, the percentages of non-malformed adults were 66.67, 26.67, 0.0 and 0.0%% after one day old of the 5th nymphal instars of *S. gregar* with rice bran extract concentrations (5, 10 and 20) ×10³ ppm, respectively compared with the control (100%).

Table (2): Effect of Rice bran extract on some biological aspects of the desert locust, *Schistocerca gregaria* treated as 1-day old of the 5th nymphal instar females.

Conc. (ppm)	Nymphal instar			Adult stage					
	Failure to ecdysis to adults Mean ± SE	%	Duration of the 5th nymphal instar/day Mean ± SE	Adult mortality Mean ± SE	%	Malformed adult Mean ± SE	%	Non malformed adult Mean ± SE	%
Control	0.0±0.0 c	0.0	8.0±0.0 c	0.0±0.0 e	0.0	0.0±0.0 d	0.0	100±0.0 a	100.0
5×10³	0.0±0.0 c	0.0	8.37±0.152 c	3.33±0.272 d	16.67	3.67±0.272 c	18.33	13.33±0.272b	66.67
10×10³	1.67±0.272 b	8.33	9.33±0.144b	5.67±0.272 c	28.33	7.33±0.272 b	36.67	5.33±0.272 c	26.67
15×10³	2.67±0.272 a	13.33	9.73±0.191ab	7.33±0.272 b	37.67	9.67±0.272 a	48.33	0.0±0.0 d	0.0
20×10³	3.33±0.272 a	16.67	10.07±144 a	9.33 ±0.272 a	46.67	7.33± 0.272b	36.67	0.0±0.0	0.0
LSD 0.05	0.814		0.548	1.231		0.939		0.664	

LSD: Least Significant Deference
Means followed with the same letter within the column were not significantly difference.

3-Prolongation Effect of Lufenuron and *Oryza sativa* Bran Extract Compounds on the Desert Locust, *Schistocerca gregaria* Females:

The average duration of the 5th nymphal instars of *S. gregaria* is about 8-day. The application of both compounds (lufenuron and rice bran extract) affected this duration. Results indicated significant prolongation in the duration of the treated nymphs with lufenuron, the duration reached 8.50, 9.18, 9.61 and 10.50 days after treatment with 25, 50, 100 and 200 ppm, respectively (Fig 1). Also, the treatment with rice bran extract caused significant prolongation in the duration of treated nymphs to be 8.37, 9.33, 9.73 and 10.07 days with (5, 10 ,15 , 20) ×10³ ppm, concentrations, respectively (Fig 2) .

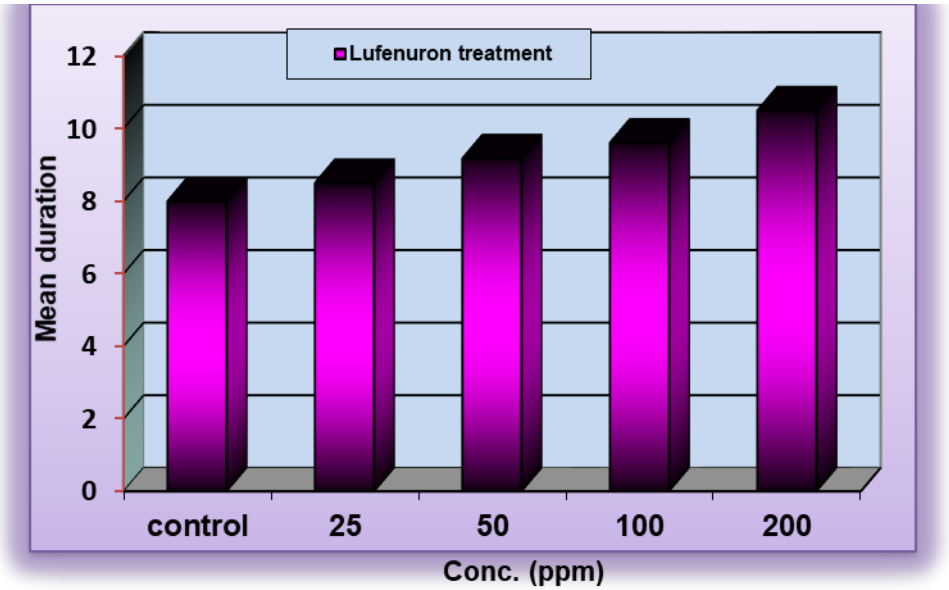

Fig. (1): Effect of Lufenuron on the duration of the 5th nymphal instars of *Schistocerca gregaria* females.

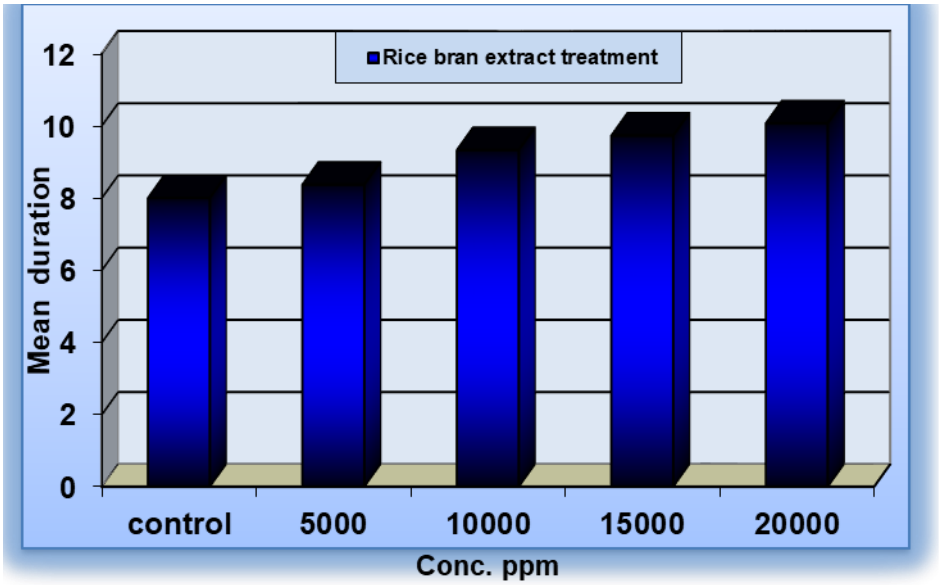

Fig. (2): Effect of *Oryza sativa* bran extract on the duration of the 5th nymphal instars of *Schistocerca gregaria* females**.**

Total Protein Content:

Table (3) showed that the total protein content of normal eggs collected at 0-day old. It was 81.07 mg protein/gm eggs, after one- day of oviposition the total protein increase significantly $p<0.05$ which was 93.80 mg protein/ gm eggs. Then the protein content during the early incubation period (blastoderm, gastrulation and the beginning of organogenesis stages), to the 4th day post oviposition, the protein content significantly $p<0.05$ declined sharply to 38.74mg protein/gm eggs. While in the 7th,11th&12th was decreased significantly $p<0.05$ reaching 50.80, 46.77&73.19 mg protein/gm eggs, post oviposition during late organogenesis stage.

Lufenuron affected eggs have significant reduction ($p<0.05$) in total protein content after the 0, 1st and 12th day of oviposition these were 60.24, 43.52 and 46.36 mg protein/ gm eggs, respectively compared with 81.07, 93.80 and 73.19 at normal eggs. On the other hand, the total protein increased significantly ($p<0.05$) at 4th & 7th day of oviposition to record 62.19 & 61.72 mg protein /gm eggs as compared with 38.74 & 50.80 mg protein / gm eggs at normal eggs. At the 11th day of egg oviposition, there was no significant difference between control and Lufenuron treatment.

The total protein content of rice bran extract affected eggs (R), increased significantly ($p<0.05$) at 0 & 4th days old, which were 107.86 & 65.64 mg protein / gm eggs as compared with 81.07&38.74 mg protein /100mg eggs in normal eggs. While the total protein content decreased significantly ($p<0.05$) after 1, 11 and 12 days of oviposition, the total protein levels were 51.56, 41.17 and 49.65 mg protein / gm eggs, respectively compared with 93.80,46.77 and 73.19 mg protein/gm eggs in normal eggs. At 7th day of oviposition, there was no significant difference in total protein, it was 51.08 mg protein/ gm eggs compared with 50.80 mg protein/gm eggs in normal eggs.

Table (3): Total protein content in control and affected *Schistocerca gregaria* eggs with LC_{50} of Lufenuron and Rice bran extract treatment.

Incubation days	mg Protein / gm eggs (Mean±S.E)		
	Control	Lufenuron	Rice bran extract
0	81.07 ±0.098 b B	60.24± 0.093 a C	107.86±0.294 a A
1	93.80±0.076 a A	43.52± 0.119 c C	51.56±0.192 c B
4	38.74±0.055 e B	62.19 ± 0.230 a A	65.645±0.0 b A
7	50.80±0.196 d B	61.72± 0.086 a A	51.08±0.388 c B
11	46.77±0.054 d A	50 .58± 0.224 b A	41.174±0.249 d B
12	73.19±0.132 c A	46.36± 0.046 b B	49.65 ±0.045 c B

Means followed with the same small letter within the same column were not significantly different. While means in the same row followed by the same capital letter were not significantly different.

Electrophoretic Analysis of Protein:

Changes in the total protein profile of normal and affected *Schistocerca gregaria* eggs with LC_{50} of Lufenuron and Rice bran extract are represented in Tables (4-9) and Figures (3-4).

- At 0-day Old Egg:

The electrophoretic mobility of the various proteins which measured enabled comparison of various fractions through successive embryonic developmental stages.

Control eggs, that were just oviposited (0-day old egg), showed 12 distinct bands that have different electrophoretic mobility, the protein fractions with MWs (250, 107.7, 67.7, 58.5, 48.8, 37, 23 & 20 KDa) were characteristic for control (C_0). At lufenuron-affected eggs (L_0), 14 protein fractions were detected. Newer protein bands with % amount (3.9, 8.8, 7.4, 6.2, 3.0, 4.5, 6.3, 3.6, 5.5 & 5.0 %) were evident as the development of embryo proceeds. Rice bran extract affected eggs (R_0) showed 12 protein fractions. Five protein bands (of MWs 325, 115.3, 96, 64 & 39 KDa) appear to be formed new. The protein band of 13 KDa was observed through control (C_0) and also (L_0) & (R_0) treatment, there were three protein bands of MWs (84, 26 & 16 KDa) appeared in both control (C_0) and (R_0) treatment. Also, there was a synchronized occurrence of the three protein banding patterns of MWs (52, 30 & 25 KDa) at both (L_0) and (R_0) treatment (Table 4).

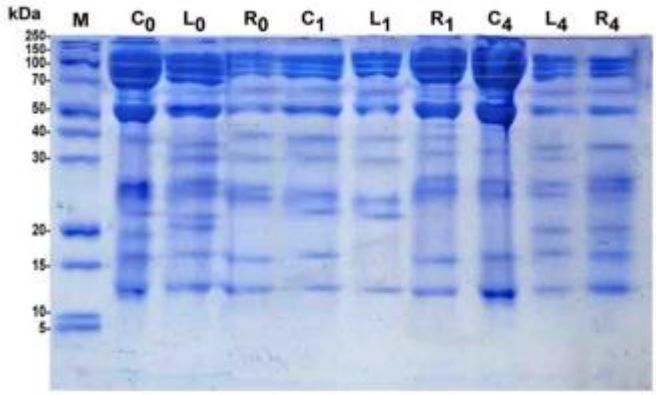

Fig. (3): SDS electrophoretic protein patterns of *S. gregaria* eggs control and affected samples with LC_{50} of lufenuron and rice bran extract at 0 hr and after 1 and 4 days of ovoposition.
M = Marker
C_0, C_1, C_4= Control eggs at 0 hr and after 1, and 4 days of oviposition, respectively.
L_0, L_1, L_4= Lufenuron affected eggs at 0 hr and after 1, and 4 days of oviposition, respectively
R_0, R_1, R_4= Rice bran extract affected eggs at 0 hr and after 1, and 4 days of oviposition, respectively

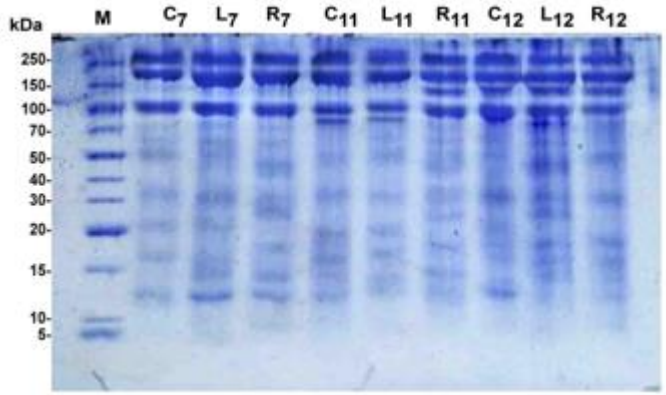

Fig. (4): SDS electrophoretic protein patterns of *S. gregaria* eggs control and affected samples with LC_{50} of lufenuron and rice bran extract after 7 ,11 and 12 days of ovoposition.
M = Marker
C_7, C_{11}, C_{12}= Control eggs after 7, 11, and 12 days of oviposition, respectively.
L_7, L_{11}, L_{12}= Lufenuron affected eggs after 7, 11, and 12 days of oviposit respectively.
R_7, R_{11}, R_{12}=.Rice bran extract affected eggs after 7, 11, and 12 days of oviposition, respectively.

Table (4): Molecular weight and % amount of eggs protein patterns of females *Schistocerca gregaria* control and treated as one day old of the 5th nymphal instars with LC_{50} of lufenuron (CSI) and rice bran extract (waste product) at 0 hr of oviposition.

Band number	MW (KDa)	Control (C_0)	Lufenuron (L_0)	Rice bran extract (R_0)
		%amount	%amount	%amount
1	325	-	-	4.009
2	275	-	3.927	-
3	250	2.699	-	-
4	115.3	-	-	8.123
5	107.7	8.996	-	-
6	104	-	8.883	-
7	96	-	-	5.389
8	90	-	7.461	-
9	84	10.404	-	4.502
10	70	-	6.268	-
11	67.7	6.869	-	-
12	64	-	-	5.966
13	63	-	3.009	-
14	58.5	2.794	-	-
15	52	-	9.678	6.807
16	48.8	11.361	-	-
17	39	-	-	6.243
18	37	4.625	-	-
19	35	-	4.559	-
20	30	-	5.869	6.439
21	27	-	6.350	-
22	26	8.181	-	5.097
23	25	-	4.712	6.236
24	23	3.256	-	-
25	22	-	3.622	-
26	21	-	5.567	-
27	20	5.349	-	-
28	17	-	5.057	-
29	16	5.103	-	4.508
30	13	6.038	5.226	5.098
Total number of bands		12	14	12

- At 1-day Old Egg:

The control (C_1), one - day old eggs showed 12 protein bands, of which six bands of % amount (3.1, 9.1, 4.0, 5.3, 5.7 & 3.7 %) appear to be formed new. It is interesting to note the occurrence of five protein bands of MWs (52, 37, 30, 25 & 13 KDa) consistently in the control (C_1) and lufenuron treatment (L_1). The protein banding pattern of molecular weight (16 KDa) was appeared in both (C_1) and (R_1) and was missing at (L_1) treatment.

The (L_1) eggs that were oviposited (1-day old) showed1 (10) distinct bands, five of

which that had significantly different electrophoretic mobility, namely bands (3, 6, 9, 14 & 27) with % amount (8.9, 6.6, 6.3, 6.2 & 4.4 %), respectively. The 1-day old egg (R_1) showed 14 protein bands of MWs (142.3, 107.7, 92, 72, 65, 60, 50.8, 43.5, 39, 32, 27, 26,16 & 12 KDa) appear to be formed new through early development (table 5).

Table (5) : Molecular weight and % amount of eggs protein patterns of females *Schistocerca gregaria* control and treated as one day old of the 5th nymphal instars with LC_{50} of lufenuron (CSI) and rice bran extract (waste product) after one day of oviposition (at 24 hrs).

Band number	MW (KDa)	Control (C_1)	Lufenuron (L_1)	Rice bran extract (R_1)
		%amount	%amount	%amount
1	325	3.144	-	-
2	142.3	-	-	3.335
3	115.3	-	8.939	-
4	107.7	-	-	9.296
5	104	9.141	-	-
6	96	-	6.632	-
7	92	-	-	9.297
8	90	4.056	-	-
9	82	-	6.377	-
10	80	5.399	-	-
11	72	-	-	6.486
12	65	-	-	2.193
13	64	5.703	-	-
14	63	-	6.259	-
15	60	-	-	2.136
16	52	9.021	7.916	-
17	50.8	-	-	11.132
18	43.5	-	-	2.347
19	39	-	-	3.496
20	37	6.277	4.936	-
21	32	-	-	3.297
22	30	5.528	4.557	-
23	27	-	-	4.015
24	26	-	-	5.223
25	25	6.917	5.291	-
26	23	3.757	-	-
27	22	-	4.444	-
28	16	4.635	-	5.657
29	13	5.479	4.848	-
30	12	-	-	5.922
Total number of bands		12	10	14

- At 4-day Old Eggs:

The protein fractions were separated into 27 protein banding patterns fluctuated from (12-14) bands. (C4) lane showed nine specific protein bands of MWs (142.3, 90, 72, 60, 53.8, 47, 32, 16 & 12 KDa). . However, two bands with MWs (107.7 & 25 KDa) were detected in (C_4) & (L_4) and were missing at (R_4).

The protein fraction of MW (27 KDa) was represented at all lane as the development of embryo proceeds. The protein fractions at (L4) were separated into thirteen protein banding patterns, the protein fractions with % amount (4.4 & 3.8%) were recorded only at (L4). It comes clear that, the protein fractions of MWs (104, 92, 26, 24 & 18 KDa) were specific for (R4). Egg development reveals a synchronized occurrence of eight protein bands of MWs (325, 80, 63, 50.8, 35, 21, 17 & 13 KDa) in both (L_4) and (R_4) treatment (Table 6).

Table (6): Molecular weight and % amount of eggs protein patterns of females *Schistocerca gregaria* control and treated as one day old of the 5th nymphal instars with LC_{50} of lufenuron (CSI) and rice bran extract (waste product) after 4 days of oviposition (at 96 hrs).

Band number	MW (KDa)	Control (C_4)	Lufenuron (L_4)	Rice bran extract (R_4)
		%amount	%amount	%amount
1	325	-	1.966	1.669
2	142.3	2.671		
3	107.7	7.112	7.581	-
4	104	-	-	6.138
5	94		4.478	-
6	92	-		4.426
7	90	8.771	-	
8	80	-	6.289	4.615
9	72	10.468	-	
10	63	-	5.615	4.885
11	60	2.438	-	-
12	53.8	4.085	-	
13	50.8	-	6.967	6.539
14	47	10.1	-	-
15	35	-	4.072	5.427
16	32	4.306	-	-
17	30	-	3.816	
18	27	4.595	4.617	4.984
19	26	-		5.939
20	25	3.879	4.4879	
21	24	-		3.516
22	21		5.846	5.245
24	18	-		1.498
26	17		6.457	6.881
27	16	6.235		-
28	13	-	5.511	8.075
29	12	7.506	-	-
Total number of bands		12	13	14

- At 7-day Old Eggs:

Control eggs (C_7) , 7-day old of oviposition showed seven specific protein bands of Mws (296.4 , 188.2 , 144 , 102 , 73 , 32&16 KDa). There were two protein fractions (50.8 & 22 KDa) clear in (C_7) and (L_7) but were missing at (R_7).

The protein bands of MWs (60 & 12 KDa) were identical in (C_7), (L_7) and (R_7) as embryonic development process proceeds. Four specific protein fractions of % amount (14.2, 9.3, 3.6 & 9.8 %) were detected at lufenuron affected eggs (L_7). The protein bands of MWs (277.2 & 15 KDa) were appeared only at (L_7) and (R_7) affected eggs and were missing at control (C_7), seven protein bands of % amount (15.9, 9.7, 3.5, 5.2, 6.1, 6.3 & 4.9) were detected only at (R_7) (Table7).

Table (7): Molecular weight and % amount of eggs protein patterns of females *Schistocerca gregaria* control and treated as one day old of the 5th nymphal instars with LC_{50} of lufenuron (CSI) and rice bran extract (waste product) after 7 days of oviposition (at 168 hrs).

Band number	MW (KDa)	Control (C_7)	Lufenuron (L_7)	Rice bran extract (R_7)
		%amount	%amount	%amount
1	296.4	12.95	-	
2	277.2	-	11.601	11.246
3	188.2	10.718	-	-
4	173	-	-	15.966
5	158.8		14.266	-
6	144	3.160	-	
7	102	10.373		-
8	98	-	9.391	
9	96			9.714
10	77.8	-	3.612	-
11	73	3.508	-	
12	70	-		3.512
13	60	3.260	4.003	4.584
14	50.8	7.346	4.589	
15	46	-	-	5.294
16	32	7.656	-	
17	31	-	9.8107	-
18	30			5.134
19	27	-		6.332
20	22	6.218	5.692	-
21	18	-	-	4.903
22	16	6.911		-
23	15	-	5.284	6.403
24	12	6.568	6.329	5.970
Total number of bands		11	10	11

- At 11-day Old Eggs:

Eggs that were oviposited (11-day old) showed four distinct bands of % amount (8.3, 5.6, 5.7 & 4.9 %) were detected at (C_{11}). It is interesting that two protein bands of

MWs (177.8 & 32 KDa) were observed only at (C_{11}) and (R_{11}), while the protein bands of MWs (50.8 & 14 KDa) were detected only at (C_{11}) and (L_{11}) but protein banding patterns of MWs (272.2 & 20 KDa) revealed a synchronized occurrence at (C_{11}), (L_{11}) and (R_{11}) .Six protein fractions of % amount (15.2, 8.8, 5.6, 4.5, 6.2 & 6.5 %) were obvious only at (L_{11}) affected eggs. Only protein fraction of MW (13 KDa) were observed in both (L_{11}) and (R_{11}). Eight protein bands of MWs (135.5, 96, 73, 61, 46, 25, 18 &15 KDa) were characteristic to (R_{11}) affected eggs, (Table 8).

Table (8): Molecular weight and % amount of eggs protein patterns of females *Schistocerca gregaria* control and treated as one day old of the 5th nymphal instars with LC_{50} of lufenuron (CSI) and rice bran extract (waste product) after 11 days of oviposition (at 264 hrs).

Band number	MW (KDa)	Control (C_{11})	Lufenuron (L_{11})	Rice bran extract (R_{11})
		%amount	%amount	%amount
1	272.2	12.931	9.224	10.565
2	182.9	-	15.226	-
3	177.8	14.902	-	10.401
4	135.5	-	-	6.896
5	104.	-	8.888	-
6	98	8.323	-	-
7	96	-	-	10.343
8	84	-	5.613	-
9	81	5.676	-	-
10	73	-	-	4.173
11	62	-	4.523	-
12	61	-	-	3.746
13	50.8	5.191	6.757	-
14	46	-	-	6.235
15	32	7.668	-	7.082
16	31	-	6.203	-
17	25	-	-	4.717
18	20	5.005	6.250	2.284
19	18	-	-	5.74
20	17	-	6.571	-
21	16	5.722	-	-
22	15	-	-	5.187
23	14	4.345	3.555	-
24	13	-	3.940	7.013
25	12	4.913	-	-
Total number of bands		10	11	13

- At 12-days-Old Eggs:

Seven specific protein bands of MWs (280, 173, 141.2, 92, 64, 43.5 & 21 KDa) were evident at (C_{12}) as development of the embryo proceeds. The 12-day old egg showed three identical protein bands of MWs (31, 18 & 16 KDa) through all lanes (C_{12}), (L_{12}) and (R_{12}). It is interesting to note the occurrence of two protein bands of MWs (50.8 & 12 KDa) only at (C_{12}), (R_{12}) and were missing at (L_{12}). Six protein fractions with % amount (6.2, 8.4, 5.4, 3.8, 10.9 & 7.5 %) were evident in the lufenuron affected eggs. The

12 - day old egg showed 12 protein bands, of which 3 bands with MWs (272.2, 177.8 & 14 KDa) appear to be formed new at (L_{12}) and (R_{12}) only. The protein bands with MWs (135.5, 98, 74.5 & 61 KDa) were produced only at (R_{12}), (Table 9).

Table (9): Molecular weight and % amount of eggs protein patterns of females *Schistocerca gregaria* control and treated as one day old of the 5th nymphal instars with LC_{50} of lufenuron (CSI) and rice bran extract (waste product) after 12 days of oviposition (at 288 hrs).

Band number	MW (KDa)	Control (C_{12})	Lufenuron (L_{12})	Rice bran extract (R_{12})
		%amount	%amount	%amount
1	280	10.048	-	-
2	272.2	-	7.213	10.531
3	177.8	-	7.935	9.087
4	173	8.529	-	-
5	141.2	6.257	-	-
6	138.3	-	6.215	-
7	135.5	-	-	6.583
8	98	-	-	8.078
9	96	-	8.467	-
10	92	13.702	-	-
11	77.8	-	5.357	-
12	74.5	-	-	7.251
13	64	5.161	-	-
14	61	-	-	2.889
15	59	-	3.855	-
16	50.8	5.715	-	7.603
17	46	-	10.998	-
18	43.5	3.648	-	-
19	31	6.603	7.165	7.146
20	25	-	7.511	-
21	21	7.086	-	-
22	18	4.116	5.588	5.695
23	16	2.883	4.442	6.083
24	14	-	4.697	3.212
25	12	4.556	-	4.002
Total number of bands		12	12	12

DNA Level:

Quantitative changes in the DNA level during embryogenesis control and affected eggs of *S. gregaria* were determined in table (10). At control, DNA level at 0 day old eggs, which were 20.4 µg /100 mg eggs exhibited a significant increase ($p<0.05$) during further embryonic development. The 1st, 11th & 12th days old (C) eggs showed a significant increase ($p<0.05$) which recorded 35.5 µg /100 mg eggs, 30.9 µg/100 mg eggs and 38.7µg /100 mg eggs, respectively. The highest level was recorded in the eggs of the 4th & 7th days old (44.2 µg/100 mg eggs & 42.3 µg/100 mg eggs), respectively.

At 0 day old (L) eggs, DNA level was low which registered 16.2 µg /100 mg eggs increased significantly ($p<0.05$) as successive stages of development proceed. The DNA highest level was recorded at the 12th, 11th & 4th days old, being 83.4 µg/100 mg eggs, 76.4 µg/100 mg eggs & 61.8 µg/100 mg eggs, respectively. At the 1 day & 7th day post oviposition, DNA level reached 29.0 µg/100 mg eggs & 35.0 µg / 100 mg eggs.

Table (10): DNA level in control and affected eggs of *Schistocerca gregaria* females resulted from treated 1 day old of the 5th nymphal instars with LC_{50} of lufenuron and rice bran extract.

Incubation days	µg DNA /100 mg eggs (Mean±S.E)		
	Control	Lufenuron	Rice bran extract
0	20.4 ± 0.374 e B	16.2 ±0.141 f C	34.13 ± 0.216 c A
1	35.5 ± 0.216 c B	29 .0 ± 0.432 e C	63.2 ± 0.216 a A
4	44.2 ±0.753 a B	61.8 ± 0.511 c A	43.1 ± 0.216 b B
7	42.3 ± 1.41 a A	35.0 ± 0.216 d B	28.1 ± 0.294 d C
11	30.9 ± 0.021 d C	76.4 ± 2.631 b A	33.5 ± 0.355 c B
12	38.7± 0 .141 b B	83.4 ± 0.282 a A	22.52 ± 0.268 e C

Means followed with the same small letter within the same column were not significantly different.
While means in the same row followed by the same capital letter were not significantly different.

At rice bran extract affected eggs (R), DNA level showed 34.13 µg / 100 mg eggs, which increased significantly ($p<0.05$) at 1 day old & the 4th day old, recording 63.2 µg / 100 mg eggs & 43.1 µg / 100 mg eggs, respectively then showed a significant reduction ($p<0.05$) at the 7th & 12th days old, where DNA level recorded 28.1 µg/100 mg eggs & 22.52 µg / 100 mg eggs, respectively. However, no significant change in DNA content occurred between 0 &11th day old, where DNA level recorded 34.13 & 33.5 µg /100 mg eggs.

At 0 day old post oviposition, DNA level was recorded 20.4 µg /100 mg eggs which decreased significantly ($p<0.05$) at (L_0), being 16.2 µg /100 mg eggs and increased highly significantly at (R_0), being (34.13 µg /100 mg eggs).

Also, DNA level at (C_1) was 35.5 µg /100mg eggs showed a significant reduction at (L_1) which was 29.0 µg /100 mg eggs. On the contrary, it showed high significant increase at (R_1) which was 63.2 µg /100 mg eggs.

At the 4th day old of embryogenesis (the earliest stages of cleavage, gastrulation and early organogenesis), the DNA content recorded 44.2 µg /100 mg eggs which exhibited a highly significant increase ($p<0.05$) at (L_4) which being 61.8 µg /100 mg eggs. It showed more or less similar level at (R_4), being 43.1 µg /100 mg eggs where no significant difference was recorded.

At 7 days old of incubation days, the DNA level was 42.3 µg /100 mg eggs. It showed a significant decrease ($p<0.05$) at both (L_7) & (R_7), which is recorded 35.0 µg /100 mg eggs & 28.1 µg/100 mg eggs, respectively.

During the course of embryonic development, DNA level recorded (30.9 µg/100 mg eggs) at (C_{11}), which increased significantly ($p<0.05$) and registered (76.4 µg /100 mg eggs) as peak value at (L_{11}), while the DNA level was being (33.5 µg /100 mg eggs) at (R_{11}).

At the last process of embryogenesis, DNA level registered (38.7 µg /100 mg eggs) at the 12th-day old egg, which showed a significant increase ($p< 0.05$) at (L12), which being (83.4 µg /100 mg eggs). While DNA level was noticed decline significantly ($p<0.05$) at (R_{12}) was 22.52 µg /100 mg eggs.

DISCUSSION

Biological Studies:

The present study indicated that, chitin synthesis inhibitors, (CSIs) and plant waste product had significant toxic and growth inhibitory effects against the desert locust, *Schistocerca gregaria.Lufenuron* is a chitin synthesis inhibitor of the benzoylphenyl ureas (BPUs) group that inhibits insect chitin biosynthesis. Lufenuron was used against the 5th nymphal instar of S. gregaria during one day by feeding technique. The present study showed that, the treatment with different concentrations of lufenuron against the 5th nymphal instar females of *S. gregaria* caused the failure of ecdysis to adults. In addition, adults mortality increased significantly with the increase of lufenuron concentrations. Also, a significant prolongation in the mean duration of the treated 5th nymphs reached 10.50 days compared with 8 days in the control.

Some studies have suggested that CSIs inhibit the action of chitin synthase, which is an integral protein that aids in the synthesis of N-acetyl glucosamine (Merzendorfer, 2006), or inhibit the incorporation of N-acetyl glucosamine into insect chitin during the molting process (Matsumura, 2010). The present results are agreed with those of several other chitin synthesis inhibitors against the same Acridide species such as diflubezuron, which interferes with the chitin synthesis during the nymphal ecdysis of *Schistocerca gregaria* causing failure of some treated instars to moult and morphological abnormalities (Mariy *et al.*, 1981; Taha & El-Gammal, 1985; Roa and Mehrotra, 1986 &1987). The greatest mortality was recorded during ecdysis of early the 4th nymphal instar to the 5th nymphal instar of treated *S. gregaria* with chlorfluazuron (Abo-El-Ela *et al.*, 1993). Also chlorfluazuron induced appreciable failure in ecdysis to adult stage when applied on the last nymphal instar (El-Gammal *et al.*, 1993). The prolongation effect reached 14 days when Azam and Seegh (1993) treated the 2nd nymphal instars of *S. gregaria* with diflubenzuron. Likewise, the durations of the 2nd and 3rd instar of *S. gregaria* were significantly prolonged when they exposed to three benzoylphenylureas; diflubenzuron, hexaflumuron and teflubezuron (Coppen and Jepson 1996b). Triflumuron caused mortality rates up to 80% after 5-15 days of the barrier application on *S. gregaria* in Mauritania (Wilps and Diop, 1997). Higher mortality was recorded within 4-9 days in the treated 4th nymphal instar of oriental migratory locust *Locusta migratoria manilensis* with Flufenoxuron and chlorbenzuron **(**Zhongren *et al.*, 2002). Mortal activity of the chitin synthesis inhibitors (CSIs): Flufenoxuron (CAS-101463) and Lufenuron (CGA184699) on the penultimate instar nymphs of the desert locust *Schistocerca gregaria* increased proportionally to the concentration level of tested compounds (Bakr *et al.*, 2008).

Many plant species have been reported to have phytochemicals with insecticidal property in leaves, flowers, and roots. These chemicals exhibit repellent, antifeedant, growth disrupting, insecticidal and ovicidal properties on insects (Isman, 2006 and Devi & Devi 2011). Some plant extracts, that have active substances, were used as botanical insecticides to protect the plants from insects (Regnault-Roger *et al.*, 2012 and Pavela, 2015). Rice bran extract like other natural plant extracts had many activities against *S. gregaria*.

The present observations showed that, feeding application of rice bran extract against females of the newly moulted 5th nymphal instar *S. gregaria* caused failure of

ecdysis to adults and also adult mortality. These effects had a positive relationship with rice bran extract concentrations, also a significant prolongation in the average duration of the treated 5th nymphs reached 10.07 days compared with 8 days in the control.

The present results are in conformity with several authors **(**Schmutterer & Freres, 1990; Nicol & Schmutterer, 1991; Wilps *et al*., 1992; Nicol *et al.* 1993; Mohamed & El-Gammal, 2002; Mohamed *et al.,* 2006 and Ghazawy *et al*., 2010)**.** They reported that treatment of *S. gregaria* with neem plant extracts caused high mortality which often occurred during moults, prolonged nymphal development and disturbance of metamorphosis, the latter resulted in morphogenetic defects of eyes, antennae, legs and wings. Extracts of *Calotrppis procera* and *Zygpphyllum simplex* induced different morph genic abnormalities in the adult stages as well as in nymphal instars of *Schistocerca gregaria* **(**Abdullah 2000). The treatment of penultimate and last nymphet instar of *Schistocerca gregaria* with extracts of *Fagonia bruguieri* induced mortality in the emerged adult and affected the adult morphogenesis (Aly *et al*., 2010).

Biochemical Studies:

During the embryonic development process, protein expression is active. Biosynthesis and catabolism programs are performed. Some identified proteins might be directly correlated to the biological characteristics of the eggs at each stage of embryonic development (Hu *et al.,* 2018). Generally, changes in nucleic acid level reflect the synthetic activities of the developing embryo and the degree of embryonic dependence on the material of maternal origin. Also changes in protein content probably reflect the balance between synthesis, storage, transport and degradation of structural and functional nutrients as well as response to particular physiological conditions (Shoukry *et al*., 2003).

Findings of the present work indicated disturbance in the amount of DNA and total protein content (quantitative and qualitative) during embryogenesis in the affected eggs, of maturated female adults *S. gregaria* treated as one day old of the fifth nymphal instar with LC_{50} of the two tested compounds (lufenuron and *Oryza sativa* bran extract) comparing with control eggs which resulted from untreated (normal) females.

The total protein content of normal eggs increased significantly from 0 day to day (1) post oviposition (pop), then declined sharply at the 4th day. The total protein increased ($p<0.05$) at the 7th ,11th and 12th days (pop) compared with the 4th day, but decreased significantly compared to 0-day (pop).

The quantitative changes in the total proteins during embryogenesis of *Atractomorpha crenulata* increased after 4 days of incubation until just prior to hatching; there was a slight fall (Sanjayan *et al*. 1988). Nemec (2002) found that the amount of protein increased progressively during embryogenesis of locust eggs. Slogget & Lorenz (2008) found that protein content declines weakly over embryonic development in ladybird beetles and in *Musca domestica*. Also the total protein of *Musca domestica* decreased gradually reaching their minimum levels at 7 hrs post oviposition (late embryogenesis) (Guneidy *et al.,* 2011).

At lufenuron affected eggs, total protein content had a significant reduction ($p<0.05$) after the 0, 1st and 12th days of oviposition, and a significant increased ($p<0.05$) at the 4th & 7th days of oviposition, compared with normal eggs. But on the 11th day of egg oviposition, there was no significant difference between control and lufenuron treatment.

The total protein content in (R) increased significantly ($p<0.05$) at the 0 and 4th days old. While it decreased significantly ($p<0.05$) after 1, 11 and 12 days of oviposition, compared with normal eggs. But on the 7th day of oviposition, there was no significant difference in total protein, compared with normal eggs. There are fluctuations of total protein in the both treatment of selected compounds.

These results are in line with (El-kerim, 2002) when applied pyrazole, an aromatic juvenile hormone analogue (JHA), on embryo of *Schistocerca gregaria.* He found depression in total protein level during the last embryonic developmental stages. Guneidy *et al.* (2011) recorded a significant decrease in total protein of rice bran extract-treated eggs of *Musca domestica* compared with the control except at 0 hr old eggs (early embryogenesis) and also recorded fluctuation between increase and decrease of total protein in lufenuron treated eggs compared with the control.

Embryonic development is a sequential and complex process controlled by genes. Some proteins are constitutively expressed throughout the developmental process. These proteins are indispensable for egg development. The existence of proteins expressed in specific stages of the egg suggests that the different developmental stages need specific proteins to proceed correctly (Fang and Li, 2010).

Qualitative electrophoretic analysis in the present study exhibited many differences in protein patterns (mobility & number of bands) in affected eggs with lufenuron and rice bran extract compared with control group. New bands appeared and others disappeared in affected eggs. In addition, there were differences in concentrations of common bands between control and the affected groups.

These results are in agreement for other tested IGRs and botanical extracts on locust and other insects. In *Rhodnius prolixus,* embryonic disturbance which resulted from treatment with fenoxycarb, might be due to alterations in normal molecular events accompanying development. Three proteins bands normally absent were expressed in fenoxycarb-treated embryos (Kelly and Huebner 1987). In *Schistocerca gregaria*, new protein bands appeared and others disappeared during embryogenesis in treated eggs with azadirachtin compared to the control group (Ghazawy *et al.*, 2010). Also Guneidy *et al*. (2011) explained that treatment with lufenuron and waste product rice bran extract against *Musca domestica* during embryogenesis resulted in the appearance and disappearance of certain protein fractions.

Quantitative changes in DNA level during embryogenesis of control and affected eggs of *S. gregaria* were determined. At control, DNA level exhibited a significant increase ($P<0.05$), during further embryonic development. The level of DNA content at normal embryos was explained by several scientists.

The DNA level was increased rapidly up to gastrulation in the developing embryos of the milkweed bug, *Oncopeltus fasciatus* (Dallas) (Harris and Forrest 1967). Also, the total DNA in the house fly, *Musca domestica*, increased gradually ($P<0.05$) during embryonic development (AL-Adil *et al*. 1972; Srinivasan and Kesavan 1979; Guneidy *et al.* 2011).

In affected eggs, which produced from maturated female *S. gregaria* treated as newly moulted 5^{th} nymphs with LC_{50} of lufenuron and *O. sativa* bran extract, exhibited significant differences ($P<0.05$) in the DNA content during embryogenesis compared with normal eggs. The changes in DNA level by several agents on other insects were observed by Kilgore and painter (1964) when they applied the chemosterilant antapholate to house fly eggs, the DNA concentration increased considerably in the eggs deposited by treated flies compared with that of the normal eggs during incubation period. Also treatment of *Musca domestica* eggs with a chitin synthesis inhibitor (Lufenuron) and a waste product from rice bran resulted in a significant decrease in DNA content throughout embryogenesis (0-7 hr postoviposition) in contrast with normal eggs (Guneidy *et al*., 2011). Lufenuron is a chitin synthesis inhibitor (CSI) of the benzoylphenyl ureas (BPUs) group that inhibits insect chitin biosynthesis. Additionally, it is worthy to note that BPUs, were reported as potential genotoxic agents to *Drosophila melanogaster* (Eid *et al*., 2017), these compounds have multiple electrophilic sites that

are suggested to have the ability to form a ducts with DNA through its nucleophilic sites. Lufenuron caused different morphological and histological malformations in *Schistocerca gregaria* embryo as result of a potential genotoxicity, generating mutations in regulatory and structural genes (Abdel Rahman, 2017).

In conclusion, the lethal concentration LC_{50} of the tested compounds (lufenuron and Oriza sativa bran extract) showed significant changes in affected eggs protein (quantitative and qualitative) and DNA level during embryogenesis. Where DNA and protein synthesis are necessary, the alteration of both or one of them affecting the biological processes during embryogenesis and this may cause the blockage of embryonic development. So, the tested compounds may be considered a valuable tool for the control of *S. gregaria* as a component of an IPM program.

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ARABIC SUMMARY

التأثيرات السمية والبيوكيميائية لمركب ليوفنيرون ومستخلص نخالة الأرز على الجراد الصحراوي *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae)

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تم تقيم التاثيرالسمى لكل من مركب الليوفنيرون (مثبط تكوين الكيتين) ومستخلص نخالة الارز على اليوم الاول من عمرالطور الخامس لحوريات اناث حشرة الجراد الصحراوى و لقد اسفرت النتائج على ان كلا من المركبين لهما تأثير سمى على الطور الحورى والحشرة الكامله .فقد نتج عنهما نسبه اماته لكل من الطورين وكذلك تشوهات مورفولوجية فى الحشرة الكاملة بعد الانسلاخ من العمر الخامس المعالج كما ادت الى اطالة عمر الحوريات. وكذلك عند معاملة الطور الخامس لاناث الحوريات فى اول يوم بالجرعه النصف مميته لكل من المركبين اتضح تاثيرهما على الكفاءة التناسلية و كذلك على حيوية البيض للحشرات البالغة الناتجة من هذة المعاملة .كما تم تقدير المحتوى البروتينى (الكمى والكيفى) وكميه الحمض النووى فى البيض الغير معامل (ناتج من حشرة غير معاملة) والبيض المتأثرالناتج من الحشرة الكاملة التى تم معاملة عمر اليوم الاول للطورالخامس من الحوريات بالجرعة النصف مميتة من مركب الليوفنيرون ومستخلص قش الارز. لقد اوضحت النتائج عن وجود فروق معنوية فى كل من المحتوى البروتينى والحمض النووى لكل من البيض الغير معامل والبيض المتأثر من المعاملات السابقة .وبالتحليل الكيفى للبروتين قد وجد بعض انواع من البروتين فى البيض المتأثر من المعاملة واختفاء انواع اخرى مقارنة بالبيض الغير معامل.