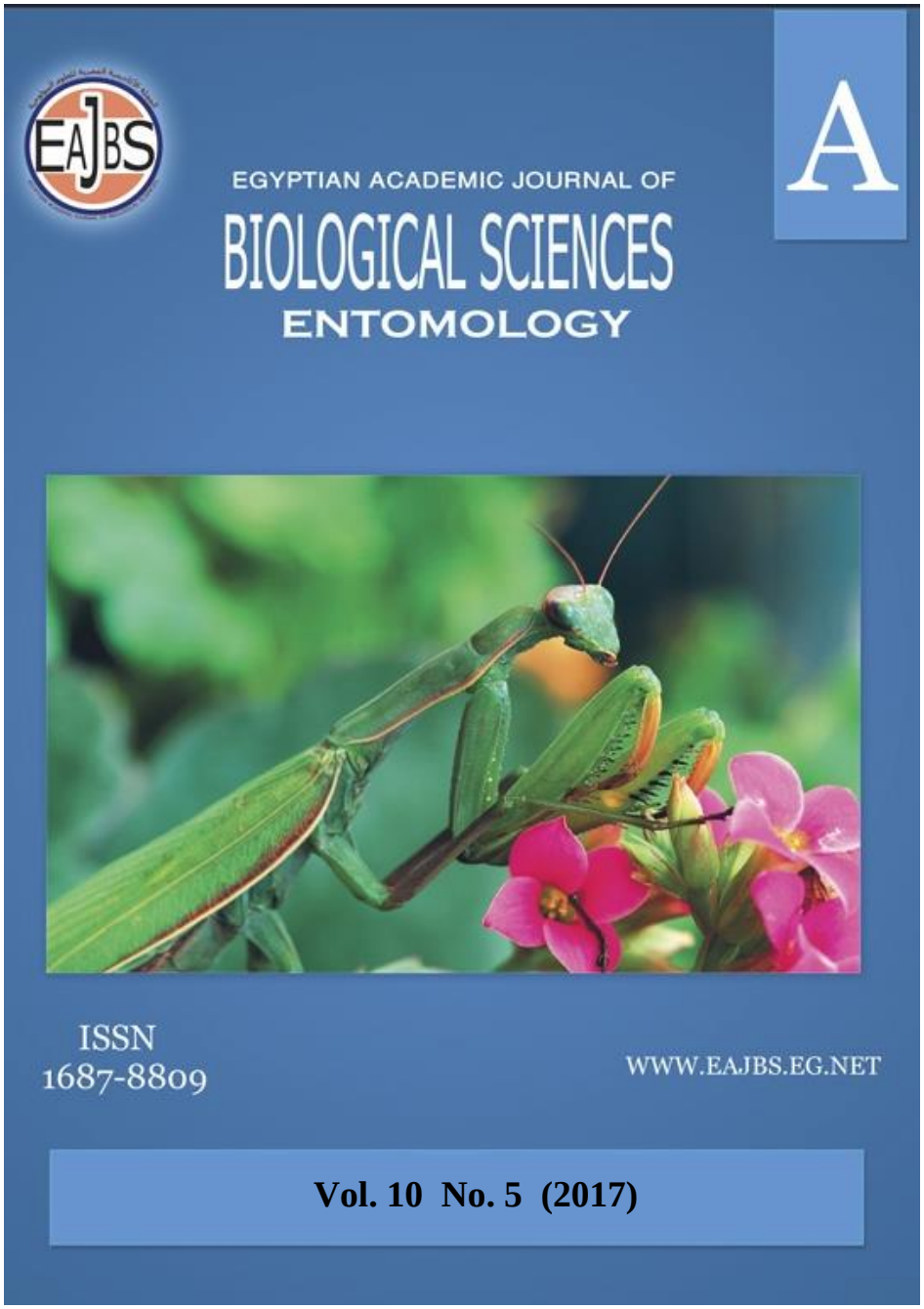

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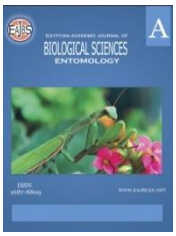

Effect of Lufenuron and *Oriza sativa* Bran Extract on Fraction Protein and Acid Phosphatase Pattern in Haemolymph of *Schistocerca gregaria*.

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ABSTRACT

The extensive use of insecticides has significant negative aspects such as the development of insect resistance, great threat to environmental health, and increased costs. IGRs and compounds from natural origin considered environmentally safe and less hazardous to non-target biota. So, the current study aimed to evaluate the insecticidal activity of the match (lufenuron) and rice bran (*Oriza sative*) extract and their effect on fraction protein and acid phosphatase (ACP) pattern of haemolymph of treated *Schistocerca gregaria*. The present findings cleared that, LC_{50}s estimate for one-day old of 5th nymphs of *S. gregaria* were 35.66 and 541.71 ppm for lufenuron and *O. sativa* bran extract, respectively. As to fraction protein pattern, a maximum number of 65 bands were detected, at molecular weight ranged from 6.73 to158.31and 18 unique bands were scored. The epigenetic distance accounted for 1 for lufenuron and 0.76 for *Oriza sativa* bran extract, 20 days post-treatment. In addition, seven acid phosphatase bands were recognized in treated and untreated 12 samples, with Rf values ranged between 0.02 and 0.86. Three unique bands were scored. In conclusion, the treated nymph with the lethal concentration LC_{50} of the tested compounds (lufenuron and *O. sativa* bran extract) showed a significant change in protein fraction and acid phosphatase patterns of haemolymph, which was clearly reflected in high epigenetic differences between control and treated *S. gregaria* samples.

INTRODUCTION

The desert locust *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididiae) is considered one of the most injurious pests to many crops, which are the main food for both human and animal in many Asian and African countries. This is due to the ability of this locust voracious feeding swarms to fly rapidly across large distances. In addition, this pest has 2-5 generations per year (Bakr *et al*., 2009; Ghoneim *et al*., 2014).Current locust control system is mainly based on organophosphorus insecticides (Lecoq, 2001; Ghoneim *et al*., 2014). The extensive use of such pesticides has significant negative aspects such as the development of insect resistance, great threat to environmental health, and increased costs (Garriga and Caballero, 2011). However, scientists intended for the use of insect growth regulators (IGRs) (Bakr *et al*., 2009) and plant extracts (El-Maghraby *et al*., 2012) control methods to pass up the risk of insecticides.

Insect growth regulators (IGRs) are selective in activity towards arthropods mainly insects, secure to non-target organisms (Fournet *et al*., 1993).

They are called "the third-generation of insecticides". IGRs comprise jeuvenile hormone (JH) mimics and chitin synthesis inhibitors (CSIs) like (lufenuron (match), diflubenzuron, hexaflumuron) (Anwar and Abd El-Maged, 2005). The lufenuron (match) can interfere with chitin synthesis process, disrupt hormonal balance at exchanging in molting, and inhibits the growth of insect (Oberlander and Silhacek, 1998). IGRs have been estimated successfully against many insect species (Glancey and Banks, 1988; Fournet *et al*., 1993; Tunaz, 2004; Anwar and Abd El-Mageed, 2005; Bakr *et al*., 2008 a; 2010; Zibaee *et al*., 2011; Abd El Bar *et al*., 2013; Bakr *et al*., 2013; Aziz, 2017).

Several compounds from plants have prospective insecticidal or repulsive properties and have no hurtful effect (Din *et al*., 2011). Bakr *et al*. (2006; 2008 b) studied the effect of the *O. sativa* bran extract against the 4th*Spodopteralittoralis* instar larvae and the newly moulted 5th instars of *S. gregaria*. While Salem *et al*. (2012) evaluated its effect against the viability of the house fly *Musca domestica* eggs. However, many other waste plant product compounds were tested successfully on several insects (Helmy *et al*., 2010; El-Maghraby *et al*., 2012; Hassan *et al*., 2012; Abd El-Bar *et al*., 2013).

The current study aimed to evaluate the insecticidal activity of the match (lufenuron) and rice bran (*Orizas ative*) extract and their effect on fraction protein and acid phosphatase (ACP) pattern of haemolymph of treated *S. gregaria*.

MATERIALS AND METHODS

Toxicology evaluation:

Origin of stock culture and rearing in the laboratory:

The stock colony of *Schistocerca gregaria* was provided from the Locust Research Department, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza. The insects were reared and handled under the following technique described by Abbassi *et al.* (2003).

Experimental insects:

The experimental nymphs were segregated from the gregarious stock colony at the beginning of the first nymphal instar and held up in cages (30x30x30 cm) in diameter. The cages were a wooden farmed equipped with zinc bottom covered by 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). Leaves of *Medicago sativa* were daily placed as feeding material. Unconsumed food and faces 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.

Insecticides used:

One of insect growth regulators (IGRs), Lufenuron (EC.10%) and agricultural waste product extract, *Oriza sativa* bran were used.

Rice bran extract (*Oriza sativa*):

Oriza sativa bran was exhaustively extracted with ethanol solvent. The extraction was accomplished by a Soxhlet apparatus. Then, it was evaporated and dried under vacuum using a rotary evaporator with water bath adjusted at 40-60^{o} C. The resulted crude extract yield from 100 gm of *O. sativa* bran was weighted before storage at 4^{o} C in screw capped vials, until use.

Treatment of experimental insects:

Both sexes of one-day old 5thnymphal instars of *S. gregaria* were treated by feeding technique with one of insect growth regulators (IGRs) and waste extract as the following:

Leaves of *Medicago sativa* were dipped in 25, 50, 75 and 100 ppm of the Lufenuron and 250, 500,700 and 1000 ppm of *O. sativa* bran extract for two minutes. Then leaves were air dried before being offered to the nymphs for feeding on it. Three replicates of 20 nymphs were subjected to each of the treated leaves. After feeding for 24 hours on the treated leaves, the alive nymphs were transferred onto untreated leaves. Mortality or malformed individuals were recorded once adults emerged.

Biochemical studies:

For biochemical analysis, ten healthy and treated newly emerged adult, treated as 5thnymphal instar with LC_{50} of each tested compounds, were weighted and kept under freezing conditions at -5°C.

Electrophoresis conditions:

The gel glasses were fixed to both lower and upper chambers of the electrophoresis apparatus. Both electrode chambers were filled at electrode buffer pH 8.6. The apparatus was connected to the power supply. The run was performed at 45 volt until the tracking dye has reached the separating gel and then the voltage was increased to 70 volt. The temperature of the gel was maintained at 7 °C by refrigerating the gel chamber during the run.

Fraction protein SDS- PAGE:

SDS-PAGE was performed in 12% acrylamide slab gel according to the method of Laemmli (1970).

Enzymes Assay:

After electrophoresis, the gels were stained according to their enzyme system at the appropriate substrate and chemical solutions then incubated at room temperature in dark for complete staining. In most cases incubation for about 1 to 2 hours is enough.

Acid phosphatase (Acph):

After electrophoresis, the gel was soaked in 100 ml of 50 mM Na-acetate buffer pH 5.0 containing 100 mg Fast blue BB salt, 100 mg α-naphthyl phosphate, 100 mg $MgCl_2$ and 100 mg $MnCl_2$ (Wendel and Weeden, 1989).

Gel mentioned above was incubated for 30 to 60 min. in the incubator at 50^{o} C until the appearance of bands.

Gel fixation:

After the appearance of the enzyme bands, the reaction was stopped by washing the gel two or three times at tap water. This was followed by adding the fixative solution, which consists of ethanol and 20 % glacial acetic acid (9: 11 v/v). The gel was kept in the fixative solution for 24 hours and then was photographed.

Calculations and data analysis:

The mortality percentages of treated nymphs were corrected against those of the control by using Abbott, formula, (1925). Probit analysis was determined to calculate LC_{50} and slope values of the tested compounds (Finney, 1971), through software computer program.

The similarity index and genetic distance were determined according to (Nei and Li, 1979).

$S = 2N / (Nx + Ny)$

Where, S = similarity value & Nx and Ny are the number of bands in individual x and y & N is the number of shared bands &Epigenetic distance (D) = 1- S.

RESULTS

Insecticidal activity of Lufenuron (IGR) and *Oriza sativa* bran extract (plant waste product) against one-day old of 5th nymphs of *Schistocerca gregaria*was recorded in Table (1). Data showed that Lufenuron was more effective compound than *Oriza sativa* extract. The LC_{50} of Lufenuron accounted for 35.66 ppm while that of *Oriza sativa* bran extract was 541.71 ppm.

Table1: Insecticidal effect of Lufenuron and *Oriza sativa* bran extract against *Schistocerca gregaria*

LC	**Lufenuron**			***Oriza sativa* bran extract**		
	Concentration (ppm)	Lower limit	Upper limit	Concentration (ppm)	Lower limit	Upper limit
LC_5	18.04	10.98	29.59	329.32	262.12	384.38
LC_{50}	35.66	28.50	44.60	541.71	509.82	575.59
LC_{95}	70.49	62.23	79.87	891.09	804.32	967.27
Slope	5.551±1.391			7.609±0.823		

Effect of Lufenuron and *Oriza sativa* bran extract on fraction protein pattern in haemolymph of *Schitocerca gregaria*

SDS protein pattern banding of haemolymph of *Schistocercagregaria* females treated with **Lufenuron** and ***Oriza sativa* bran** extract and their control was shown (Table 2, Fig. 1). A maximum number of 65 bands were detected, at molecular weight ranged from 6.73-158.31. The maximum number of bands was detected in sample no. 4 (control 20 days post-treatment) and sample no. 8 (females treated with lufenuron, 20 days post-treatment), and was accounted for 15 bands.

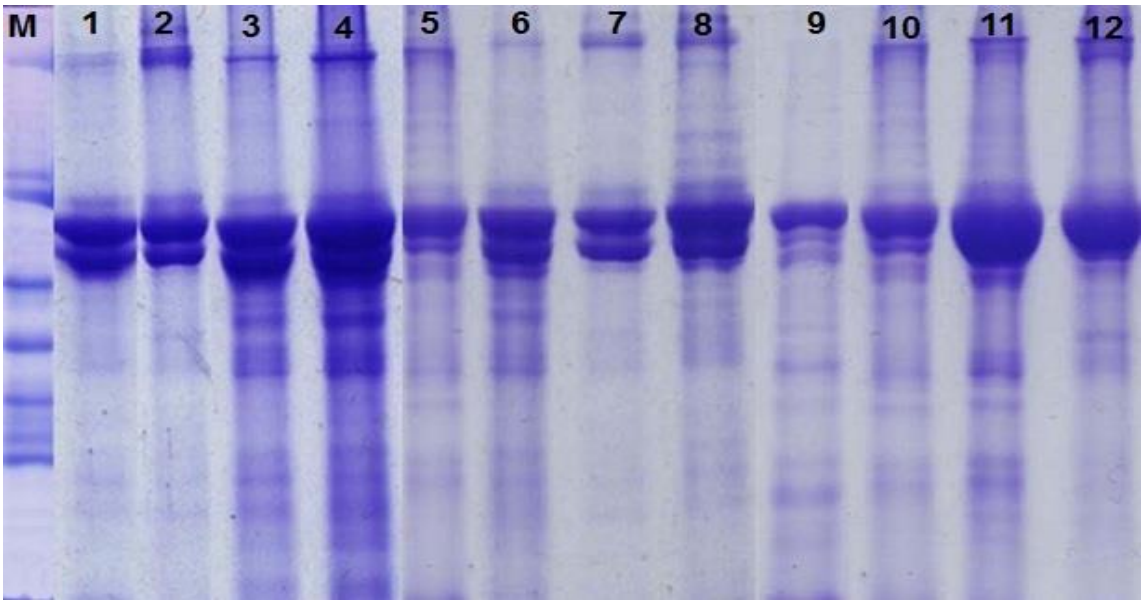

Fig. 1: SDS protein electrophoretic pattern of haemolymph of *S. gregaria* control and treated samples with Lufenuron and *Oriza sativa* bran extract. (For abbreviations, see the footnote of Table 2).

Table 2: Molecular weight (Mw) and % amount of haemolymph refractionation protein pattern of *Shistocerca greagaria* control and treated females with lufenuron and *Oriza sative* bran extract at different time intervals post-treatment.

Parameter:		Marker		1		2		3		4		5		6		7		8		9		10		11		12	
Row	Rf	Mw	% am.	Mw	% am.	Mw	% am.	Mw	% am.	Mw	% am.	Mw	% am.	Mw	% am.	Mw	% am.	Mw	% am.	Mw	% am.	Mw	% am.	Mw	% am.	Mw	% am.
r_1	0.05															156.79	13.83	155.34	5.36							158.31	6.68
r_{2*}	0.07																					147.83	6.1				
r_3	0.08					143.67	15.4			142.71	7.37													141.78	4.55	143.67	7.49
r_4	0.09																	138.32	4.01								
r_{5*}	0.13																					126.8	6.49				
r_6	0.14							126.24	3.51															125.14	7.93	125.14	4.84
r_{7*}	0.15									124.09	4.49																
r_{8*}	0.16																					121.59	4.42				
r_9	0.17			119.28	4.25							119.28	4.47					120.19	3.45	119.28	4.53						
r_{10*}	0.18							116.72	3.79																		
r_{11}	0.19									115.52	5.23											115.91	6.05				
r_{12}	0.2			114.74	5.7																			114.36	7.52	113.98	8.34
r_{13}	0.21											113.24	4.3					112.51	3.7								
r_{14*}	0.22							111.46	3.11																		
r_{15}	0.23																					110.1	5.72				
r_{16*}	0.24											109.12	4.08														
r_{17**}	0.25																	106.95	6.61								
r_{18}	0.26											106.36	3.96											106.36	6.26		
r_{19}	0.3																	101.72	4.88								
r_{20}	0.31									100.48	7.34	100.48	7.67	100.97	6.87	100.73	10.52					100.48	4.6	100.73	4.76	100.48	5.66
r_{21}	0.32	100	10.7					98.29	6.99																		
r_{22}	0.33			97.17	7.03	97.17	6.96																				
r_{23}	0.36											89.28	12.92					90.79	18.62	90.28	22.23	90.79	16.03				
r_{24}	0.37					87.3	20.66							87.79	16.83	88.28	23.06										
r_{25}	0.38			85.86	13.66			86.34	14.28	86.34	13.31													86.34	26.71	86.82	30.88
r_{26*}	0.39																			83.97	9.41						
r_{27}	0.41																	79.9	10.9			80.34	7.62				
r_{28}	0.42					77.72	20.95					79.46	8.75	79.46	12.29	79.46	14.89										
r_{29}	0.43			76.03	13.78			77.3	17.58											76.45	12.5						
r_{30}	0.44									75.2	14.29	74.37	6.81														
r_{31}	0.45													73.56	10.56	73.97	7.18									72.75	5.87
r_{32*}	0.56																			53.07	8.42						
r_{33}	0.57	50	16.91	50	8.13	51.31	5.93																			50.87	7
r_{34}	0.58							48.77	5.01	49.02	4.51									48.77	5.07						
r_{35*}	0.59													48.05	5.84												
r_{36}	0.6																	47.57	8.87								
r_{37}	0.61					46.4	4.9									46.16	6.85									46.63	11.07
r_{38}	0.62			45.7	6.75					45.47	4.69	45.47	4.74	45.47	6.01					45.24	6.3			45.24	8.73		
r_{39}	0.63							44.56	6.88													44.56	8.3				
r_{40}	0.67									41.27	3.47									40.42	6.6						
r_{41}	0.68	40	16.31									40	6.72					39.51	4.58			40	4.85	40	5.5		
r_{42*}	0.71									34.67	4.22																
r_{43}	0.73	30	12.33															29.4	5.68								
r_{44}	0.76			21.73	7.29	22.88	6.02	23.47	3.63	21.73	3.51	22.88	7.15	22.3	8.42												
r_{45}	0.77	20	15.94																					19.76	3.48		
r_{46}*	0.78																			19.51	5.99						
r_{47}	0.79																	17.82	5.64			18.3	5.21				
r_{48}	0.8											16.88	5.78	16.88	6.19												
r_{49}	0.81							16.41	6.76	16.64	3.59																
r_{50}	0.82			15.48	6.65	15.71	5.88																	15.25	6.04		
r_{51}	0.83																			14.1	14.42	14.33	5.2				
r_{52*}	0.84																	13.65	4.24								
r_{53}	0.86			11.2	6.02	11.2	3.81	11.64	5.63																		
r_{54}	0.87									10.98	6.9					10.98	11.41							10.11	4.68		
r_{55*}	0.89	9	7.02																								
r_{56}	0.91							7.15	6.16															7.77	4.12		
r_{57}	0.92									6.73	4.49																

* Asterisks indicator to characteristic band

Key for Table 3 (For Fraction Protein haemolymph).

M Marker
1- Control after 48 hrs. Post-treatment
2- Control after 10 days Post-treatment
3- Control after 15 days Post-treatment
4- Control after 20 days Post-treatment
5- Sample treated with lufenuron48 hrs. Post-treatment
6- Sample treated with lufenuron after 10 days Post-treatment
7- Sample treated with lufenuron after 15 days Post-treatment
8- Sample treated with lufenuron after 20 days Post-treatment
9- Sample treated with *O. sativa* bran extract 48 hrs. Post-treatment
10- Sample treated with *O. sativa* bran extract 10 days Post-treatment
11- Sample treated with *O. sativa* bran extract 15 days Post-treatment
12- Sample treated with *O. sativa* bran extract 20 days Post-treatment

Whereas the minimum number of bands was observed in sample no. 7 (females treated with lufenuron, 15 days post-treatment) and was 7 bands. The haemolymph SDS protein pattern revealed 18 unique bands and scored as follows: three unique bands for sample no. 10 at Mw values of 147.83, 126.8 and 121.59. Two unique bands for sample no. 4 at Mw value of 124.09 and 34.67. Two unique bands for control sample no. 3 at Mw values of 116.72 and 111.46. There are one unique band for sample 5 at Mw value of 109.12, two unique bands for sample no. 8 at Mw of 106.95 and 13.65, three unique bands for sample no. 9 at Mw of 83.97, 19.51 and 53.07, three unique bands for sample no. 6 at Mw of 68.43 and 59.99, 48.05, one unique band in sample no. 7 at Mw value of 53.52. The last unique band was observed for the marker at Mw value of 9.0.

Similarity index and hence epigenetic distances were calculated between control and treated samples as shown in Table 3. The latent effect of either match or rice bran extract was obvious since epigenetic distances accounted for 1 for Lufenuron and 0.76 for *Oriza sativa* bran extract, 20 days post-treatment.

Table 3: Epigenetic distances between treated and untreated samples in refraction protein pattern haemolymph of females *Schistocerca gregaria*.

Samples post-treatment	**Lufenuron**	***Oriza sativa* bran extract**
48 hrs	0.68	0.81
10 days	0.69	1
15 days	1	0.85
20 days	1	0.76

Effect of Lufenuron and *Oriza sativa* bran extract on acid phosphatase pattern in haemolymph of *Schitocerca gregaria.*

Seven acid phosphatase bands were recognized in treated and untreated samples, with Rf values ranged between 0.02 and 0.86 (Table 4, and Fig. 2). The acid phosphatase pattern revealed 3 unique bands for sample no. 12 (haemolymph of female *Schitocerca gregaria* treated with LC_{50} of rice bran extract after 20 days from treatment); one of them at Rf value of 0.02, the second at Rf value of 0.11 and the third at Rf value of 0.17.

Table 4: Relative fragmentation (Rf) and amount % of acid phosphatase pattern of *Schistocerca gregaria* haemolymph for control and samples treated with LC_{50} of tested compounds at different time intervals post-treatment.

Samples		**1**		**2**		**3**		**4**		**5**		**6**		**7**		**8**		**9**		**10**		**11**		**12**	
Lane	**Rf**	+	**% am.**	+	**% am.**	+	**% am.**	+	**% am.**	+	**% am.**	+	**% am.**	+	**% am.**	+	**% am.**	+	**% am.**	+	**% am.**	+	**% am.**	+	**% am.**	+	**% am.**
r_{1*}	**0.02**	-		-		-		-		-		-		-		-		-		-		-		+	9.15
r_2	**0.03**	-		-		-		-		-		-		-		-		+	23.25	+	16.67	+	15.73	-	
r_{3*}	**0.11**	-		-		-		-		-		-		-		-		-		-		-		+	8.59
r_{4*}	**0.17**	-		-		-		-		-		-		-		-		-		-		-		+	13.18
r_5	**0.72**	-		-		-		-		-		-		+	100			+	76.75	+	50.67	+	43.3		
r_6	**0.73**	+	100	+	100	+	100	+	100	+	100	+	100			+	100	-		-		-		+	34.93
r_7	**0.86**	-		-		-		-		-		-		-		-		-		+	32.65	+	40.97	+	34.14

* Asterisks indicator to characteristic band (+) = Present band (-) = Absent band

Key for Table 2 (For Acid Phosphatase)

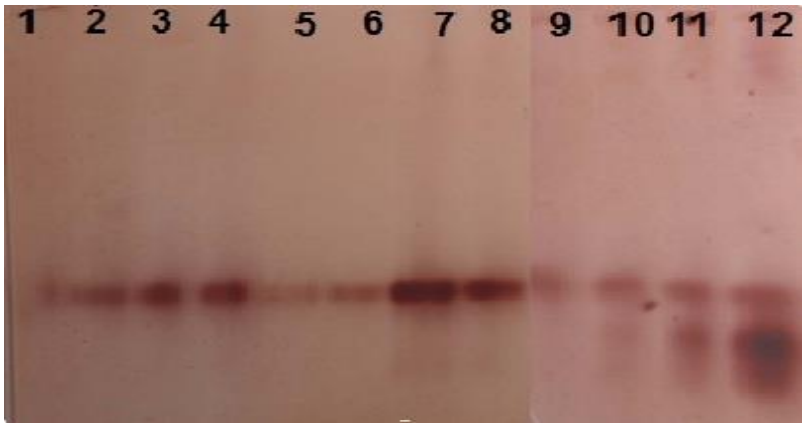

Fig. 2: Electrophoretic acid phosphatase pattern of haemolymoh of *S. gregaria* control and treated samples with Lufenuron and *Oriza sativa* bran.(For abbreviations, see the footnote of table (2).

All control samples revealed each only one band with Rf values of 0.73. Also, all treated samples with Lufenuron recorded each one band. Three of them with Rf values of 0.73 and one of them with Rf value of 0.72. While samples treated with *O. sativa*bran extract recorded different bands as follows: sample no. 9(48 hrs post-treatment) revealed two bands with Rf values of 0.03, 0.72. Sample no. 10 and sample no. 11 (10 and 15 days post-treatment) recorded each three different bands with Rf values of 0.03, 0.72 and 0.86. Sample no. 12 (20 days post-treatment) revealed five bands with Rf values of 0.02, 0.11, 0.17, 0.73 and 0.86.

DISCUSSION

The present investigation revealed that, the treatment with insect growth regulators has toxic effects on the desert locust, *Schistocerca gregaria.* These effects depend on the concentrations of the compound and the age of the treated insects. In the present study, Lufenuron act as chitin synthesis inhibitor (CSI), was used against the 5thnymphal instar of *S. gregaria* during one day by feeding technique. The present study showed that, the treatment with different concentrations against one day old of the 5thnymphal instar of S. gregaria caused nymphal mortality and failure to ecdysis to adult increased with the increase of Lufenuron concentrations. More or less, the present results are agreed with those finding by several chitin synthesis inhibitors against the same acridide species, *S. gregaria* such as: diflubezuron, which interfered with the chitin synthesis during the nymphal ecdysis to the last instar causing some mortalities (Taha and El-Gammal, 1985), the greatest mortality was recorded during ecdysis of early the 4thnymphal instar to the 5th nymphal instar of *S. gregaria*when treated with chlorfluazuron (Abo El-Ela *et al.*,1993), also chlorfluazuron induced appreciable failure in ecdysis to adult stage when applicated on the last nymphal instar (El-Gammal *et al.*, 1993), Coppen and Jepson (1996a) when treated the 2nd nymphal instars of *S.gregaria* with diflubenzuron, hexaflumuron and teflubenzuron, they recorded mortality after all other treatments. Triflumuron caused different mortalities after 5 to 15 days of the barrier application in Mauritania (Wilps and Diop, 1997). diflubenzuron and teflubenzuron caused abortive moult, and most survivors developed twisted wings (Wakgari 1997), also, lufenuron exhibited an inhibitory effect on the adult emergence after treatment of last instar nymphs , regardless of the timing of treatment (Bakr, R.F. *et al* 2008).

In the present study on *S. gregaria*, the treatment of 5$_{th}$nymphal instar with

Oriza sativa bran extract, resulted in retarded development since the developmental duration was prolonged and developmental rate was regressed parallelly to the concentration level. More or less, prolonged developmental duration was a good indicator to the inhibited development of the migratory locust *L. migratoria* after treatment with azadirachtin of the desert locust *S. gregaria* after treatment with the seed oil from *Az. Indica* (Nicol and Schmutterer, 1991), of *S. gregaria* after treatment with azadriachtin (Wilps *et al*. 1992), of *S. gregaria* after treatment with neem extracts (Freisewinkel 1993), of 3rdnymphal instar of *Schistocerca gregaria* treatment with neem seed oil (Nicol *et al* 1993), of *S. gregaria* after treatment with three plant species, *Calotrpis procera, Zygophyllum gaetulum* and *Peganumhar mala*. Abbassi *et al*. (2003), of *S. gregaria* after treatment with extracts of *Melia azaedarach, Azadirachtaindica* and *Eucalyptus globules* (Mohamed *et al.* (2006), of *S. gregaria* after treatment with various neem products (Al-Fifi ,Z.I.A. (2009), of *S. gregaria* after treatment with the extracts of four plants, namely *Mucunapruriens* (Fabaceae), *Adenium obesum* (Apocynaceae), *Azadirachta indica* (Meliaceae) and *Calotropis procera* (Asclepiadaceae (Abdalla A.M, *et al*. (2009), of *S. gregaria* after treatment with *F. bruguieri* extracts (Hamadah, *et al*. (2010), of *S. gregaria* after treatment with jatropha seed oil (Ebtisam M. Bashir, *et al.* (2013).

The haemolymph fractioned protein pattern recorded diversity between control and treated *S. gregaria* samples as well; since G. d accounted for 1 for lufenuron and 0.76 for *O. sativa* bran extract, 20 days post-treatment. These data are in agreement for other tested IGRs on other insects (Anita *et al*., 1998; El Bermawy *et al*., 2005; Etebari *et al*., 2007; Bakr *et al*., 2009; Abd El-Bar *et al*., 2013) and also for other tested botanical extracts (Bakr *et al*., 2002; El Bermawy *et al*., 2002; Abd El-Bar *et al*., 2013).

Changes in pattern of protein can mirror specialization and adaptation in different organisms. Each protein views the activity of a specific gene during the production of enzymes which act as catalysts for production of the needed proteins, which respectively are responsible for specific biological character (Hassan and Abd El-Hafez, 2009). Indeed, in the present work, the differences between control and treated samples were translated to epigenetic distances which accounted to 1 in samples treated with match and to 0.76 in samples treated with rice bran extract, even at 20 days post-treatment. This may due to the production of other types of proteins responsible for the production of some characters in treated samples (sterility, malformed adults).

The study of Krishnayya and Rao (1995) showed that plumb again decreased the protein concentration in haemolyph and fat body in last instar of *H. armigera* (Lepidoperta). Etebari *et al*. (2007) reported that pyriproxyfen (IGR) decreased the amount of protein in treated silkworm. While De Kort *et al*. (1991) showed that pyriproxyfen could induce an inhibition of synthesis of protein in haemolymph of *locustami gratoria*.

The present data showed differences between *S. gregaria* control and treated samples in haemolymph acid phosphatase pattern. These results are in harmony with the findings of Abd El-Bar *et al*. (2013) who estimated the effect of three waste products of urea-derivative (which considered CSIs) and rice straw on acid phosphatase pattern of tissues of treated cotton leaf worm *S. littoralis.*

ACP comprise group of enzymes that are non-specific and can hydrolyze many organic esters, which liberate phosphate ions. The optimum pH ranges are from 4.5 to 6 (Essner, 1973). Many researchers have distinct ACP activities with the death of cells, they are considered lysosomal markers (Gregorc and Bowen, 1997). In flying

insects, ACP correlate with tissue hydrolysis, metamorphic molts, gonad maturation and nutrition (Radha and Priti, 1969). This enzyme hydrolyzes many orthophosphate ester and hence activate transphosphorylation reactions to multiply phosphate pool to synthesize higher activity (ATP compounds), ATPase and DNA or RNA (Hollander, 1971). Hence, the high diversity between treated samples and control may due to the lower metabolic rate in treated *S. gregaria* samples in this study. Senthil Nathan *et al*. (2005) suggested that "decreased levels of ACP activity reduced liberation of phosphate ions for energy needed for metabolism as well as the reduced rate of enzyme regulation transport". ACP has an imperative role in detoxification. Enzymes included in detoxification in insects are considered the enzymatic defense towards foreign materials enter insect's bodies and play a major role in maintaining their normal physiology (Li and Liu, 2007).

Ghoneim *et al*. (2014) studied the effect of n-butanol (botanicl extract) against ACP in *S. gregaria* haemolymph and reported that the decreased ACP may be due to strong inhibition of ecdysone, which have been followed by subsequent decline in lysosomal number and decreased levels of ACP. However, authors stated contradictory effects of IGRs and plant extracts on ACP activity. Assar *et al*. (2012) for example showed that Cyromazine (IGR) highly increased the ACP activity. Also Anwar and Abd El-Mageed (2005) concluded that change in response against some tested IGRs can be correlated with increase in ACP activity in treated *S. littoralis*. However, in the current study, it was noted that the tested compounds induced low mortality rates in treated nymphs, followed by great malformed rates of the adults (unpublished data) and high total inhibition of adults, which strongly suggested a strong inhibitory effect either for match or rice bran extract on ACP in haemolymph of treated *S. gregaria*.

In conclusion, the treated nymph with the lethal concentration LC_{50} of the tested compounds (lufenuron and *Oriza sativa* bran extract) showed a significant change in fraction protein andacid phosphatase patterns of haemolymph, which was clearly reflected in high epigenetic differences between control and treated *S. gregaria* samples. So, the tested compounds may be considered a valuable tool for the control of *S. gregaria* as a component of an IPM programme.

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ARABIC SUMMARY

تأثير ليوفينيورون ومستخلص غلاف حبة الارز على الانماط البروتينية والفوسفات الحمضى للجراد الصحراوى

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أجريت هذه الدراسة المعملية لتقييم الأثر البيوكيميائي لمركبين هما ليفينيورون ومستخلص غلاف حبة الأرز على هيموليمف حشرة الجراد الصحراوي *شيستوسيركاجريجاريا*. كانت الجرعة نصف المميتة 35.66 pppmل ليفينيورون و 541.71 pppmلمستخلص غلاف حبة الأرز. وأظهر الفصل الكهربي للأنماط البروتينية عدد 65 حزمة بروتينية مع وزن جزيئي تراوح بين 158.31 و6.73 مع 18 حزمة مميزة.، كما أظهر الفصل الكهربي أن التباعد الوراثي بين الكنترول والعينات كان 1و 0.76لكل من ال ليوفينيورون و مستخلص غلاف حبة الأرز على التوالي للعينة (عشرين يوم بعد المعاملة). في حالة الفوسفات الحمضي وجد أن عدد الحزم التي وجدت نتيجة الفصل الكهربي كانت سبع خمائل مع معامل هجرة يتراوح بين 0.02 و0.86. و كانت هناك ثلاث حزم مميزة. مما سبق يتضح أن المركبين تحت الاختبار قد أحدثا تأثيرا في الأنماط البروتينية و الفوسفات الحمضي و ل هيمولمف حشرة الجراد الصحراوي و الذي انعكس في التباعد الوراثي المذكور.