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Do Egg Pods in the Desert Locust *Schistocerca gregaria* Display as Oogenesis Limiting Factor? IV-The Effects of Egg Pod Factors on Haemolymph Main Metabolties

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Abstract: The haemolymph of treated ovipositing adult females treated with egg pod factors, was collected during the periods of vitellogenesis, oogenesis and egg laying, after 10, 17, 19 and 21 days of the adult life. The exposure of solitary and gregary adult females to contaminated ovipositing sites resulted in an obvious disturbance in the concentration of the haemolymph main metabolites during vitellogenesis and oogenesis. These results support the biological effects of the two extracts on hatching percentages. It could be attributed to the inhibitory action of the tested extracts on Juvenile hormone levels during these periods.

Key words: *Schistocerca gregaria*, haemolymph, egg pod factors, vitellogenesis, oogenesis- Juvenile hormone

INTRODUCTION

It is well-documented fact that the ovarian development in the adult female of *Locusta migratoria* is essentially dependent on the corpora allata[1,2]. The JH is suggested as a gonadotrophic factor for stimulation of synthesis and release of vitellogenic proteins, from the fat body[3]. Although there are several reports on the presence of moulting hormones in adult insects[4,5]. It was surprising to observe that ovarian maturation in female adults (never in males) of *Locusta migratoria* was concomitant to the production of ovary large amounts of ecdysteroids active in the classical bioassay for moulting hormones. According to Lagueux[6], considerable amounts of ecdysteroids are produced during each ovarian cycle in adult female of *Locusta migratoria* when vitellogenesis is almost completed The hormonal molecules are synthesized at the end of maturation of the terminal oocyte during each cycle, at the time when vitellogenesis is almost completed. Ovariole yield of *Schistocerca gregaria* were higher by using hexane extract of froth or egg mass than by ethanol[7]. The objective of this study aims to discuss the mechanism of controlling egg production in the desert locust which may introduce an unprecedent phenomenon in this respect.

MATERIALS AND METHODS

Evaluation of the biochemical response of *S. gregaria* to egg pod extract during ovipositing of adult females was carried out as the method was designed by Saini *et al.*[8] to study their effects on haemolymph main metabolites. Two components of the egg pod (froth and egg) were extracted separately in hexane and ethanol. Each extract was added to the sterilized sand in the treated cups as ovipositing sites for solitary and gregary phases. The ovipositing 8 days old adult females with adult males of the same age were exposed to these cups till egg laying. Untreated males and females caged in another cups with normal sand under the same conditions far from treated ones. The haemolymph of solitary and gregary females exposed to non-polar and polar extracts of froth and eggs; were collected for analysis The haemolymph was sampled in the 10th day (representing the pre-oviposition period), the 17th day and 19th day (representing the start of oviposition) and 21st day (representing the oviposition period). The concentration of haemolymph main metabolites (proteins, lipids, carbohydrates and cholesterol) were determined.

Chemical analysis: As general practice, all the tested metabolites in each treated group were extracted and estimated separately. Three pools for each treatment were utilized and each pool consisted of 8 locusts. Each tissue pool was divided into four equal samples. Each for assay of one metabolite. Each sample was immediately subjected, when still fresh, to the chemical analysis. The metabolites were colourimetrically determined presuming the following steps:

Samples collection and purification: The haemolymph was collected through a fine puncture in the hind leg membrane and transferred into clean dry centrifuge tubes. Few crystals of phenyl thiourea were added to prevent

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menalization before analysis. A known volume of the collected haemolymph (0.1 mL) was diluted up to 2 mL with saline solution and purified by centrifugation to remove blood cells and pigments. Then the filtrate was collected for haemolymph analysis.

Determination of protein: Protein content was determined by Biuret reagent according to the method described by Gornall *et al.*[9].

Determination of carbohydrate: Total carbohydrates were estimated by the method of Trinder[10].

Determination of total lipid: Total lipids were estimated by modified methods of Knight *et al.*[11].

Determination of cholesterol: Total cholesterol was determined by the enzymatic colorimetric method[12].

Statistical analysis: All data were subjected to analysis of variance. Means were compared using least significant difference (LSD) at $\alpha = 0.05$ by using Microsoft Excel software Program.

RESULTS AND DISCUSSION

Effects on total haemolymph protein: The early studies on the reproductive potential of locust indicate that the juvenile hormones (JH) are effective agents during oocyte maturation[13]. During vitellogenesis in locust (parallel to the time of treatment with pod factors during the pre-ovipositing period), JH pod factors switches general protein synthesis in fat bodies to vitellogenin, which increases in parallel to a total protein content. When the eggs are released into the oviduct (parallel to the time of treatments during the oviposition period), the second generation of oocytes is already in need for vitellogenin nourishment[14]. On the other hand, suppression of JH (production or function) prevents vitellogenin synthesis completely and diminished the production of other proteins[15].

Four dates for sampling were practiced based on expected physiological states; pre-oviposition and oviposition : 10 and 17 days before egg laying, 19 days after laying the first egg pod and 21 days during the ovipositing period, respectively.

Obvious phase dependant variation could be detected in control females. The total haemolymph protein was higher in solitary phase, in all days of sampling, than gregary phase. In both phases the content of 21st day was highest followed by 10th day followed 17th day and lastly 19th days, thus coincided with egg maturation (Table 1).

The treatment with egg pod extracts caused remarkable reduction in solitary females haemolymph protein content. The haemolymph protein content was lowered extremely and thus explaining the down rate of egg pod, eggs per pod, fecundity and reproductive performance shown with respect to solitary females (in the first paper of this series). It may be of concern to note that this trend was proper in case of using extracts of solitary pods (Table 1).

However, in gregary females, by ethanol and the treatment by extracts of gregary egg pods this tendency was reversed and treatments increased the protein haemolymph content. It thus reflected the relatively faint effect of treatments with respect to reproduction potential. It also explain the qualitative improvement of gregary laid eggs.

The extracts of solitary egg pods were more effective than those of gregary phase. The gregary ethanol extracts of gregary pods caused higher haemolymph protein content than control reaching sometimes seven folds (gregary ethanol/froth with solitary females; 8.11 g/100 mL. Compared with 1.39).

In conclusion, the total haemolymph protein as the key metabolite for production of eggs and determining its quality give the explanation of previous results. The non-polar and polar compounds in the extracts and used in contamination of sand may interfere with the humeral balance of treated females causing existence of anti juvenile hormone action. The effect of these factors must be premiring and not releasing owing to its prolonged effect. As found by Highnam *et al.*[16] JH is the most effective agent during egg maturation and any interference with its function induce confusion of ovarian maturation and metamorphosis. The increase in haemolymph protein contents shown in the present study may be considered according to Highnam *et al.*[16], an indicator for blocking yolk deposition in the oocytes. This assumption was previously thrown in a precedent paper concerning weight of eggs. Normal solitary females have high reproductive potential than the normal gregary ones and consequently the haemolymph protein content reflected such phase variation[17]. However, the gregarious contain high content of JH when compared with solitaries[18].

In the light of these results it was tried to carry on biometrics studies on the corpora allata of treated and control females. Regretfully, the results were not promising and no dependable correlation was found, probably because the effect of pod factors may be releasing working on the JH directly rather on the corpora allata. Anyhow, this point is promising to peruse the mode of action of the pod factors. In line with this explanation, Giraridie *et al.*[19] working on the time

Table 1: The effects of non-polar and polar of egg pod extracts, on the haemolymph total protein (g/100 mL) of the ovipositing *S. gregaria* females

	Hexane extracts				Ethanol extracts			
	Solitary female		Gregary female		Solitary female		Gregary female	
Type of extract and days of sampling	Cont.±S.D.	Treat±S.D.	Cont.±S.D.	Treat±S.D.	Cont.±S.D.	Treat±S.D.	Cont.±S.D.	Treat±S.D.
Solitary froth								
10	1.34±0.02	0.05±0.01	0.12±0.02	0.14±0.03	1.34±0.02	0.09±0.01	0.12±0.02	0.08±0.03
17	1.17±0.02	0.54±0.02	1.15±0.03	0.21±0.01	1.17±0.02	0.15±0.01	7.69±0.02	0.87±0.02
19	1.07±0.02	0.08±0.01	0.93±0.02	0.09±0.02	1.07±0.02	0.63±0.04	1.28±0.01	45.35±0.02
21	1.39±0.03	0.43±0.04	1.29±0.10	0.11±0.02	1.39±0.03	0.78±0.06	7.69±0.04	49.35±0.03
Solitary egg								
10	1.34±0.02	0.16±0.04	0.12±0.02	0.07±0.02	1.34±0.02	0.44±0.04	0.12±0.02	01.00±0.02
17	1.17±0.02	0.58±0.03	1.15±0.03	0.04±0.01	1.17±0.02	0.41±0.02	7.69±0.02	3.46±0.10
19	1.07±0.02	0.23±0.05	0.93±0.02	0.48±0.06	1.07±0.02	0.48±0.03	1.28±0.01	22.51±0.05
21	1.39±0.03	0.10±0.02	1.29±0.10	1.05±0.04	1.39±0.03	0.41±0.01	7.69±0.04	25.11±0.09
Gregary froth								
10	1.34±0.02	0.43±0.03	0.12±0.02	2.06±0.08	1.34±0.02	2.12±0.30	0.12±0.02	1.71±0.11
17	1.17±0.02	0.95±0.03	1.15±0.03	1.97±0.20	1.17±0.02	6.40±0.11	7.69±0.02	15.71±0.12
19	1.07±0.02	0.84±0.04	0.93±0.02	1.89±0.07	1.07±0.02	7.22±0.09	1.28±0.01	3.95±0.04
21	1.39±0.03	0.66±0.05	1.29±0.10	2.53±0.30	1.39±0.03	8.11±0.05	7.69±0.04	7.89±0.30
Gregary egg								
10	1.34±0.02	0.82±0.12	0.12±0.02	2.06±0.33	1.34±0.02	2.04±0.12	0.12±0.02	1.11±0.11
17	1.17±0.02	1.22±0.10	1.15±0.03	1.11±0.10	1.17±0.02	4.18±0.12	7.69±0.02	12.86±0.03
19	1.07±0.02	1.67±0.01	0.93±0.02	0.92±0.11	1.07±0.02	2.23±0.04	1.28±0.01	10.00±0.10
21	1.39±0.03	1.42±0.04	1.29±0.10	1.20±0.30	1.39±0.03	1.98±0.01	7.69±0.04	12.86±0.10

Table 2: The effects of non-polar and polar of egg pod extracts, on the haemolymph total lipid (g L^{-1}) of the ovipositing *S. gregaria* females

	Hexane extracts				Ethanol extracts			
	Solitary female		Gregary female		Solitary female		Gregary female	
Type of extract and days of sampling	Cont.±S.D.	Treat±S.D.	Cont.±S.D.	Treat±S.D.	Cont.±S.D.	Treat±S.D.	Cont.±S.D.	Treat±S.D.
Solitary froth								
10	2.20±0.04	0.69±0.02	5.57±0.07	4.50±0.07	2.20±0.04	3.81±0.11	5.57±0.07	4.17±0.09
17	1.67±0.01	0.40±0.02	10.53±0.07	9.08±0.06	1.67±0.01	2.92±0.1	10.53±0.07	11.22±0.07
19	2.67±0.02	1.40±0.07	6.84±0.10	5.22±0.01	2.67±0.02	2.86±0.02	6.84±0.10	8.32±0.10
21	2.00±0.30	0.40±0.02	7.84±0.03	4.52±0.10	2.00±0.30	6.19±0.02	7.84±0.03	8.32±0.16
Solitary egg								
10	2.20±0.04	0.29±0.52	5.57±0.07	1.67±0.07	2.20±0.04	1.43±0.11	5.57±0.07	5.42±0.50
17	1.67±0.01	6.00±0.50	10.53±0.07	5.42±0.07	1.67±0.01	4.76±0.02	10.53±0.07	9.58±0.04
19	2.67±0.02	3.60±0.20	6.84±0.10	3.44±0.20	2.67±0.02	6.19±0.03	6.84±0.10	5.82±0.07
21	2.00±0.30	2.50±0.10	7.84±0.03	3.44±0.20	2.00±0.30	4.76±0.05	7.84±0.03	6.85±0.06
Gregary froth								
10	2.20±0.04	2.08±0.08	5.57±0.07	13.16±0.30	2.20±0.04	2.92±0.06	5.57±0.07	23.16±0.16
17	1.67±0.01	7.00±0.60	10.53±0.07	21.05±0.07	1.67±0.01	2.83±0.08	10.53±0.07	10.53±0.14
19	2.67±0.02	4.58±0.20	6.84±0.10	14.74±0.02	2.67±0.02	3.33±0.04	6.84±0.10	15.26±0.11
21	2.00±0.30	5.42±0.03	7.84±0.03	12.63±0.03	2.00±0.30	4.63±0.10	7.84±0.03	10.53±0.14
Gregary egg								
10	2.20±0.04	3.42±0.12	5.57±0.07	3.67±0.08	2.20±0.04	10.42±0.2	5.57±0.07	4.33±0.80
17	1.67±0.01	2.77±0.02	10.53±0.07	3.00±0.04	1.67±0.01	5.83±0.18	10.53±0.07	4.33±0.06
19	2.67±0.02	4.58±0.30	6.84±0.10	4.33±0.12	2.67±0.02	7.50±0.30	6.84±0.10	3.00±0.06
21	2.00±0.30	5.82±0.10	7.84±0.03	4.50±0.07	2.00±0.30	6.25±0.04	7.84±0.03	5.67±0.11

dependent variation in the activity of ovary maturating neurohormone from the nervous corpora cardiaca during oogenesis in *Locusta migratoria*, showed that the action of the neurohormone on vitellogenesis is probably not mediated through the corpora allata. The ovary maturating neurohormone appeared to be a previously reported gonadotropic juvenile hormone independent factor.

Effects on total lipids: Gregary females have had haemolymph total lipid exceeding those of solitaries many folds (solitaries ranged from 1.67-2.67 g L^{-1}, gregarious from 5.57-10.53 g L^{-1}). The contents of the two phases showed also variations in the days of sampling. In solitaries the 19th day was the highest (2.67), followed by 10th day (2.20), followed by 21st day (2.00) and lastly the 17th day (1.67). In gregarious the 17th day was the highest (10.53), followed by the 21st day (7.84), followed by 19th day (6.84) and lastly the 10th day (5.57) (Table 2).

In most cases the 19th day lipid content, contrary to protein content, increased after treatments. The gregary extracts was more increasing the lipid content.

Table 3: The effects of non-polar and polar of egg pod extracts, on the haemolymph total carbohydrate (mg mL^{-1}) of the ovipositing *S. gregaria* females

	Hexane extracts				Ethanol extracts			
	Solitary female		Gregary female		Solitary female		Gregary female	
Type of extract and days of sampling	Cont.±S.D.	Treat±S.D.	Cont.±S.D.	Treat±S.D.	Cont.±S.D.	Treat±S.D.	Cont.±S.D.	Treat±S.D.
Solitary froth								
10	5.92±0.09	0.92±0.05	3.80±0.06	4.60±0.08	5.92±0.09	2.76±0.06	3.80±0.06	2.31±0.15
17	3.35±0.10	5.46±0.04	28.75±0.60	12.90±0.20	3.35±0.10	13.36±0.09	28.75±0.60	33.33±0.40
19	1.22±0.03	5.99±0.20	15.42±0.30	14.16±0.05	1.22±0.03	11.84±0.04	15.42±0.30	22.37±0.10
21	7.32±0.06	9.22±0.08	12.50±0.01	8.21±0.10	7.32±0.06	10.60±0.12	12.50±0.01	15.50±0.12
Solitary egg								
10	5.92±0.09	2.74±0.04	3.80±0.06	0.59±0.09	5.92±0.09	13.82±0.22	3.80±0.06	0.6±0.040
17	3.35±0.10	0.92±0.04	28.75±0.60	1.79±0.03	3.35±0.1	3.69±0.01	28.75±0.60	0.81±0.05
19	1.22±0.03	0.04±0.92	15.42±0.30	2.56±0.04	1.22±0.03	2.30±0.10	15.42±0.30	0.95±0.07
21	7.32±0.06	0.04±0.92	12.50±0.01	10.50±0.06	7.32±0.06	26.27±0.02	12.50±0.01	1.52±0.01
Gregary froth								
10	5.92±0.09	1.92±0.21	3.80±0.06	2.08±0.08	5.92±0.09	4.84±0.22	3.80±0.06	0.83±0.13
17	3.35±0.10	0.46±0.03	28.75±0.60	2.29±0.14	3.35±0.1	2.98±0.10	28.75±0.60	32.08±0.08
19	1.22±0.03	10.12±0.40	15.42±0.30	4.17±0.20	1.22±0.03	22.83±0.21	15.42±0.30	16.83±0.06
21	7.32±0.06	5.95±0.20	12.50±0.01	5.00±0.07	7.32±0.06	13.69±0.02	12.50±0.01	15.42±0.01
Gregary egg								
10	5.92±0.09	4.80±0.11	3.80±0.06	1.25±0.05	5.92±0.09	11.29±0.28	3.80±0.06	4.27±0.20
17	3.35±0.10	5.94±0.10	28.75±0.60	6.63±0.11	3.35±0.1	13.69±0.02	28.75±0.60	26.23±0.11
19	1.22±0.03	0.29±0.01	15.42±0.30	3.32±0.13	1.22±0.03	12.50±0.11	15.42±0.30	18.90±0.20
21	7.32±0.06	5.95±0.20	12.50±0.01	3.52±0.06	7.32±0.06	3.57±0.05	12.50±0.01	18.29±0.13

Table 4: The effects of non-polar and polar of egg pod extracts, on the haemolymph total cholesterol (mg 100 mL^{-1}) of the ovipositing *S. gregaria* females

	Hexane extracts				Ethanol extracts			
	Solitary female		Gregary female		Solitary female		Gregary female	
Type of extract and days of sampling	Cont.±S.D.	Treat±S.D.	Cont.±S.D.	Treat±S.D.	Cont.±S.D.	Treat±S.D.	Cont.±S.D.	Treat±S.D.
Solitary froth								
10	7.69±0.09	1.28±0.28	8.15±0.13	7.44±0.10	7.69±0.09	2.59±0.09	8.15±0.13	4.25±0.09
17	7.69±0.02	10.91±0.06	8.20±0.30	6.43±0.11	7.69±0.02	0.87±0.02	8.55±0.30	15.79±0.10
19	1.28±0.01	5.10±0.30	6.67±0.10	4.32±0.09	1.28±0.01	45.35±0.02	6.67±0.10	13.04±0.70
21	7.69±0.04	11.46±0.40	3.33±0.04	2.95±0.13	7.69±0.04	49.35±0.03	3.33±0.04	13.16±0.20
Solitary egg								
10	7.69±0.09	8.92±0.09	8.15±0.13	6.57±0.07	7.69±0.09	12.59±0.09	8.15±0.13	1.43±0.10
17	7.69±0.02	3.82±0.10	8.20±0.30	2.86±0.03	7.69±0.02	3.46±0.10	8.55±0.30	10.00±0.40
19	1.28±0.01	5.10±0.05	6.67±0.10	2.63±0.02	1.28±0.01	22.51±0.05	6.67±0.10	9.50±0.05
21	7.69±0.04	1.27±0.02	3.33±0.04	1.32±0.05	7.69±0.04	25.11±0.09	3.33±0.04	6.40±0.05
Gregary froth								
10	7.69±0.09	1.57±0.06	8.15±0.13	13.88±0.70	7.69±0.09	12.86±0.08	8.15±0.13	11.38±0.35
17	7.69±0.02	6.58±0.40	8.20±0.30	1.54±0.02	7.69±0.02	15.71±0.12	8.55±0.30	13.79±0.02
19	1.28±0.01	2.88±0.03	6.67±0.10	3.08±0.03	1.28±0.01	3.95±0.04	6.67±0.10	20.69±0.03
21	7.69±0.04	4.29±0.07	3.33±0.04	1.54±0.03	7.69±0.04	7.89±0.30	3.33±0.04	13.19±0.02
Gregary egg								
10	7.69±0.09	8.72±0.11	8.15±0.13	1.54±0.09	7.69±0.09	14.08±0.25	8.15±0.13	7.69±0.12
17	7.69±0.02	8.58±0.4	8.20±0.30	1.28±0.05	7.69±0.02	12.86±0.03	8.55±0.30	12.82±0.11
19	1.28±0.01	7.14±0.05	6.67±0.10	2.42±0.08	1.28±0.01	10.00±0.10	6.67±0.10	3.85±0.07
21	7.69±0.04	8.52±0.30	3.33±0.04	2.32±0.04	7.69±0.04	12.86±0.10	3.33±0.04	2.56±0.04

Effects on total carbohydrates: Table 3 revealed that the phase dependant variation of lipid contents was properly reflected in carbohydrate contents.

In most cases the carbohydrate contents were remarkably increased by ethanol extracts and decreased by hexane extracts.

Effects on total cholesterol: A phase dependant variation was also found here. Solitary control contained 1.28-7.69 mg/100 mL and gregary one contained 3.33-8.20 mg/100 mL . The order of sampling period content was 10th, 17th , 21st (7.69) and lastly 19th (1.28) in solitary females, while in gregarious the order was 17th following by 10th, following by 19th and lastly 21st (Table 4).

Ethanol extracts exited very pronounced increase in both phases, but this increase was much more in solitaries than in gregarious. Hexane caused increase in some treatments and decrease in others without obvious tendency.

Finally, it may be valid that the answer of the question labeling these papers is Yes egg pods do. Under

this experimental study the egg pod factor limited the oogenesis, more and above it affected other aspects of the reproductive potential. The effects were extended to some aspects of biological significance and other concerning the offspring.

The current results are indeed in contradiction with those of Saini *et al.*[8]. They were interested in studying the attractant effect of egg pods and their experiments were different from the experiments of this study in the following principals:

- They used gravid females which are physiologically toned to express the oviposition behaviour. Their threshold to express the action pattern was in wait for the token stimuli to release the mechanism of egg laying. In this study the treated females have had no eggs yet in their ovaries and the factors affected, consequently, the egg production. When females were used in the oviposition period, they were about to start the second peritrophic period and during maturation of oocytes. In other words, when these factors were tested during vitellogenesis and oogenesis their adverse effects on egg production were able to be expressed.
- They used only the egg pods of gregary phase as a source for extraction and tested only the gregary females. It must be recalled that present work revealed phase dependent responses towards these factors; the adverse effect was lower for the quantitative characters in gregarious (hatching, fertility and ovariole yield). In solitaries the quantitative characters (number of egg pods, number eggs per female and fecundity) were less affected when compared with gregarious. This potency of gregarious may be in agreement with Saini *et al.*[8] who worked only on gregarious and showed that egg pods of gregarious gravid females increased in response to egg pod factors.
- They mixed the polar and non-polar fractions in some experiments. It was shown by the different responses towards the egg pod factors that the non-polar and polar fractions each have had its specific pointing to probable dissimilarity.
- They used number of egg pods as a parameter to evaluate the effect, which is logic according to the objective of their work. In the present work numerous aspects were evaluated and perused to the offspring to evaluate the controlling effects of egg pods rather than the behaviour of aggregation to oviposit.

For these differences in the experimental procedure and objectives and parameters, it may be accepted that the present work revealed the control effect of the egg pod factors, while the experimental work of Saini *et al.*[8] revealed the attractant effect of these factor.

The identification of such factors has very considerable implications for locust control and there is very possibility that agonists or antagonists of these factors or its production might provide the basis of new strategies to control locust. Such work was initiated by Rai *et al.*[20] but a lot of work still be needed. Finally, it may be concluded that the answer of the question labeling these papers is Yes egg pods do. Under this experimental study the egg pod factor limited the oogenesis, more and above it affected other aspects of the reproductive potential.

REFERENCES

1. Joly, L., 1960. Function des corpora allata chez *Locusta migratoria*. These, Strasborg, France.
2. Minks, A.K., 1967. Biochemical aspects of Juvenile hormone action in the adults *Locusta migratoria*. Arch. Neerl. Zool., 17: 175-258.
3. Bar-Zev, A., E. Wasje, E. Cohen and H. Emmerich, 1975. Vitellogenin accumulation in the fat body and haemolymph of *Locusta migratoria* in relation to egg maturation. J. Insect Physiol., 27: 1257-1263.
4. Schlaeger, D.A., M.S. Factts and S.H. Kang, 1974. Ecdson mediated stimulation of carboxlase activity and its relationship to ovarian development in *Aedes aegypti*. J. Cell. Biol., 16: 454-465.
5. Hagedorn, H.H., J.O. Connor, M.S. Fuchs, B. Sage and M.K. Bohm, 1975. The ovary as a source of ecdysone in an adult mosquito. Proc. Not. Acad. Sci. USA, 72: 3255-3259.
6. Lavuex, M., C. Sall and J.A. Hoffman, 1997. Ecdysteroid during embryogensis of *Locusta migratoria*. Am. Zool., 21: 715-726.
7. Eid, M.A., S.A.S. Elmaasarawy, H.A. Elshabrawy, G. Elsayed, A.M. Elgammal and G.A. Mohamed, 2004. Do egg pods in desert locust Schistocerca gregaria display as oogenesis limiting factor? 2- Effects of females treated during the oviposition period. Pak. J. Biol. Sci., 7: 589-592.
8. Saini, R.K., M.M. Rai, A. Hassanali, J. Wawiye and H. Odongo, 1995. Semiochemical from froth of egg pods attract ovipositing females *Schistocerca gregaria*. J. Insect Physiol., 41: 711-716.
9. Gornall, A., C. Bardwell and M. David, 1949. Determination of serum protein by means of biuret reaction. J. Biochem., 177: 751-766.
10. Trinder, P., 1969. Enzymatic determination of sugars in serum and plasma. Ann. Clin. Biochem., 6: 24.

11. Knight, J.A., S. Anderson and J.M. Rawle, 1972. Chemical basis of the sulfophosphovanillin reaction for estimating total lipids. Clin. Chem., 18: 19-202.
12. Richmond, W., 1973. Preparation and properties of a cholesterol oxidase from *Noctidia* sp. and its application to the enzymatic assay of total cholesterol in serum. Clin. Chem., 19: 1350-1356.
13. Loher, W.L., 1960. The chemical acceleration of the maturation process and its hormonal control in the male of the desert locust. Proc. Roy. Soc. London. Ser., B., 153: 308-397.
14. Ferenzy, H.J., E.L. Esther and L. Lubzens, 1981. Vitellin and vitellogenin incorporation by isolated oocytes of *Locusta migratoria*. J. Insect Physiol., 27: 869-875.
15. Gellisson, G. and G.R. Wyatt, 1981. Air borne factor affecting the grouping behaviour of locust. Nature, 218: 782-783.
16. Highnam, K.C., P.T. Hasked and L. Hill, 1964. The endocrine system of isolated and crowded *Locusta* and *S. gregaria* in relation to oocytr growth and the effects of flying upon maturation. J. Insect Physiol., 10: 849-864.
17. Uvarov, B.P., 1977. Grasshoppers and Locusts. Center for Overseas Pest Research. London.
18. Tawfik, A.J., K. Treiblamyr, A. Hassanali and E.O. Osir, 2000. Time-cours haemolymph Juvenile hormone titers in solitaries and gregarious adults of *S. gregaria* and their reaction to pheromone emission, CA volumetric changes and oocyte growth. J. Insect Physiol., 46: 1143-1150.
19. Giraridie, J., J.C. Huet and Z. Atay-Kadiri, 1995. Lom OMP, a putative ecdysiotropic factor for the ovary in *Locusta migratoria*. J. Insect Physiol., 42: 215-221.
20. Rai, D.M., A. Hassanali, R.K. Saini, H. Odongo and H. Kahoro, 1997. Identification of component of the ovipositing aggregation pheromone of the gregarious desert locust, *Schistocerca gregaria*. J. Insect Physiol., 43: 83-87.