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Sterilizing Activity of the Insect Growth Regulator, Lufenuron on *Drosohpila melanogaster* (Meigen)

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ABSTRACT

Due to the complicated problems coming from excessive applications of insecticides, searching of safe substitutes to these insecticides has become necessity. Thus, the insect growth regulators are candidated to be used in such concern. The insect growth regulator, lufenuron was applied against *Drosophila melanogaster* (Meigen) as mixed with the diet of the larvae, with concentrations of 5, 10 and 20 ppm to test its efficacy as a sterilizer. Data showed that 5ppm treatment pushed up the larvae to speed pupation by the first two days after treatment. Overall results showed that 20 ppm recorded the highest pupation (94%) followed by 5, 10 ppm and control treatments with values of 87.5, 85.3 and 82.4%, respectively. The top concentration also caused the lowest adult emergence recording 39.4% then, 10, 5 ppm and control with values of 44.8, 71.4 and 85,7%, resp. The mortalities were arranged in descending order as follows 76.9, 35.0, 7.7 and 4.2% at 20, 5, 10 and control respectively. There were no dead adults recorded except on the ninth day at 10 ppm concentration and control. Regarding the sex ratio, it was greatly affected by lufenuron. It tended to increase the number of males. The number of males was four times the number of females at 5 ppm (1:4). There were no females at 20 ppm (0:12). Number of females was similar to that of males at 10 ppm (1:1). According to these results, the number of the output generation recorded 16 and 80 individuals after 10 and 15 days resp., compared to 96 and 220 individuals respectively in control. Females put eggs on the diet surface at 10 ppm, but it did not hatch. Uncompleted emergence was recorded at 5 and 20 ppm (21.4 and 52.9 %, respectively). Adults with deformed wings were recorded at 20 ppm as 11.8%. Total protein analysis and phenoloxidase activity were carried out. The reduction in total protein occurred in females due to lufenuron treatment. The highest reduction was 16.67 mg/ 1000 insects at 10 ppm concentration that affected on female fecundity. Phenoloxidase activity was high in males. It recorded 1153.33 M O.D./1000 insects at 10 ppm, which affected in male fertility. This may explain why the eggs did not hatch.

Keywords: Sterelizing , Insect Growth Regulator (IGR), lufenuron, *Drosohpila melanogaster*, Sex ratio

INTRODUCTION

One of the objectives of sustaining ecological balance is to preserve the presence of all living organisms, even harmful species. The insects are characterized by their high offspring to resist adverse conditions. Recent trends in the control of insect pests have been based on reduction of offspring without using the traditional pesticides. The insect growth regulators (IGR's) are selective and safe control agents. They are defined as substances which act within an insect to accelerate or inhibit regulatory physiological processes essential to normal development or producing progeny (Siddall, 1976).

To solve the problem of insect resistance to pesticides, it has become important to test levels of effectiveness of the IGRs, both registered and under development, at different concentrations on susceptible insect strains. In the first place, such data should provide a better understanding of these products with regard to their ovicidal or larvicidal properties in order to delay the appearance and spreading of resistance as long as possible (Charmillot *et. al.,* 2001).

IGRs specifically interfere with chitin deposition which was only discovered in insect cuticle or work as specific hormones influencing insect maturity and reproduction mediation (Wright, 1976). They act on insect physiological processes (Hejazi and Jeffrey, 1986& King and Bennett, 1989). Lufenuron (LFN) is a chitin synthesis inhibitor for numerous insect pests (Mosson *et al*. 1995). Also, the insect growth regulators (IGRs) have been prepared in baits to control trypetid and some dipteran pests as these chemicals inhibit adult reproduction (Alam *et. al.,* 2000; Moya *et. al.,* 2010 and Sánchez-Ramos *et.al*., 2012). One more advantage is the insect growth regulators are less harmful to the natural enemies than pesticides. They caused a low reduction (35.54 – 40.51%) in *Chrysoperla carnea* and true spiders populations as important predators in sugar beet fields, while the conventional insecticide caused a high reduction (93.39%) in these predators (Ibrahim, 2014).

Therefore, this research aimed to study the sterilizing activity of lufenuron (IGR) on *Drosophila melanogaster* (Meigen), with the possibility of applying thise substance within safe chemical sterilizer.

MATERIALS AND METHODS

1- Culturing *Drosophila melanogaster* :

The adults of *D. melanogaster* were collected from nature. The flies were allowed to put their eggs on ripe banana in a glass bottle. When larvae appeared, the artificial diet was prepared as that described by Wilson and Cryan (1997). It was consisted of corn meal, sugar cane molase , yeast, agar and propionic acid as an antifungal agent. Larvae were transferred into other glass bottles (4 cm diameter) which was one third full of diet. The bottles were closed by thin cloth and rubber band and were incubated at 25C˚(Nunaatuk and Intoch, 2009) . The diet was renewed every 10 days to avoid bacteria and mold growth. *D. melanogaster* was reared for six generations at the laboratory of Economic Entomology Dept., Faculty of Agriculture, Kafrelsheikh University.

2-Insect growth regulator (IGRs):

Product name: lufenuron 96% TC.

Chemical name : (RS)- 1- [2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)phenyl]-3-(2,6-difluorobenzoyl)urea(IUPAC)

Chemical formula: $C_{17}H_8CL_2F_8N_2O_3$

It is a chitin synthesis inhibitor (CSI) Produced by Dezhou Luba Fine Chemical Company, Dezhou, China.

Imported by Kafr El-Zayat Pesticides and Chemicals Company.

3-The laboratory test:

Three concentrations; 5, 10 and 20 ppm of lufenuron were prepared using acetone as a solvent. The concentrations were determined to be less than $LC_{50.}$ The tests were based on treating the artificial diet with

lufenuron. Food was treated by mixing with each concentration at a ratio of 1 ml lufenuron/ 10 ml semi liquid diet (1:10) according to Ali *et al*., 2016.

The fresh semi liquid diet was poured into petri dishes (5 ml/dish) as four dishes were assigned for each concentration, in addition to four dishes as control (acetone only). Thirty five larvae of second-third larval instars were introduced into each dish, and left feeding for three hours. Then, the larvae were transferred individualy into lufenuron-free glass tubes (1×5 cm) with fresh artificial diet. The number of larvae was varied in each treatment because they were difficult to distinguish from the diet. The tested larvae were kept in the tubes for nine days and were observed every 6 hours (four times /day). The percentage of pupation, adult emergence, sex ratio and the deformed adults (incomplete emergence and/or deformed wings) were recorded. Mortality data concerning larvae and pupae were corrected using Abbott's formula (Abbott, 1925).

Statistical analysis

Probit regression estimates and lethal concentrations and times including 50 and 99% mortality were calculated using a complementary log-log (CLL) regression model, using IBM SPSS Statistics software, in which percentage mortality (y) was transformed to the loge (_loge [1 _ y/100]) scale, and exposure time (x) was transformed to the log10 scale. The goodness-of-fit of the CLL model to the data was compared using a chi-square statistic (Abbar et al., 2016). Differences between any two lethal or effective values were considered to be significantly different ($P < 0.05$) if the 95% CI for the ratio did not include 1 (Robertson et al., 2007).

To estimate the sterilization activity of lufenuron, enclosed adults were picked up from all treatments and control at the end of ninth day. After sex determination, females and males were kept alive separately for each treatment at -3c^{o}. They became ready to total protein and phenoloxidase activity analysis according to Bradford (1979) and Ishaaya (1971) respectively. Numbers of emerging first generation adults were recorded.

RESULTS AND DISCUSSION

The obtained results in Tables (1 and 2) showed that the maximum pupae number after six hours from treatment was 32 pupae at 5ppm concentration followed by 10, 20 ppm and control (28, 16 and 12, respectively). At the end of the second day, the highest pupae percentage was achieved in control (70.6%), then 20, 5 and 10 ppm concentrations, respectively. Results at the 3rd ,4th and 5th days revealed that 20 ppm ranked first in the pupation percentage with values of 88.6, 94.3 and 94.3%, respectively, while control ranked last (73.3, 79.4 and 82.4%, respectively).

By estimating the percentage of daily pupation, it was clear that 5 ppm treatment (the least IGR concentration) pushed up *D. melanogaster* larvae to pupate speedy in the first two days. By increasing lufenuron concentration (10 or 20 ppm), pupation percentage were lower. On the other hand, the highest lufenuron concentration (20 ppm) induced the maximum pupation in the last three days of pupation period. By the end of the fifth day, 20 ppm treatment resulted in 94% pupation followed by 5 ppm, 10 ppm treatments and control with values of 87, 85 and 82%, respectively.

Table 1. The accumulative numbers of *Drosophila melanogaster* pupae after lufenuron treatment with tested concentrations

Day	Hours after treatment	The tested concentrations/ppm						Control No. (4)	Control %
		5 ppm		10 ppm		20 ppm			
		No.(1)	%	No.(2)	%	No. (3)	%		
1	6	32	25.0	28	20.6	16	11.4	12	8.8
	12	36	28.1	36	26.5	24	17.1	20	14.7
	18	40	31.3	40	29.4	28	20.0	24	17.6
	24	60	46.9	48	35.3	48	34.3	44	32.4
2	30	76	59.4	60	44.1	76	54.3	60	44.1
	36	80	62.5	68	50	80	57.1	64	47.1
	42	84	65.6	72	52.9	88	62.9	68	50.0
	48	84	65.6	76	55.9	96	68.6	96	70.6
3	54	88	68.8	88	64.7	100	71.4	100	73.5
	60	92	71.9	88	64.7	112	80.0	100	73.5
	66	96	75	100	73.5	120	85.7	100	73.5
	72	96	75	100	73.5	124	88.6	100	73.5
4	78	104	81.3	108	79.4	124	88.6	100	73.5
	84	104	81.3	112	82.4	128	91.4	104	76.5
	90	108	84.4	112	82.4	132	94.3	108	79.4
	96	108	84.4	116	85.3	132	94.3	108	79.4
5	102	112	87.5	116	85.3	132	94.3	112	82.4
	108	112	87.5	116	85.3	132	94.3	112	82.4
	114	112	87.5	116	85.3	132	94.3	112	82.4
	120	112	87.5	116	85.3	132	94.3	112	82.4

(1) Out of 128 larvae.
(2) Out of 136 larvae.
(3) Out of 140 larvae.
(4) Out of 136 larvae.

Table 2. The daily pupation percentage at the three tested lufenuron concentrations throughout five days after treatment

Lufenuron Concentration/ppm	Accumulative Pupation % (days after treatment)				
	1	2	3	4	5
5	32.8	63	72	82	87
10	27	50	69	82	85
20	20	60	81	92	94
control	18	52	73	77	82

Results arranged in Table (3) show that the adults appeared at 5 ppm concentration on the fourth day as 60 adults. Then, the other concentrations (included control) showed individuals on the fifth day. As shown in Table(3) and Fig. (1), the order was descending as follows; control, 20 ppm, 10 ppm (52, 40 and 16 individuals). The numbers of pupae were close at both 5 ppm concentration and control during the five days. The result was also close in the other two concentrations (10 and 20 ppm) in the same period. At the end of enclosing period, the adult emergence was recorded as 85.7, 71.4, 44.8 and 39.4% for control, 5, 10, and 20 ppm, resp. Fig.(2) illustrated that 20 and 10 ppm concentrations had the highest effect of non- pupae hatching (73% each) compared to 5 ppm concentration or control (27%).

Concerning the adult mortality, Table (3) and Fig.(3) showed that half of the enclosed adults died on the first day of the beginning of hatching (the fifth day of the whole experimental period) at 20 ppm concentration. The mortality was calculated as 62.5%. However, mortality was 6.7% at 5 ppm concentration and final mortality was 76.9 and 35% resp. On the other hand, at the concentration of 10 ppm and control, no adults died up to the ninth day, when mortalities were 7.7 and 4.2%, resp.

Table 3. Effect of lufenuron treatments on the *Drosophila melanogaster* adult emergence

Day	5 ppm			10 ppm			20 ppm			control		
	A.E.	D	M%	A.E.	D	M%	A.E.	D	M%	A.E.	D	M%
5th	60	4	6.7	16	0	0.0	40	20	62.5	52	0	0.0
6th	76	12	15.8	44	0	0.0	48	30	62.5	84	0	0.0
7th	80	16	20.0	52	0	0.0	52	32	61.5	88	0	0.0
8th	80	24	30.0	52	0	0.0	52	34	65.4	96	0	0.0
9th	80	28	35.0	52	4	7.7	52	40	76.9	96	4	4.2
Adult emergence%		71.4			44.8			39.4			85.7	
Non adult emergence%		28.6			55.2			60.6			14.3	

A.E.: number of adult emergence.
D: number of dead adults.
M %: adult mortality %.

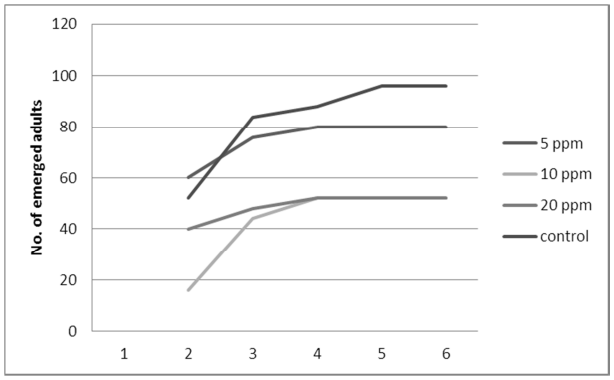

Fig 1. Effect of lufenuron treatments on *Drosophila melanogaster* adult emergence

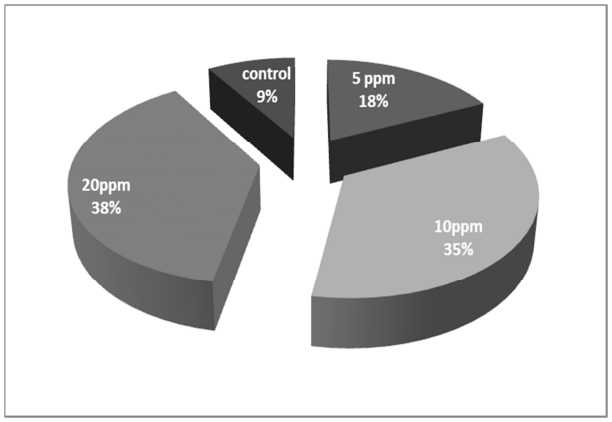

Fig. 2. Percentage of non-emerged *Drosophila melanogaster* adult due to lufenuron treatments

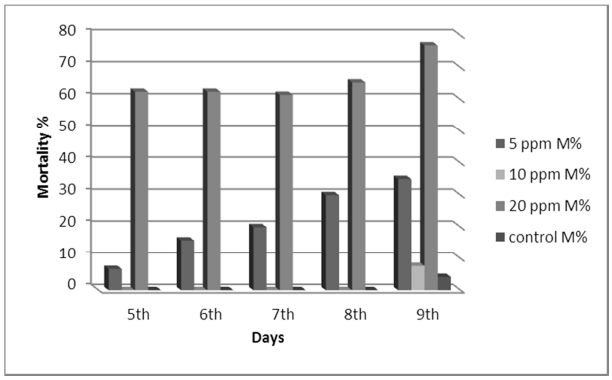

Fig 3. Effect of lufenuron treatments on *Drosophila melanogaster* adult mortality

The linear regression with concentration–mortality curves for the five exposure time of *D. melanogaster* adults were used to confirm the resistance of the target insect to lufenuron. The X2 values for goodness-of-fit were not significant ($P > 0.05$) indicating the suitability of the probit model for the intended estimates (Table 4). The treatments were not considered significant when there was an overlap in the 95% CL of lethal time values. In all treatments, mortality percentage increased with the increase in concentrations and with the passage of time (Table 4). The lowest LC50 and LC99 values were 18.57 and 33.66 ppm, respectively, when D. melanogaster adults were exposed to lufenuron after 6 days. In contrast, after 9 days the highest LC50 and LC99 values were recorded (14.71 and 40.48 ppm, respectively).

Table 4. Probit regression estimates and concentrations required for 50 and 99% reduction of *Drosophila melanogaster* adults progeny production based on mortality assessment conducted 5, 6, 7, 8 and 9 d after exposure to lufenuron at concentration of 5, 10 and 20 ppm .

Exposure time (days)	Regression equation	Slope ± SE[b]	LC (95% CL)[c] (day)		X^{2d}	*df*	*P* -value
			LC_{50}	LC_{99}			
5	*y*=-2.17+0.13 *x*	0.13±0.27	18.57 (23.21-14.86)	33.66 (42.07-26.93)	24.26	1	0.0001
6	*y*=-1.73+0.11 *x*	0.11±0.01	19.72(24.65-15.78)	39.26(49.08-31.41)	36.93	1	0.0001
7	*y*=-1.47+0.09 *x*	0.09±0.01	18.86(23.58-15.09)	42.57(53.21-34.06)	41.94	1	0.0001
8	*y*=-0.93+0.07 *x*	0.07±0.01	18.03(22.54-14.420)	46.49(58.11-37.19)	57.61	1	0.0001
9	*y*=-2.33+0.17 *x*	0.11±0.01	14.71(18.39-11.77)	40.48(50.61-32.38)	49.87	1	0.0001

[a] N ¼ Total number of adults used to generate the probit regression estimates; [b] Slope of the probit mortality line; [c] LC_{50} values and 95% confidence limits (CL); [d] Goodness-of-fit test.

Sex ratios of enclosed adults as a result of larval treatment with lufenuron are shown in Table (5). The number of males (36 males) was about quadruple the number of females (8 females) in the rate of 1:4 at 5 ppm concentration. In 10 ppm concentration, the numbers of females to males were equal (24:24) by 1:1. There were no females at 20 ppm concentration but the numbers of males were 12 (0:12). Normally, the sex ratio was 4:1 in control. Incomplete adult emergence occurred at 5 ppm and 20 ppm as 21.4 and 52.9%, respectively. The percentage of deformed wings (11.8%) was recorded only at 20 ppm concentration.

Table 5. Sex ratio of emerged *Drosophila melanogaster* adultsand deformed different lufenuron concentrations

Lufenuron Concentration(ppm)	♀	♂	Sex ratio	Incomplete adult emergence		Deformed wings	
				No.	%	No.	%
5	8	36	1:4	12	21.4	-	-
10	24	24	1:1	-	-	-	-
20	-	12	0:12	18	52.9	4	11.8
control	53	37	1.4:1	-	-	-	-

According to the abovementioned results, the recorded number of next-generation is clarified in Table (6) 10 and 15 days later. The minimum number of individuals was recorded at 5ppm concentration. They were 16 and 80 individuals after 10 and 15 days compared to control (96 and 220 individuals, resp.). However, there were no individuals despite egg occurrence on the diet surface at 10 ppm concentration. This means that this concentration caused 100% sterilizing. This result may be explained by the analysis of total proteins and phenoloxidase activity (Tables 7and 8).

The different concentrations of lufenuron had greater effects on the mean of total proteins in females than in males. The largest reduction occurred at 10 ppm concentration (16.67 mg/1000 insects) that led to egg reduction. On the contrary, phenoloxidase activity was higher in males in the same concentration (1153.33 m O.D./1000 insects) that affected egg fertility. This may explain why egg hatching did not occur. While at 20 ppm concentration, the mean of total protein deficiency (18.47 mg/1000 insects) with increased enzyme activity (2078.33m O.D./1000 insects) in females probably explain the presence of males only in the output generation.

Table 6. Effect of lufenuron on *Drosophila melanogaster* offspring production

Concentration (ppm)	Individuals /10 days	Individuals /15 days
5	16	80
10	-	-
20	-	-
Control	96	220

Table 7. Protein content in female and male emerging adults of *Drosophila. melanogaster* treated with lufenuron

Concentration(ppm)	mg /1000 insects mean±S.D
	Female
5	30.90
10	16.67
20	18.47
Control	37.80
	Male
5	29.63
10	30.47
20	36.33
Control	33.80

Table 8. phenoloxidase activity in female and male emerging adults of *Drosophila melanogaster* treated with lufenuron

Concentration(ppm)	M O.D./1000 insects Mean±S.D.
	Female
5	1771.69
10	937.67
20	2078.33
Control	1634.33
	Male
5	961.00
10	1153.33
20	1074.33
control	930.00

Van De Wouw *et al*. (2006) reported that the insecticide cyromazine (IGR) caused earlier emergence in *Drosophila melanogaster*. Peleg (1983) agreed with these findings that the insect growth regulators inhibited egg hatching of the coccinellid *Chilocorus bipustulatus* (L). Chang *et. al*. (2014) found that the fruit fly, *Bactrocera latifrons (*Hendel*)* adults exposed to LFN treated medium after mating led to reduced egg hatch. Sampson *et. al*.(2016) reported that lufenuron mixed with diet media induced female sterilization of *Drosophila melanogaster*. Similar results were obtained by Zhou *et. al*.(2016) concerning the onion flies.

Boshra (1992) found that the reduction of protein amino acids in the irradiated females of the Indian meal

moth, *Plodia interpunctella* (Hubner) led to reduction in producing and hatching eggs. Sachdev *et al*. (2014) mentioned that the irradiation affected on phenoloxidase activity in *Helicoverpa armigera* (Hubner) male causing sterility. Also, the sex ratio in F1 progeny skewed towards males.

Conclusion from the above, the biochemical effects of lufenuron (CSI) on *Drosophila melanogaster* (as an example) are similar to those obtained by using irradiation, as both led to sterilization, except that the insect growth regulators are less expensive and easier to be applied. These substances may be considered safe sterilizers.

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النشاط التعقيمي لمنظم النمو الحشري ، لوفينورون على ذبابة الفاكهة *Drosophila melanogaster*(Meigen)

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نظرًا للمشاكل المعقدة الناشئة عن التطبيقات المفرطة للمبيدات الحشرية ، أصبح البحث عن بدائل آمنة لهذه المبيدات الحشرية أمرًا ضروريًا. لذلك فإن منظمات النمو الحشرية يمكن استخدامها لهذا الهدف. تم تطبيق منظم النمو الحشري ليوفنيورون ضد ذبابة الدروسوفيلا *Drosophila melanogaster* مخلوطا مع غذاء اليرقات بتركيزات 5, 10 و20 جزء في المليون. اظهرت النتائج ان تركيز 5 جزء في المليون دفع اليرقات الى الإسراع في التعذير في اليوم الأول والثاني بعد المعاملة. حقق تركيز 20 جزء في المليون أعلي نسبة تعذير (94 %) يليها 5 ثم 10 جزء في المليون ثم المقارنة بالنسب التالية 87,5, 85,3 و82,4 % علي الترتيب. كما أن التركيز الأعلى أدى إلى أل نسبة خروج للأطوار الكاملة. فقد سجل 39,4 % ثم 10 ,5 جزء في المليون والمقارنة بنسب 44,8 ,71,4 و85,7 % على الترتيب. كان الترتيب التنازلي لنسب الموت كالتالي: 76,9 ,35,0 , 7,7 و4,2 % في تركيزات 20 ,5 ,10 جزء في المليون والمقارنة على الترتيب. لم تسجل أطوار كاملة ميتة إلا في اليوم التاسع في حالة تركيز 10 جزء في المليون و المقارنة. فيما يتعلق بالنسبة الجنسية, فكان لمركب ليوفنيورون تأثير كبير حيث اتجهت النسبة الجنسية لزيادة عدد الذكور على حساب أعداد الإناث. ففي تركيز 5 جزء في المليون كانت أعداد الذكور أربعة أضعاف عدد الإناث (1:4). بينما لا يوجد إناث في تركيز 20 جزء في المليون (0:12). تساوت أعداد الذكور مع الإناث في تركيز 10 جزء في المليون (1:1). طبقا للنتائج السابقة, كانت أعداد الأفراد في الجيل التالي 16 و80 فرد في معاملة 5 جزء في المليون مقارنة ب 96 و220 فرد في المقارنة بعد 10 و15 يوم من على الترتيب. أما في تركيز 10 جزء في المليون , وضعت الإناث البيض على سطح البيئة الغذائية لكن لم يفقس. الخروج غير الكامل سجل في تركيزات 5 و20 جزء في المليون (21,4 و52,9% على الترتيب). الأطوار الكاملة ذات الأجنحة المشوهة كانت في تركيز 20 جزء في المليون بنسبة 11.8 %. تم إجراء تحليل البروتين الكلي ونشاط انزيم الفينول اوكسيديز. فقد سبب مركب ليوفنيورون نقص في البروتين الكلي في الإناث حيث كان اعلى نقص 16,67 ملجم/ 1000 حشرة في تركيز 10 جزء في المليون مما يؤثر على خصوبة الإناث. اما نشاط انزيم فينول اوكسيديز كان أعلى في الذكور فقد سجل 1153,33 م.و.د/1000 حشرة في تركيز 10 جزء في المليون مما يؤثر على خصوبة الذكور. وربما تفسر التغيرات في تركيز الإنزيم عدم فقس البيض .