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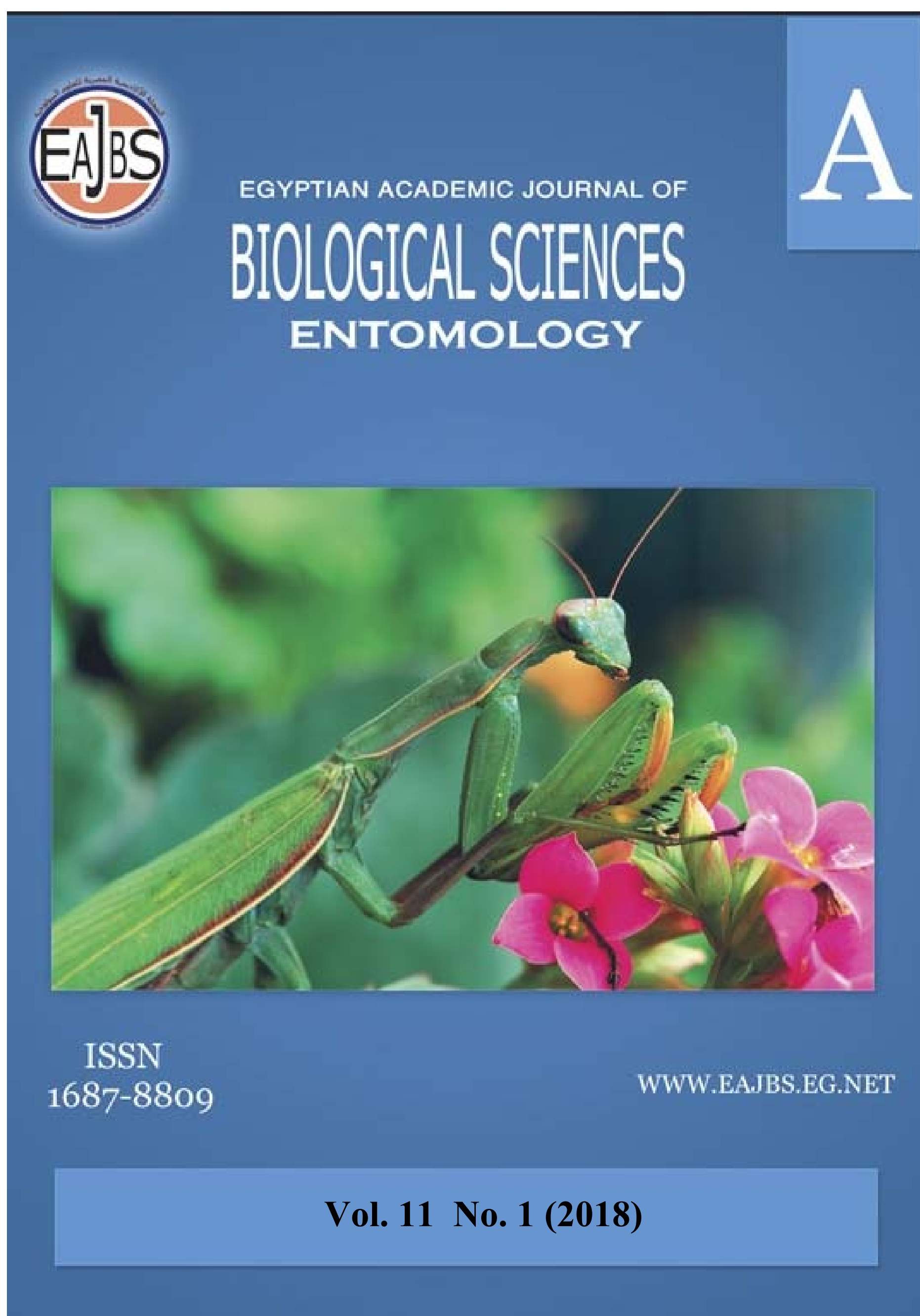

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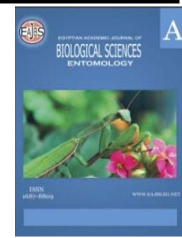

Effect of the Insect Growth Regulator " Lufox" on Some Biological Aspects of the Soft Tick *Argas persicus (Oken)*

Reda F. A. Bakr[1&2]; Dalia A.M. Salem[1]; Wafaa A. Radwan[1]; Nadia Helmy[1]; Nawal M. Shanbaky[1] and Amira E. Abd El Hamid[1]

1- Department of Entomology, Faculty of Science, Ain Shams University, Cairo, Egypt
2- Department of Biology, College of Science, University of Bisha, Bisha ,KSA
Email: redabakr55@gmail.com

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ABSTRACT

Lufox interfered with the reproduction and development of *Argas persicus* when fed adult female and second nymphal instar were topically treated with a single dose (10 *ul*/tick) of Lufox at three different concentrations (10, 50, and 150 ppm). Generally, the effects of Lufox were dose- dependent, increased by increasing dose concentration, and temporally dependent on the physiological state of the treated tick.

Lufox treatment of the adult female *A. persicus* at different periods of its reproductive cycle reduced fecundity and fertility of the female tick by decreasing the number and percent hatching of the deposited eggs, respectively. Lufox treatment prolonged the pre-oviposition, oviposition, and the egg incubation periods. The effects were most prominent when females were treated during vitellogenesis (3rd day after feeding).

Lufox was toxic to *A .persicus,* induced mortality of the treated newly moulted fed second nymphal instar to reach 100% at 150 ppm. Application of Lufox prolonged the premoulting periods of the treated 2nd and the resulted 3rd nymphal instars, delayed, and reduced adult emergence. Also, Lufox treatment led to production of different deformities in legs and inability to shed exuvia of the resulted 3rd instar nymphs and an inhibition of adult emergence. The probable physiological bases of the observed effects of Lufox on adult female and nymphs were discussed.

INTRODUCTION

The fowl tick *Argas persicus (Oken)* infests poultry and wild birds in Egypt and several other countries around the world (Hoogstraal *et al.*, 1975). This species is of a considerable veterinary importance as a parasite of poultry and may act as a limiting factor in poultry production (Khalil, 1979). *A .persicus* is an efficient reservoir and vector of several agents causing diseases such as: *fowl spirochactosis* (Diab and Soliman, 1977), *fowl aegyptianellosis* (Ahmed and Soliman,1966), *fowl tuberculosis,* and *chicken cholera or fowl plague* (Khalil 1979). Also occurrence of

Rickettsia of the spotted fever group, and isolation of viruses of Crimean-Congo hemorrhagic fever (CCHF) and west Nile virus (WNV) from *A.persicus* in USSR have been reported by (Rehaceek *et al.*, (1977) and Chumakov *et al.,* (1974)). Antibodies to CCHF and WNV have been demonstrated in sera of humans and lower animals in Egypt (Taylor *et al.*, 1956; Darwish *et al.*, 1977), respectively. Thus, it is important to look for an efficient controlling method of the tick but safe to the animal hosts and environment.

Recent studies sought an alternative vector control approach to avoid and overcome problems associated with the use of old acaricides. New controlling methods of medically important arthropods focus on key or specific physiological, biochemical, and biological events that can be inhibited, altered, or influenced (Mayer *et al.*, 1990). Hormones and chitin in arthropods affect development and reproduction (Novak, 1969; Engelman, 1979; Solomen *et al.*, 1982) have distinct mechanisms and sites of action and may represent specific and potential targets for the arthropod control. Insect Growth Regulators (IGRs) including juvenile hormone analogues (JHAs) and Chitin Synthesis Inhibitors (CSIs) (Graff, 1993) have been widely studied and suggested as promising insecticides and acaricides.

Administration of juvenile hormones and their analogues (JHAs) to certain insect stages can directly affect oogenesis, embryogenesis, metamorphosis, fecundity, and fertility without any harm to beneficial animals (Slama & Williams, 1966; Sehnal, 1976; and Riddiford 1984).

Most chitin synthesis inhibitors are benzoylurea compounds that interfere with insect development, disturbing the moult resulting in deformations in the cuticle, and mortality or adult emergence inhibition (Mian&Mula, 1982; Wilson & Cryan, 1997; and Mulla *et al.*, 2003).

A mixture of the CSI (Lufenuron) and JHA (fenoxycarb) named "Lufox" proved to be effective against insect pests such as stored product pests (Kavallieratos *et al.*, 2012); fruit pests as codling moth *Eupoecilia ambiguella* & *Cydiapemonella* (Dolzhenko *et al.*, 2011), and grape moths (Charmillot *et al.*, 2006). It is suggested that Lufenuron and fenoxycarb are suitable substitutes for organophosphate compounds for insect pest control on fruits (Sechser, 1996).

In the present study, we evaluated the effect of the IGRs mixture Lufox (Lufenuron plus fenoxycarb) on fecundity, fertility, and oviposition of adult female *A .persicus* treated at different periods of its reproductive cycle. Also effects on moulting, mortality, and induction of abnormalities in the treated and resulted life stages were evaluated when Lufox was applied topically on fed 2nd instar nymph of the tick.

MATERIALS AND METHODS

Rearing of the Tick:

The soft tick, *Argas persicus* (Oken) was collected from a domestic chicken house at Banisweif Governorate, Egypt. To establish a laboratory colony, ticks were maintained at 27 ± 1°C and 75 % RH and 16 hrs light. The ticks were held in transparent polyethylene tubes, which were sealed at one end and covered at the other end with muslin cloth securely held by rubber bands (Kaiser, 1966).

Domestic pigeons, (*Columbia livia*) from a commercial breeder in Cairo, were used as host to start a laboratory colony. The pigeon host was tied to a wooden board with one wing stretched laterally. The inner wing feathers were plucked and ticks (adults and nymphs) were placed to feed on the wing for 15- 20 minutes. Following

engorgement, ticks were transferred to a Petridish containing a filter paper disk and observed until coxal fluid was emitted. Pairs of males and females were placed separately in each rearing tube. Females were observed until they completed oviposition and egg hatch.

The hatched larvae were collected using an aspirator and allowed to feed on the wing of a pigeon, after being plucked from feathers, within a cloth sleeve. Most larvae fed and detached after 7 days. Engorged larvae were collected and held in rearing tubes until moulted within 5-7 days to give the nymphal stage.

Insect Growth Regulator Used (Lufox):

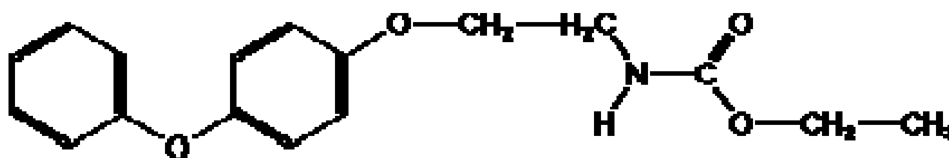
Lufox is a mixture of the JHA fenoxycarb and CSI lufenuron.

a) **Juvenile hormone analogue (fenoxycarb**):

Trade name: Fenoxycarb.

Chemical name: Ethyl [2-(4-phenoxyphenoxy) ethyl] carbamate.

Structure formula:



Emulsion concentration (EC): 7.5%

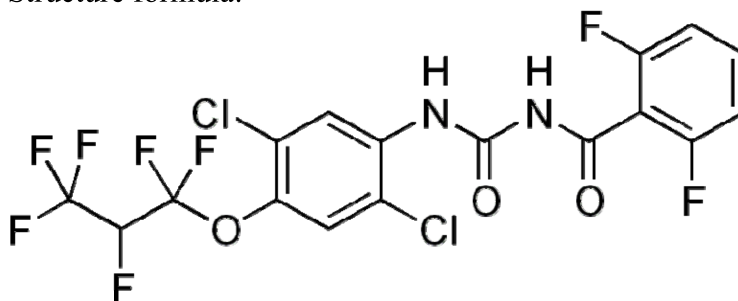
b)**Chitin synthesis inhibitor (lufenuron**):

Trade name: Axor.

Chemical name: Lufenuron.

Chemical name: N-[2,5-dichloro-4-(1,1,2,3,3,3 hexafluoropropoxy) phenyl] amino] carbonyl] -2,6-difluorobenzamide.

Structure formula:



Emulsion concentration (EC): 3.5%

Application of Lufox:

1. **Female Treatment:**

Three concentrations of the Lufox mixture (10, 50, and 150 ppm) in dist. water were used. It was administered topically, with a micro-syringe, on the ventral side of the posterior half of female body. Ten µl of Lufox was used per each female. It was applied to female ticks at different periods of the reproductive cycle (Radwan *et al.,* 2009). Mated unfed and fed females untreated (control) and topically treated on the 1st day after feeding (daf) (early previtellogenesis), 3rd daf (early vitellogenesis), 7th daf just before egg laying (ovulation), and on 20 daf. Pairs of treated females and normal males were kept at 27 ± 1°C, 75% RH in an insectary.

Eggs were collected daily to follow their hatchability and to study the effect of Lufox on the female fertility and the viability of eggs.

2. **Evaluation of Lufox Effect:**

The effect of Lufox on the female fecundity and the viability of the eggs were determined by counting the total number of eggs laid by each female and the number of hatched and unhatched eggs during the oviposition period after females' treatment. Also, effects on the preoviposition, oviposition, and incubation periods were recorded in the treated and untreated female.

Newly moulted fed second instar nymphs were treated with three different concentrations of Lufox (10, 50, and 150 ppm) were placed in rearing tubes in the incubator to determine percentage mortality and other effects on the subsequent third nymphal and adult stage.

4) Statistical Analysis:

The obtained data were manipulated statistically with SPSS version 16. While probabilities (p) were carried out using $P<0.01$, ANOVA, Duncan's multiple range tests.

RESULTS

Effect of Lufox on fecundity of female *Argas persicus* and viability of their deposited eggs:

1. Effect of Lufox on Fecundity:

Results in Table (1) show that female treatment with Lufox on the 1st daf caused insignificant decrease ($p>0.01$) in the fecundity "number of eggs laid" of female *A. persicus* at all the three concentrations tested. The number of eggs laid by the females treated with Lufox on the 1st daf were 95.2±6.09, 93±5.7, and 89.8±5.21 eggs/female for 10, 50, and 150 ppm, respectively, compared with 96.2±6.41 eggs/female for control.

Results in Table (2) show that female treatment with Lufox on the 3rd daf caused significant decrease ($p<0.01$) in the fecundity of female *A. persicus* at the two higher concentrations. The number of eggs laid by females treated with Lufox on the 3rd daf decreased gradually ($p<0.01$) by increasing concentration of Lufox and were 84.2±4.26, 66.6±3.13, and 23.2±6.31 eggs/female for 10, 50, and 150 ppm, respectively, compared with each other and with 94.6±6.02eggs/female for control.

Results in Table (3) show that female treatment with Lufox on the 7th daf caused insignificant decrease ($p>0.01$) in the fecundity of female *A. persicus* at all the three concentrations tested. The number of eggs laid by the females treated with Lufox on the 7th daf were 92.4±6.16, 90±5.63, and 86.2±4.65 eggs/female for 10, 50, and 150 ppm, respectively, compared with 95.4±7.44 eggs/female for control.

Data in Tables (1-3) show that there is a significant decrease ($p<0.01$) in the fecundity (number of eggs laid) of females treated with Lufox on the 3rd daf compared with 1st and 7th daf treated females at each concentration studied. The effect was most prominent at 150ppm where the average number of eggs laid were 23.2±6.31, 86.2±4.65, and 89.8±5.21 eggs/female for 3^{rd,} 7th, and 1st daf treated females, respectively.

2. Egg Viability:

Results in Table (1) show that female treatment with Lufox on the 1st daf caused a significant decrease ($p<0.01$) in the viability (hatching percent) of the deposited eggs at all the three concentrations used. The percentage of hatched eggs were 88.97±2.10, 85.95±2.12, and 83.4±1.07% for 10, 50, and 150 ppm, respectively, compared with 98.97±0.21% for control.

Results in Table (2) show that female treatment with Lufox on the 3rd daf caused a significant decrease ($p<0.01$) in the viability of the deposited eggs at the two higher

concentrations. The percentage of hatched eggs decreased gradually ($p<0.01$) by increasing concentration of Lufox, where the percentages of hatching were 82.89±2.23, 65.47±2.78, and 40.6±14.92% for 10, 50, and 150 ppm compared with each other and with 98.52±0.399% for control.

Results in Table (3) show that female treatment with Lufox on the 7th daf caused a significant decrease ($p<0.01$) in the viability of the deposited eggs at all the three concentrations used. The percentage of hatched eggs decreased gradually ($p<0.01$) by increasing concentration, where the percentages of hatching were 86.69±0.99, 82.74±1.01, and 79.1±2.13% for 10, 50, and 150 ppm compared with each other and with 98.65±0.42% for control.

Data in Tables (1-3) show that there is a significant decrease ($p<0.01$) in the viability (hatching percent) of eggs deposited by females treated with Lufox on the 3rd daf compared with 1st and 7th daf treated females at each concentration used. The effect was most prominent at 150 ppm where the percentages of hatching were 40.6±14.92, 79.1±2.13, and 83.4±1.07% for 3rd, 7th, and 1st daf treated females, respectively.

3. Effect of Lufox on the Preoviposition and Oviposition Periods of Female *Argas persicus* and the Incubation Period of Their Deposited Eggs:

3.1. The Preoviposition and Oviposition Periods:

Results in Table (1) show that, the preoviposition and oviposition periods of females treated with Lufox (10 μl/female) on the 1st daf were gradually insignificantly increased to reach maximum ($p<0.01$) at the highest concentration tested. The preoviposition of female treated with 10, 50, and 150 ppm were 9.6±0.51, 11.2±0.73, and 12±0.70 days, respectively, compared with 8.8±0.37 days for control. Also, the oviposition periods of females treated with 10, 50, and 150 ppm were 19.6±1.94, 26.2±2.89, and 29±2.53 days, respectively, compared with 14±2.43 days for control. The preoviposition and oviposition periods increased gradually ($p<0.01$) by increasing the concentration.

Results in Table (2) show that the preoviposition and oviposition periods of the females treated with Lufox on the 3rd daf were prolonged ($p<0.01$) at all the three concentrations tested (except at 10 ppm for oviposition). The preoviposition periods of females treated with 10, 50, and 150 ppm were 11.6±0.51, 13.4±0.39, and 14.4±0.63 days, respectively, compared with 9±0.31 days for control. Also, the oviposition periods of females treated with 10, 50, and 150 ppm were 25.8±2.89, 33.2±2.99, and 39.4±2.66 days, respectively, compared with 15.4±2.04 days for control. The preoviposition and oviposition periods increased gradually ($p<0.01$) by increasing the concentration of Lufox.

Results in Table (3) demonstrate that the preoviposition and oviposition periods of females treated with Lufox on the 7th daf were increased significantly ($p<0.01$) at the two high concentrations tested. The preoviposition of females treated with 10, 50, and 150 ppm were 10±0.31, 12±0.63, and 12.6±0.51 days, respectively, compared with 9±0.31 days for control. Also, the oviposition periods of females treated with 10, 50, and 150 ppm were 21.8±3.03, 28.2±2.94, and 32±2.65 days, respectively, compared with 15.6±1.99 days for control. The preoviposition and oviposition periods increased gradually ($p<0.01$) by increasing the concentration.

There is a significant increase in the preoviposition period of females treated with 150 ppm on the 3rd daf compared with those of females treated on the 1st and 7th daf ، where the preoviposition period were prolonged to reach 14.14±0.63, 12.6±0.51, and 12±0.70 days for 3rd, 7th and 1st daf treated females, respectively. Also, there is a significant increase ($p<0.01$) in the oviposition periods of females treated with 150

ppm on the 3rd daf compared with 1st and 7th daf treated females, where the oviposition period were prolonged to reach 39.4±2.66, 32±2.65, and 29±2.53 days for the 3rd, 7th and 1st daf treated females, respectively.

3.2. Eggs Incubation Period:

Results in Table (1) show that there is no significant increase ($p>0.01$) in the incubation period (till hatching) of eggs laid by female *A. persicus* treated with Lufox on the 1st daf at all concentrations tested. The incubation periods were 18.2±2.22, 21.6±2.22, and 23.4±1.8 days for 10, 50, and 150 ppm, respectively.

Results in Table (2) show that there is a significant increase ($p<0.01$) in the incubation period of eggs laid by female *A. persicus* treated with Lufox on the 3rd daf at all concentrations tested. The incubation periods were 24.2±1.77, 28±1.67, and 34.2±1 days for 10, 50, and 150 ppm, respectively, compared with 16.2±1.8 days for control. The incubation periods increased gradually ($p<0.01$) by increasing the concentration.

Results in Table (3) show that there is a significant increase ($p<0.01$) in the incubation period of eggs laid by female *A. persicus* treated with Lufox on the 7th daf at the two higher concentrations tested. The incubation periods were 20.6±2.11, 24.4±1.56, and 27±1.38 days for 10, 50, and 150 ppm, respectively, compared with 16.6±1.50 days for control. The incubation periods increased gradually ($p<0.01$) by increasing the concentration of Lufox.

There is a significant increase in the incubation periods of females treated on the 3rd and 7th daf with 50 and 150 ppm compared with 1st daf treated females. The effect was most prominent in eggs of females treated with 150 ppm where the incubation period were prolonged to reach 34.2±1.66, 27±1.38, and 23.4±1.8 days for the 3rd, 7th, and 1st daf treated females, respectively.

Table (1): Effect of a single dose (10µl/female) of selected concentrations of Lufox on fecundity of female *Argas persicus* and viability of eggs laid by females treated topically on the 1st daf.

Conc. (ppm)	No. of eggs laid/female mean±SE (range)	% hatched eggs mean±SE (range)	Preoviposition period (day) mean±SE (range)	Oviposition period (day) mean±SE (range)	Eggs incubation period (day) mean±SE (range)
Control	96.2±6.41[a*] (85-121)	98.97±0.21[b] (100-98.83)	8.8±0.37[b] (8-10)	14±2.43[b] (7-18)	15.4±2.13[a] (8-20)
10	95.2±6.09[a] (82-118)	88.7±2.10[a] (93.22-85.37)	9.6±0.51[ab] (8-11)	19.6±1.94[ab] (13-25)	18.2±2.22[a] (12-25)
50	93±5.70[a] (75-110)	85.95±2.12[a] (92.22-80)	11.2±0.73[ab] (9-13)	26.2±2.89[ab] (16-33)	21.6±2.22[a] (15-27)
150	89.8±5.21[a] (71-101)	83.4±1.07[a] (85.26-79.21)	12±0.70[a] (10-14)	29±2.53[a] (20-35)	23.4±1.8[a] (19-29)

* Means bearing different letters (within columns) are significantly different.

Table (2): Effect of a single dose (10µl/female) of selected concentrations of Lufox on fecundity of female *Argas persicus* and viability of eggs laid by females treated topically on the 3rd daf.

Conc. (ppm)	No. of eggs laid/female mean±SE (range)	% hatched eggs mean±SE (range)	Preoviposition period (day) mean±SE (range)	Oviposition period (day) mean±SE (range)	Egg incubation period (day) mean±SE (range)
Control	94.6±6.02 ^{a*} (80-115)	98.52±0.399 ^{c} (100-97.73)	9±0.31 ^{c} (8-10)	15.4±2.04 ^{c} (8-20)	16.2±1.82 ^{c} (10-21)
10	84.2±4.26 ^{ab} (70-95)	82.89±2.23 ^{bc} (88.89-76.47)	11.6±0.51 ^{b} (10-13)	25.8±2.89 ^{bc} (15-31)	24.2±1.77 ^{b} (19-30)
50	66.6±3.13 ^{b} (55-72)	65.47±2.78 ^{ab} (73.24-63.08)	13.4±0.39 ^{b} (12-14)	33.2±2.99 ^{ab} (22-40)	28±1.67 ^{ab} (25-34)
150	23.2±6.31 ^{c} (0-35)	40.6±14.92 ^{a} (28.57-22.58)	14.4±0.63 ^{a} (16-20)	39.4±2.66 ^{a} (30-45)	34.2±1.66 ^{a} (31-39)

* Means bearing different letters (within columns) are significantly different.

Table (3): Effect of a single dose (10µl/female) of selected concentrations of Lufox on fecundity of female *Argas persicus* and viability of eggs laid by females treated topically on the 7th daf.

Conc. (ppm)	No. of eggs laid/female mean±SE (range)	% hatched eggs mean±SE (range)	Preoviposition period (day) mean±SE (range)	Oviposition period (day) mean±SE (range)	Eggs incubation period (day) mean±SE (range)
Control	95.4±7.44 ^{a*} (80-122)	98.65±0.42 ^{c} (100-97.54)	9±0.31 ^{b} (8-10)	15.6±1.0 ^{b} (10-21)	16.6±1.50 ^{b} (12-21)
10	92.4±6.16 ^{a} (75-112)	86.69±0.99 ^{b} (89.47-84.21)	10±0.31 ^{b} (9-11)	21.8±3.03 ^{ab} (10-27)	20.6±2.11 ^{ab} (15-28)
50	90±5.63 ^{a} (71-104)	82.74±1.01 ^{ab} (84.51-78.95)	12±0.63 ^{a} (10-14)	28.2±2.94 ^{a} (18-35)	24.4±1.56 ^{a} (19-28)
150	86.2±4.65 ^{a} (70-95)	79.1±2.13 ^{a} (85.72-72.29)	12.6±0.51 ^{a} (11-14)	32±2.65 ^{a} (23-37)	27±1.38 ^{a} (22-30)

* Means bearing different letters (within columns) are significantly different.

4.1. Effect of Lufox on Immature Stages and Adult Emergence of *Argas persicus*:

Results presented in Table (4) show the effect of different concentrations of Lufox (10, 50, and 150 ppm) on the newly moulted fed 2nd nymphal instar of *Argas persicus* and subsequent stages. A significant decrease ($P<0.01$) in viability of the treated 2nd nymphal instar was observed, where percentage mortality increased gradually ($P<0.01$) to 14±2.45, 56±2.45, and 100±0.00% for nymphs treated with 10, 50, and 150 ppm of Lufox, respectively, compared with 0% for control nymphs. There was also a significant increase ($P<0.01$) in the duration of the premoulting period of the 2nd nymphal instar, where it reached 22.4±0.39 and 26.4±0.74 days for 2nd nymphal instar treated with 10 and 50 ppm of Lufox, respectively, compared with 16.8±0.58 days for control nymphs. The premoulting period increased gradually with increasing the concentration ($P<0.01$).

The 3rd nymphal instar emerged from the treated 2nd nymphal instar, showed no mortality. However, significant morphological effects ($P<0.01$) were observed (Table

4) where the percentages of the malformed 3rd nymphal instar reached 36±4 and 30±0.00% for nymphs treated with 10 and 50 ppm, respectively, compared with 0%±0.00 for control nymphs (P<0.01). Also, there was noticeable increase of the premoulting period of the 3rd nymphal instar (p<0.01) at the higher dose (50 ppm). The moulting period, were 25.4±2.16 and 29.8±0.91 days for nymphs emerged from 2nd instar nymphs treated with 10 and 50 ppm, respectively, compared with 17.4±0.67 days for control. Results in Table (4) demonstrate a significant reduction (P<0.01) of adult emergence, to 50±3.13 and 14± 2.45 % for nymphs treated with 10 and 50 ppm of Lufox, respectively, compared with 100±0.00% for control nymphs. Adult emergence was completely prevented on treatment with 150 ppm as a result of the 100% mortality of the 2nd instar nymphs treated with this concentration.

4.2. Malformations:

The treatment of the fed newly moulted 2nd nymphal instar of *Argas persicus* with a single dose (10µl/nymph) of 10 and 50 ppm Lufox, induced different malformations of development in the resulted 3rd nymphal instar. Some individuals were paralyzed and were unable to complete the moulting process; (Plate I, Figure 2). Some others had abnormal legs which hardened and had pale color, (Plate I, Figure 3). A number of the resulted 3rd nymphs were able to complete the moulting process but the old cuticle remained connected to their legs (Plate I, Figure 4). Some other individuals had a missing leg at the coxal level (Plate I, Figure 5), and some were able to complete the moulting process without any deformity in the resulting stage.

Table (4): Effect of a single dose (10µl/nymph) of Lufox on immature stages and emergence of adult of *Argas persicus* treated as 2nd nymphal instar.

Conc. (ppm)	2nd nymphal instar					Adult stage	
	% Mortality mean ±SE (range)	Premoulting period(day) mean ±SE (range)	3rd nymphal instar % Mortality mean ±SE (range)	Malformation % mean ±SE (range)	Premoulting period(day) mean ±SE (range)	Adult emergence % mean ±SE (range)	Inhibition of adult emergence % mean ±SE (range)
Control	0±0.00d** (0)	16.8±0.58c (15-18)	0.0±0.0a (0)	0.0±0.0b (0)	17.4±0.67b (15-19)	100±0.00a (100-100)	0±0.00d
10	14±2.45c (10-20)	22.4±0.39b (21-23)	0.0±0.0a (0)	36±4a (20-40)	25.4±2.16b (24-27)	50±3.13b (40-60)	50±3.87c (40-60)
50	56±2.45b (50-60)	26.4±0.74a (24-28)	0.0±0.0a (0)	30±0.0a (30-30)	29.8±0.91a (28-33)	14±2.45c (10-20)	86±2.55b (80-90)
150	100±0.00a (100)	-	-	-	-	-	-

** Means bearing different letters (within columns) are significantly different

Fig.1 Normal third nymphal instar.

Fig.2 Third nymphal instar showed paralysis in legs.

Fig.3 Third nymphal instar with abnormal legs (hardened and pale color).

Fig.4 Third nymphal instar with the old cuticle remained connected with legs.

Fig.5 Third nymphal instar with a missing leg at the coxal level.

Plate I: Malformations produced by topical treatment with Lufox of fed newly moulted 2nd nymphal instar of *A. persicus*

DISCUSSION

Effects of Lufox on Fecundity, Fertility, and Oviposition of Female *Argas persicus*:

Topical treatment of mated fed adult female *Argas persicus* with a single dose (10 *ul* /female) of Lufox (a mixture of the JHA fenoxycarb and the CSI Lufenuron) reduced its fecundity and fertility (number and % hatch of the deposited eggs).

Also, Lufox treatment of the female slowed down oocyte and embryonic development, caused prolongation of the pre-oviposition, oviposition, and the eggs incubation periods. Generally, effects were increased by increasing dose concentration of Lufox (10, 50, and 150 ppm) being most pronounced at 150 ppm.

Tick oogenesis is divided into three stages, previtellogenic, vitellogenicand, and ovulation (just before egg laying) (Diehl *et al.*,1982). Findings of the present

study indicated that female *A. persicus* was most sensitive to effects of Lufox when treated during vitellogenesis 3rd daf followed by ovulation (7th daf) period, while females treated during previtellogenesis (1st daf) were the least sensitive.

Evidence suggests that vitellogenesis in argasid ticks is under hormonal control (Shanbaky and Khalil, 1975; Shanbaky *et al.*, 1990) and that this gonadotropic hormone (Leahy and Booth, 1980) is a juvenile hormone – like compound (Pound and Oliver, 1979; Connat *et al.*, 1983).

In the present study, effects of JHA, Fenoxycarb in Lufox may add to those of the anticipated high level of the natural gonadotropic hormone during vitellogenesis (Shanbaky and Khalil, 1975; Shanbaky *et al.*, 1990) which might have led to an interference with the normal oocyte development. The high titer of the natural hormone in addition to the exogenous JHA or Fenoxycarb might have led to disturbances in the release of the natural hormone. Also, Fenoxycarb may have an antigonadotropic effect as that of some other JHA (Mansingh and Rawlins, 1977) which might have led to the reduction of fecundity, fertility, and prolongation of the pre-and oviposition periods observed in the present study.

The present results confirm with those of previous studies on ticks. Treatment of the newly engorged females of the lone star tick *Amblyomma americanum* with pyriproxyfen decreased the number of eggs deposited as dosage and exposure time increased. Also, it resulted in complete inhibition of egg hatch at all treatment levels except that of the lowest concentration and shortest exposure period (Teel *et al.*, 1996). Methoprene and hydroprene resulted in 41.7 - 79.2% reduction in the quantity of eggs laid by *B.microplus* females (Mansingh and Rawlins, 1977).

Solmon and Evans (1977) found that treatment of newly engorged females of *Boophilus microplus*, *B.decloratus*, and *Amblyomma hebraeum* with JHA (Methoprene ZR-615, ZR-515, and Altosid) caused desiccation of deposited eggs. The authors suggested that the mechanism of action of JH mimics causing egg desiccation in *Amblyomma hebraeum* may involve disruption of the water-proof properties of the egg shell. McDaniel and Oliver (1978) attributed egg hatch reduction in *Dermacentor variabilis* females treated with Altozar and R-20458 to desiccation resulting from interference with functioning of Gene’s organ, thus the waterproofing of eggs laid was modified.

In contrast to the reducing effects of Lufox on fecundity and fertility of female *A.persicus*, the present results showed that Lufox treatment of the engorged female *A.persicus*caused a general increase (prolongation) of the preoviposition, oviposition in the female and incubation (hatching) period of their deposited eggs. The present results agree with those reported by Khalil *et al.* (1996), where females *Argas hermani* from nymphs treated with 25- azocholestone before feeding have a prolonged preoviposition period.

Mohsen and Alchalabi (1989) reported that fenoxycarb delayed significantly the preoviposition period in surviving females emerged from treated 4th instar larvae of *Culex quinquefasciatus*. Reduction of fecundity, fertility and prolongation of preoviposition period by treatment with JHAs was also reported in other blood sucking insects for example in *Cimex lectularius* (Shaarawi *et al.,* 1981) and *Ctenocephalides felis* (Meola *et al.*, 2001).

The aforementioned results of the present work showed that the responses of the engorged female *A.persicus*to Lufoxtreatment were affected by the female physiological state during the different periods of the reproductive cycle. The treated females were most sensitive to Lufox application during vitellogenesis and to a less extent in ovulation (3rd and 7th daf, respectively) and were the least sensitive during

the early previtellogenesis. Similarly Radwan *et al.* (2009) reported that application of JHA, admiral on female *A. persicus* reduced viability of the deposited eggs when females were treated on 1stdaf, but the effect was highly significant when females were treated during vitellogenesis (3rddaf) and ovulation (7thdaf). Solmon and Evans (1978) found that treatment of newly engorged female *Boophilus decloratus, B. microplus* and *Amblyomma hebraeum* at low doses of JH mimics only those eggs laid early in the egg laying period desiccated and the number of eggs desiccated was dose dependent. They suggested that the mimics (Methoprene, ZR-515 and ZR-516) were inactivated by the treated ticks and that activity was dependent on both intrinsic activity and the rate of inactivation of the compound.

Differences among the aforementioned results might be attributed to differences in the tick species and the JH mimics used. Furthermore, the observed effects of Lufox on reproduction and development of oocytes and deposited eggs of the treated female *A. persicus*might be attributed to the presence of the CSI, Lufenuron, in the applied mixture (Lufox). As in other arthropods, the presence of chitin has been clearly demonstrated in the cuticle of ticks (Balashov, 1972) as well as in the egg shell (Jaskoski and Butter, 1971).

In ticks, a dose-dependent reduction in weight of total egg mass and a slight increased oviposition latency were observed following injection of the avermectin (CSI) analogue MK-243 into the haemocoele of engorged female *Amblyomma hebraeum*. Egg laying was almost inhibited at 100*ug*/Rg body weight (Lunke and Kaufman, 1992). Decreased egg production, reduced egg hatching, abnormal egg size and shape, and increased percentages of unhatched embryonated and sterile eggs were observed by Mahmood *et al.* (1991) among ivermectin treated mosquitoes, *Aedes aegypti*. Administration of lufenuron to adult *Drosophila melanogaster* slightly depressed oogenesis, but had a dramatic effect on fertility of the oviposited eggs which failed to hatch (Wilson and Cryan, 1997). Failure of the completely developed embryos to hatch was explained by their inability to perforate the surrounding membrane probably due to a weakened chitinous mouth hook assembly that was insufficiently rigid to affect hatching.

Effect of Lufox on Immature Stages of *Argas persicus*:

A single dose of Lufox (10µl/tick) induced mortality of topically treated 2nd instar nymphs of *A.persicus*, slowed down development of tick, produced malformed 3rd nymphal instar, delayed and reduced adult emergence. Treatment with Lufox prolonged the premoulting period of the treated 2nd instar nymphs and the resulted 3rd instar nymphs which delayed adult emergence. Also, the treatment of the 2nd instar nymphs resulted in different deformities in the 3rd instar emerged. Malformations included paralysis and missing legs, inability to complete moulting or to shed exuvia and changed color. Delayed effects were manifested as inhibition of adult emergence.

All the aforementioned effects may suggest an interference of the JHA, fenoxycarb (in Lufox) with hormones involved in ecdysis of immature ticks. In certain insect species, JH has been considered to lower ecdysone titer or to antagonize, inhibit or interfere with ecdysone-dependent processes (Lezzi and Wyss, 1976; Masner *et al.,* 1976). Based on experimental evidence an endogenous tick hormone similar to insect JH was considered to be involved in the ecdysone-dependent control of moulting and development processes in the argasid tick *Ornithodoros porcinus porcinus* (Solomon *et al.,* 1982).

Another probable explanation of lethality, prolongation of the premoulting

periods and other observed effects of Lufox on immatures observed in the present work, is that the application of the JHA fenoxycarb (in Lufox) on the 2nd instar nymphs of *A. persicus* might have overdosed the normal level of a natural JH-like compound (Pound& Oliver, 1979; Connat, 1988). Then, the JHA could not be metabolized before metamorphosis (3rd instar nymphs) and as a result, metamorphosis is disturbed or blocked (Liu and Chen, 2001).

Results of the present study are in accordance with those of previous studies of several insect growth regulators on ticks. Low's mixture (JHA) prolonged the premoulting period and caused high mortality when applied on detachment day to nymphal *Haemaphysalis concinna* (Ioffe and Uspensky, 1979). Hydroprene (Altozar) a JH mimic delayed *Ixodes ricinus* metamorphosis and caused high mortality of treated eggs, newly emerged larva (Ioffe & Uspensky, 1980) and nymphs treated 5-days postfeeding (Ioffe *et al.,* 1977).

Khalil *et al.,* (1996) observed that treatment of *Hyalomma dromedarii* with 10µg dose reduced the premoulting period of nymphs treated with Azacholiestane before feeding. Some adults emerged from 5-20µg dosage groups treated before feeding failed to shed their exuvia and others had deformed legs. Difference in results of previous studies might be attributed to differences in the investigated tick species, chemical compound or the IGR and method of its application in addition to the physiological state of the tick.

JHAs exhibit various effects on immature stages of blood sucking and other insects (Slama *et al.,* 1974). Fenoxycarb treatment reduced survival of larvae and pupae of different species of mosquitos (Mohsen and Al-Chalabi, 1989; and Walker and Edman, 1990)

In the present study, the observed effects of Lufox on development of *Argas persicus* might be attributed to the CSI lufenuron in the Lufox mixture. Topical treatment of the 2nd instar nymphs induced mortality in the treated nymphs, prolongation of the premoulting periods in the 2nd & 3rd nymphal instars, malformation in the 3rd nymphal instar and inability to moult or to shed exuvia, changed color and a delay and reduction of adult emergence. The interference of lufenuron with chitin synthesis and metabolism (Mayer *et al.,* 1990) might have rendered the treated 2nd instar nymphs and the resulted subsequent stages to be unable to synthesize sufficient or proper chitin which prevented normal development (Wilson & Cryan, 1997). Generally, CSI may affect different steps in chitin biosynthesis; however, the exact step that is inhibited is unclear but may include a step in chitin precursor transport (Nakagawa and Matsumura, 1994) or the polymerization stage of chitin by interacting with chitin synthase (Hajjar & Casida, 1978). Ecdysteroids and Juvenile hormones and their metabolism would seemed to be likely targets for CSIs such as diflubenzuron (Yu & Terrier, 1977).

CSIs exert various effects on immature stages of insect. Triflumuron (CSI) is available alternative for the control of *Aedes aegypti.* Beyond its effectiveness against its larvae (Martins *et al.,* 2008) it produced structural abnormalities, such as deformed wings, or easily broken legs. Mortality was observed during either larval or pupal development when *Drosophila melanogaster* larvae were fed a diet containing a low concentration (<1ppm) of Lufenuron. Survivor adults were unable to fly which was attributed to abnormal cuticular development in wing hinges. Larvae fed a higher concentration (10ppm) completed development within that instar but died during ecdysis to the next instar, which was attributed to inadequate cuticle synthesis. Third instar larvae pupariated, but the puparium was abnormal and pupation did not occur (Wilson & Cryan, 1997).

A combination of the CSI lufenuron plus the JHA fenoxycarb in "Lufox" proved to be effective against several insect pests of fruits (Gournel and Litoux, 1999; Zartaloudis *et al.,* 2009; Al-Abbar *et al.,* 2010a, b; Dolzhenko *et al.,* 2011; and Halawa *et al.,* 2013) and stored product pests (Kavallieratos *et al.,* 2012). The selectivity of Lufox ingredient towards beneficial insects allows the integration of this insecticide in integrated pest management programmes (Gournel & Litoux, 1999). Lufox was selected as acceptable, low risk product from a toxicity point of view (Dolzhenko *et al.,* 2011) and was considered as a viable grain protectant in wheat and maize (Kavallieratos *et al.,* 2012).

In conclusion, the biological studies in the present work demonstrate that a single dose of Lufox interferes effectively with reproduction and development of topically treated adult female and second nymphal instar of *A. persicus,* respectively. The tick is sensitive to Lufox effects during most of the reproductive cycle of the female (vitellogenesis and ovulation) and the response of immature involves not only the treated stage but also the resulted subsequent stages.

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ARABIC SUMMARY

بعض التأثيرات البيولوجية لمنظم النمو الحشري اللوفكس على القراد اللين *ارجاس بيرسيكس*

رضا فضيل على بكر[١&٢] - داليا عبد البديع سالم[١] - وفاء أحمد رضوان[١] - نادية حلمى أحمد[١] - نوال محمود شنبكى[١] - اميرة السيد عبد الحميد[١]

١ – قسم علم الحشرات – كلية العلوم – جامعة عين شمس- القاهرة- مصر.
٢ – قسم الأحياء – كلية العلوم – جامعة بيشه – بيشه – المملكة العربية السعودية

وجد من الدراسة أن اللوفكس يتداخل فى عملية التكاثر لإناث القراد البالغ المتغذى والمتزاوج ***آرجاس بيرسيكس*** وكذلك مع نمو الطور الثانى للحوريات عند المعالجة الموضعية (بالتلامس)بجرعة واحدة (١٠ميكروليتر/للقراد) بتركيزات مختلفة (١٠،٥٠، ١٥٠ جزء فى المليون)، وتعتمد تأثيرات اللوفكس عموما على تركيز الجرعة حيث أنه يزداد بزيادة تركيز الجرعة وكذلك وقتيا (مؤقتا)على الحالة الفسيولوجية للقراد المعالج.

لوحظ أن معالجة إناث القراد المتزاوج ***آرجاس بيرسيكس*** باللوفكس عند فترات مختلفة من دورة التكاثر يؤدى الى نقص فى خصوبة الأنثى وحيوية البيض عن طريق تقليل عدد ونسبة فقس البيض الناتج. كما أن المعالجة باللوفكس تؤدى الى بطء فى نمو البويضة والتطور الجنينى عن طريق اطالة فترة ما قبل وضع البيض وفترة وضع البيض وكذلك فترة حضانة البيض و هذة التأثيرات ابرز ما تكون عند معالجة الإناث خلال اليوم الثالث بعد التغذية .

لوحظ أن اللوفكس له تأثير سام على القراد ***آرجاس بيرسيكس*** حيث أنه أدى الى موت ١٠٠ ٪ من الطور الثانى حديث الانسلاخ المعالج بتركيز ١٥٠ جزء فى المليون، وكذلك تسببت المعالجة فى إطالة فترة ما قبل الانسلاخ للطور الثانى للحوريات المعالج والطور الثالث للحوريات الناتج كما انه يؤخر ويقلل ظهور الطور البالغ. كما تؤدى المعالجة باللوفكس إلى إنتاج عيوب(تشوهات) فى الأرجل وعدم القدرة على التخلص من الجلد القديم لطور الحورية الثالث الناتج وكذلك منع ظهور الطور البالغ.