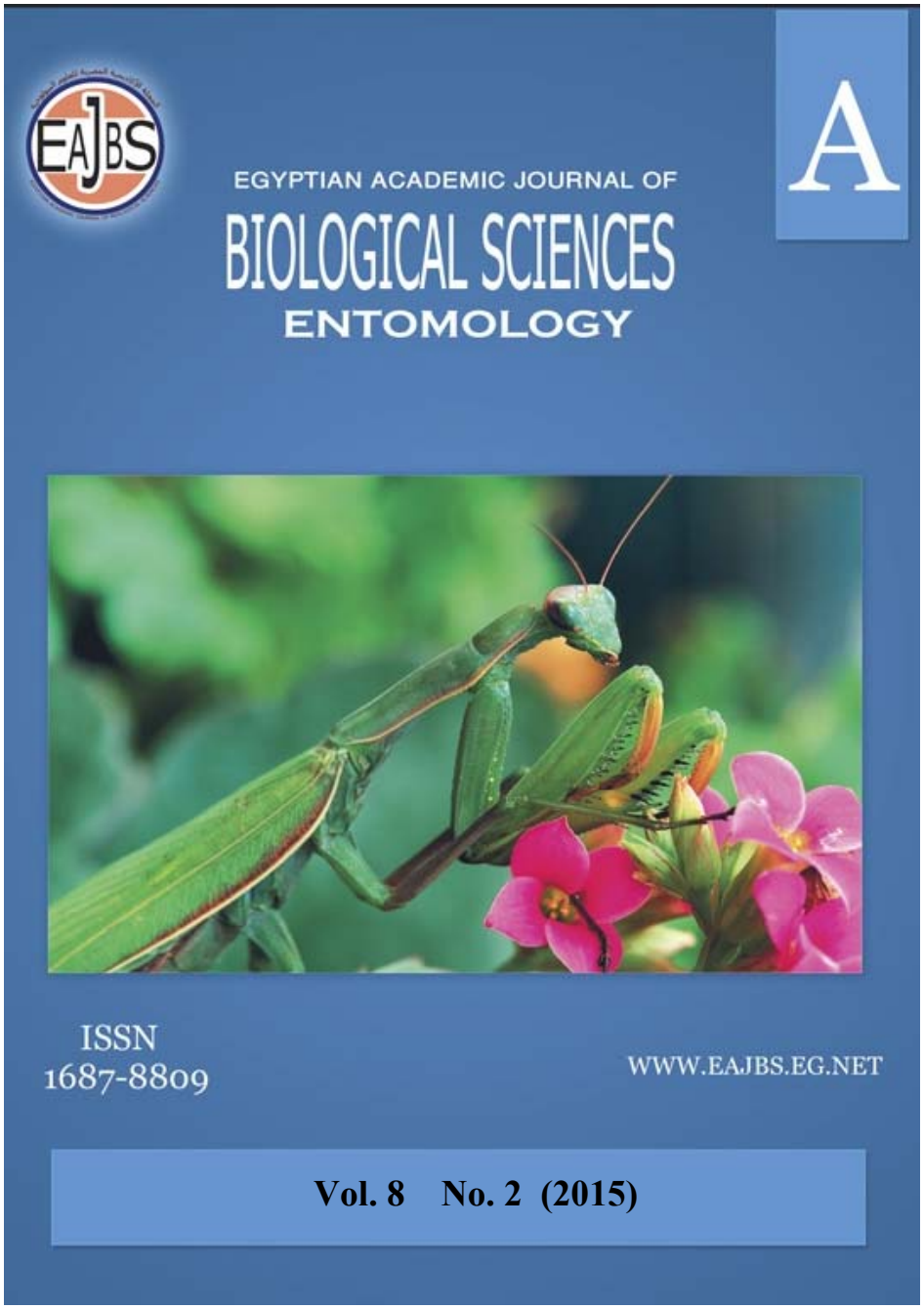

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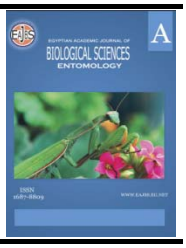

Effect of lufox on haemolymph and ovarian protein in the soft tick, *Argas persicus*.

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ABSTRACT

Changes in the total protein titer in the haemolymph (HL) and ovaries (Ov) of mated fed normal and Lufox treated female ***Argas persicus*** during the reproductive cycle and in the HL of normal male were studied. Tick engorgement was followed by an intial drop of the HL total protein concentration immediately and up to the 2nd and 3rd day after feeding (daf), then by a gradual increase on next days to reach maximum on 4-7 and 6-7 daf in the female and male, respectively. The level in the female HL was much higher than that in the male during vitellogenesis and the onset of oviposition. In the ovaries of normal female the total protein level increased gradually immediately up to 2 daf and evidently on 3 daf to reach a maximum on 5-7 daf. The protein level in the HL and ovaries decreased after the completion of oviposition (20 daf).

Topical application of a single dose of 50 ppm Lufox on the mated female *A. persicus* on the 3rd daf interfered with protein production causing a significant reduction in the total protein level in the HL (43.04-55.57%) and ovaries (36.47-46.43%) during vitellogenesis on 4-7 daf and in eggs (15.38%) freshly deposited by the treated female. Also, application of Lufox on the 3rd daf altered the normal pattern of change in the level of HL and ovary total protein during the reproductive cycle by preventing any appreciable increase in the level on next days (4-7 daf) where the level remained constant in the ovaries and even decreased in the HL (4-5 daf).

INTRODUCTION

Insect growth regulators (IGRs) such as hormones and chitin synthesis inhibitors (CSI) can disrupt reproduction and development in insects (Walker and Edman, 1990; Martins *et al*. 2008; Kavalleratos *et al*. 2012 and Halawa *et al*. 2013) and ticks (Connat and Nepa, 1990; Lunke and Kaufman, 1991; Friesen *et al*. 2003 and Radwan *et al*. 2009). As a group IGRs may represent an alternative approach for arthropod control (Engelmann, 1979) to avoid and overcome problems associated with the use of insecticides and acaricides. In arthropod vectors of disease, IGRs are considered as safe to the vertebrate host as they interfere or affect metabolic pathways that are unique to arthropods and involved in their specific physiological and biological events of moulting, metamorphosis and reproduction (Mayer *et al.* 1990).

An increase in the level of haemolymph and ovarian total protein has been found to occur during vitellogenesis of adult female ixodid (Tatchell, 1971 and Araman, 1979) and argasid ticks (Shanbaky *et al,* 1990 and Yousery, 2011).

In the fowl tick, *Argas persicus*, topical application of Lufox (mixture of a juvenile hormone analogue and a CSI) on the adult female tick reduced fecundity and fertility of the treated female and delayed their oviposition and egg hatch (Abd ElHamid, 2014).

The present work is aimed to study the effect of Lufox topical treatment of adult female *A. persicus* on the concentration of total protein in the hemolymph, ovaries and newly deposited eggs in order to evaluate the possible physiological implications of this mixture of IGRs in tick control.

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°C ± 1,75 % RH and 16 hrs light.

The ticks were held in transparent polyethylene tubes which were covered with muslin cloth securely held by rubber bands (Kaiser, 1966).

Domestic pigeons, (*Columbia livia*) from commercial breeder in Cairo, were used as host for feeding ticks. Both tick colony and host were kept in an insectary provided by Research and Training Center of disease vectors (RTC), Ain Shams University.

The pigeon host was tied to a wooden board with one wing stretched laterally. The inner wing feathers were plucked and tick 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 separately placed 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 feather within a cloth sleeve. Most larvae fed and detached after 7 days. Engorged larvae were collected and held in rearing tubes until moulting within 5-7 days to give the nymphal stage. The tick has three nymphal instars. Nymphs developed to adults after 2 moults within 11- 16 day.

Insect growth regulators used:

Lufox (mixture)

1. Juvenile hormone mimic (fenoxycarb):

Trade name: fenoxycarb.

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

Emulsion concentration (EC): 7.5%

2. Chitin synthesis inhibitor (lufenuron):

Trade name: Axor.

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

Emulsion concentration (EC): 3.5%

Application of Lufox:

Female treatment:

A single dose (10 µl/female) of 50 ppm Lufox in distilled water was topically applied on the posterior half of the ventral side of the adult female. Lufox was applied

at mated fed female on the 3rd daf, when Lufox caused the highest effect on fecundity and fertility of female *A. persicus* (Abd Elhamid, 2014). Pairs of treated females and normal males were kept separately at 27°C ± 1, 75% RH in an insectary.

Collection of samples:

Samples of HL, ovaries and eggs from mated fed normal and Lufox treated female, HL of normal male were studied at different physiological stages of the female reproductive cycle. Tissue samples were collected from five ticks and each experiment was replicated three times.

To collect HL,the first pair of legs of the tick were cut at the level of the coxa and exuding HL was taken up by means of micropipettes of 3 µl capacity. Traces of phenylthiourea were added to HL to prevent coagulation.

Ovaries of each of the control female and treated female ticks at different physiological stages were dissected out and weighed.

Freshly deposited eggs, collected from each of the untreated and treated female ticks, were weighed.

Extraction and estimation of protein:

Haemolymph, ovaries and eggs samples were homogenized in 0.1 ml of 50% of sucrose in 0.1 M tris-HCL buffer pH 7.3. The homogenate was diluted to 2 ml with the same buffer and then centrifuged at 3000rpm for 20 min. The soluble protein content of the supernatant was determined by the method of Lowery *et al.* (1951).

Procedure:

0.1ml of 2N NaoH was added to 0.1ml of sample or standard, hydrolyzed at 100°C for 10 min in a heating block or boiling water. The hydrolysate was cooled to room temperature and 1 ml of freshly mixed complex-forming reagent (a mixture in proportion of 100: 1: 1 of 2% (w/v) Na_2Co_3, 1% (w/v) $CuSo_4$. $5H_2O$ and 2% (w/v) sodium potassium tartrate in distilled water) was added and the solution was let at room temperature for 10 minutes. 0.1ml of 1N folin reagent was added and mixed thoroughly using a vortex mixer. The mixture was let at room temperature for 30-60min. The absorbance was read on a spectrophotometer at 595nm and compared to a standard curve using bovine globulin and the total protein concentration was expressed as mg/ml or mg/gm for HL, ovary and eggs, respectively.

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

Total protein concentration in the haemolymph:

1. Total protein concentration in the haemolymph of normal female and male *Argas persicus*:

Results in Table (1) and Fig. (1) show that the concentration of the total protein in the HL of mated fed female and male *A. persicus* decreased significantly ($P < 0.01$) immediately (1hr) after feeding and to a lesser extent during the next 2 and 3 daf compared to that of the unfed female and male ticks, respectively (13.37±0.33 and 10.67± 0.18mg/ml). An appreciable increase ($P < 0.01$) in the total protein level in the HL of mated females and male was observed on the 3rd and 4th daf to reach that of the unfed female and male, respectively($P > 0.01$). The level of HL protein continued increasing on the 4th and 5th daf ($P < 0.01$) to exceed the level of the unfed ticks of the corresponding sex and reach a maximum on days 4-7 and 6-7 daf in the female and

male, respectively. The protein level decreased again ($P < 0.01$) on 20 daf, but was still higher than the unfed controls.

Table 1: Changes in the total protein concentration in the hemolymph (HL) of normal mated unfed and fed female and male *Argas persicus* on different days after feeding (daf).

Tick physiological state	Mean total protein (mg/ml) ±SE (range)	
	Female	Male
Unfed	13.37±0.33^{c}** (12.2-14.3)	10.67± 0.18^{d} (10.2-11.3)
0 daf	5.4±0.25 ^{e} (4.8-6.2)	4.1±0.18 ^{h} (3.6-4.7)
1 daf	6±0.24 ^{e} (5.2-6.9)	5.03±0.17 ^{gh} (4.5-5.6)
2 daf	8.7±0.16^{d} (8.10-9.10)	7.47±0.37^{fg} (6.2-8.5)
3 daf	14.3±0.27^{c} (13.5-15.2)	8.67±0.28 ^{ef} (8-9.7)
4 daf	20.93±0.42 ^{a} (19.6-22.3)	11.81±0.3 ^{cd} (10.8-12.7)
5 daf	22.3±0.38 ^{a} (21.1-23.5)	13.7±0.28 ^{bc} (12.9-14.7)
6 daf	22.5±0.46 ^{a} (21-23.9)	18.8±0.62 ^{a} (16.9-20.8)
7 daf	23±0.35^{a} (21.9-24.1)	19.3±0.55 ^{a} (17.5-21)
20 daf	18.13±0.35^{b} (16.9-19.1)	14.6±0.37 ^{b} (13.3-15.6)

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

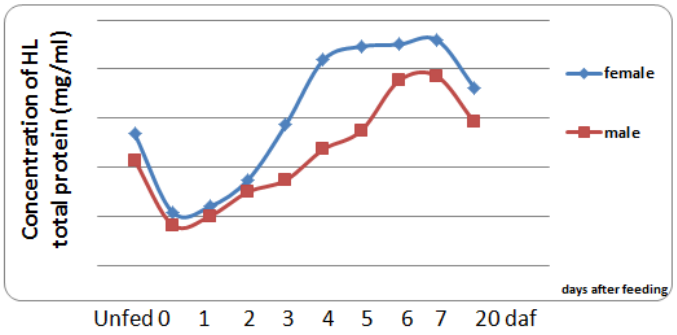

Fig. 1: Changes in the concentration of haemolymph total protein of adult normal female and male *Argas persicus* on different days after feeding (daf).

The total protein titres in the HL of the female and male *Argas persicus* were insignificantly different up to 2daf. However, on 3-7daf the level of protein in female HL was greater ($P < 0.01$) than that in the male HL. Furthermore, protein levels of both female and male approached each other on 20 daf.

2. Total protein concentration in the haemolymph of Lufox treated female *Argas persicus*:

Results in Table (2) and Fig. (2) show that the total protein concentration in the HL of mated fed female treated topically with 50 ppm lufox on the 3rd daf significantly ($P < 0.01$) decreased on the 4th and 5th daf than that on the 3rd daf (13.36±0.25 mg/ml). A slight increase in the level of HL protein of the treated female *A. persicus* was observed on the 6th daf and 7th daf to reach that on the 3rd daf. Protein level in the HL reached its maximum level on the 20th daf compared with other physiological stages tested.

Table 2: Changes in the total protein concentration in the haemolymph of Lufox treated* mated fed female *Argas persicus* on different days after feeding.

Tick physiological state	Total protein (mg/ml) mean±SE (range)	% reduction***
3 daf	13.36±0.25[b**] (12.5-14.1)	6.57%
4 daf	9.3±0.27 [c] (8.4-10.1)	55.57%
5 daf	10.06±0.22[c] (9.3-10.2)	48.89%
6 daf	11.4±0.196 [bc] (10.9-12.1)	49.33%
7 daf	13.1±0.27[b] (12.3-14)	43.04%
20 daf	16.2±0.28 [a] (15.3-17.1)	10.65%

*Mated fed female was treated topically with 10 µl/female of 50ppm Lufox on the 3rd daf.
**Means bearing different letters (within column) are significantly different.
*** Percent reduction relative to normal.

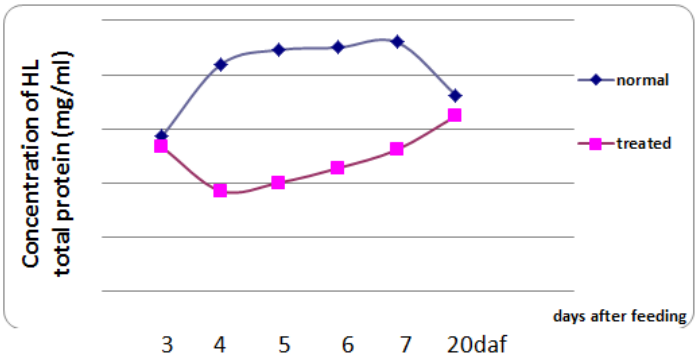

Fig. 2: Changes in the concentration of haemolymph total protein of adult normal and Lufox topically treated (on 3rd daf) female ***Argas persicus*** on different days after feeding (daf).

In comparison between the concentrations of total protein in the HL of both normal and treated mated fed female ***Argas persicus*** there was a significant decrease ($P < 0.01$) in the HL total protein of the treated female during the 4th to the 7th daf. During this period the percent reduction of the HL protein level ranged 43.04-55.57% relative to the corresponding physiological states in normal female (Table 1).

Total protein concentration in the ovary:

1. Total protein concentration in the ovary of normal female *Argas persicus* ovary:

Data in Table (3) and Figure (3), show that the concentration of the total protein in the ovary of mated fed normal female *Argas persicus* increased gradually but not significantly ($P > 0.01$) immediately (1 hr) after feeding and on days 1 and 2 after feeding compared to that of the unfed females (56.87±2.02 mg/gm). An increase ($P < 0.01$) in the total protein of ovaries of the female was observed on the 3rd daf (80±1.4 mg/gm) and continued ($P < 0.01$) on the 4th daf and next days to reach a maximum level ($P < 0.01$) on the 5th to 7th daf. A decrease ($P < 0.01$) in the level of total protein in the ovaries of normal females was observed on 20 daf , but was still higher than that in the unfed.

2. Total protein concentration in the ovary of Lufox treated female *Argas persicus*:

Data in Table (3) and Figure (3) demonstrate that the total protein concentration in the ovary of mated fed female *A. persicus* treated topically with 50ppm lufox on the

3rd daf showed no significant (P > 0.01) change up to the 7th daf. Protein concentrations ranged from 77.07±0.95 to 85.67±0.41mg/gm tissue on the 3rd daf to the 7th daf. However, an increase in the protein level (P < 0.01) was observed on the 20th daf to reach 97.8±3.52 mg/gm tissue.

Table 3: Changes in the total protein concentration in the ovaries of normal and Lufox treated* mated unfed and fed female *Argas persicus* on different days after feeding (daf).

Tick physiological state	Mean total protein normal (mg/gm)±SE (range)	Mean total protein treated (mg/gm)±SE (range)	%reduction in the treated female***
Unfed	56.87±2.02^{e**} (50.3-63.1)	-	-
0 daf	66.03±2.29^{de} (58.6-73.1)	-	-
1 daf	68.17±2.26 ^{de} (60.9-75.2)	-	-
2 daf	71.9±1.88^{de} (66.9-78.5)	-	-
3 daf	80.6±1.4 ^{cd} (75.9-84.7)	77.067±0.95^{b} (74.6-80.4)	4.38%
4 daf	122.3±3.37^{b} (110.3-130.6)	77.7±0.84^{b} (75.4-80.6)	36.47%
5 daf	147.47±2.02^{a} (140.5-153.1)	79±0.78^{b} (76.3-81.2)	46.43%
6 daf	151±1.87^{a} (145.7-157.1)	81.8±0.38^{b} (80.7-83.1)	45.83%
7 daf	139.8±1.21^{a} (135.7-143.3)	85.67±0.41a^{b} (84.6-87.1)	38.72%
20 daf	90.1±1.01^{c} (87.1-93.51)	97.8±3.52^{a} (90.2-110.6)	8.55(%increase)
Eggs	49.48±1.47 (47.03-52.1)	41.87±1.19 (40.02-44.1)	15.38%

*Mated fed female was treated topically with 10 µl/female of 50ppm Lufox on the 3rd daf.
**Means bearing different letters (within column) are significantly different.
*** Percent reduction relative to normal

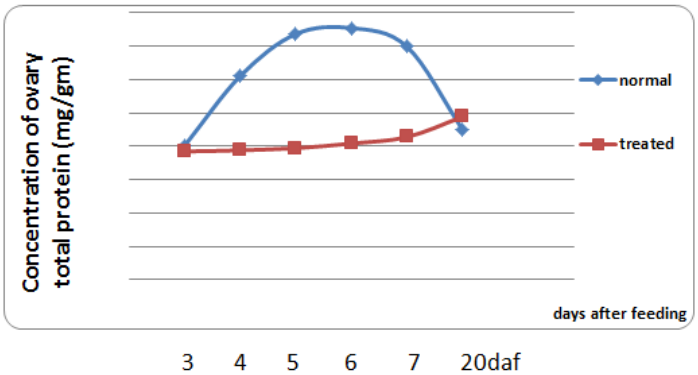

Fig. 3: Changes in the concentration of ovary total protein of adult normal and Lufox topically treated (on 3rd daf) female ***Argas persicus*** on different days after feeding (daf).

In comparison between the total protein of both normal and Lufox treated mated females *A. persicus* ovaries, there was a significant decrease (P < 0.01) in the level of total protein during the 4th to 7th daf of treated females. During this period the percent reduction ranged from 36.47 to 46.43 % relative to their corresponding state in normal female.

Total protein of the eggs:

Results in Table (3) show that the freshly laid eggs by normal mated fed adult female *A. persicus* have total protein concentration of 49.48±1.47mg/gm tissue. However, in freshly laid eggs by the Lufox treated adult female, the level of the total protein decreased ($P < 0.01$) to reach 41.87±1.19mg/gm egg.

DISCUSSION

Total protein in the HL, ovary and eggs of *Argas persicus*

The total protein concentration in the HL of mated fed adult *A. persicus* decreased immediately and up to the 2nd and 3rd daf in the females and males respectively and was insignificantly different in both sexes up to the 2nd daf. The results conform to those in other argasid ticks (Shanbaky *et al*. 1990b and Yousery, 2011). The observed decrease could be attributed to a dilution of the HL by an influx of excess water from the blood meal and the marked increase in the HL volume as was observed by Hefnawy (1972) and Hesse (1984) in the argasid*, A.arboreus and O.moubata*. In accordance with the findings of the present study, Boctor and Araman (1971) found a decrease of the concentration of free amino acids in the HL of *A. persicus* and *A. arboreus* after feeding, which was attributed to dilution of the hemolymph.

In the present work, the initial drop of total protein concentration in the HL of *A. persicus* 1 hr after feeding was followed by a gradual increase of the level to reach that of the unfed controls on the 3rd and 4th daf and to exceed it on the next days to reach maximum on days 4-7 and the 6th and 7th daf in females and males, respectively. This period coincided with the rapid phase of digestion reported in other argasids (*Tatchell,* 1964*; Aeschlimann and Grandjean,* 1973 and *Elshoura,* 1988). Changes in concentration of HL total protein in *A. persicus* followed similar patterns to those found *in A. hermanni* (Shanbaky *et al*., 1990b) and *O. erraticus* (Yousery, 2011).

The significant increase in the HL protein level in the female than in the male *A. persicus* (3-7 daf) and in previously studied argasids, *A. hermanni* (Shanbaky *et al.*, 1990) and *O. erraticus* (Yousery, 2011) reflects the increasing demands for proteins during vitellogenesis in the female (Shanbaky *et al.* 1990c). In *A. hermanni*, the increase of the concentration of the HL proteins in the females (3-6 daf) positively correlated with an increase of the ovarian protein level, weight and the number of mature oocytes which suggested an uptake of HL protein by the ovaries (Shanbaky *et al*., 1990b). Furthermore, the levels of HL protein and midgut proteases were higher in the female than male *O. erraticus* pointing to an increased digestion of protein in the female than in male tick to supply needs of the female (Yousery, 2011).

In the present work, the protein level in the HL of the female *A. persicus* decreased once more after the completion of oviposition suggesting its consumption by developed oocytes. This is in agreement with previous studies in ticks which have shown that the increase of total HL protein level during vitellogenesis and oviposition was followed by a progressive decrease in HL protein at the end of oviposition in female *B. microplus* (Tatchell, 1971), *R. sanguineus* (Araman, 1979), *A. hermanni* (Shanbaky *et al.*, 1990b) and *O. erraticus* (Yousery, 2011).

In the present work the concentration of the total protein in the ovaries of the mated females *A.persicus* gradually increased after feeding to reach the maximum level on 5-7th daf which lagged behind the maximum in the HL (4-7 daf) and might be attributed to uptake of the HL protein and/ or increased synthesis in the ovary proteins. An increase in the level of ovary protein was observed by Shanbaky *et al*.

(1990b) in the mated engorged *A. hermanni*. The authors demonstrated that the period of the increase of the ovary protein level coincided with the progress of vitellogenesis (3-6 daf) as determined by the weight of the ovaries and the number of mature oocytes. Furthermore the increase of the level of the ovarian total protein was associated with an increase of the level and the number of the exogenous vitellins during the period of vitellogenesis (3-6 daf) suggesting an uptake of protein by ovary during vitellogenesis. Also, in the ixodid *Boophilus microplu*s, the total level and the relative amounts of two hemoprotein bands in the HL increased during vitellogensis (Tatchell, 1971).

The level of the total protein in the ovary of *A. persicus* decreased after the completion of oviposition but was still higher than in the unfed. Similarly, the concentration of vitellin in the ovaries of *Ixodes scapularis* declined after oviposition (James and Oliver, 1996).

The effect of Lufox on the total protein of the haemolymph, ovary and deposited eggs of the treated female *Argas persicus*:

In comparison with the untreated controls, topical treatment of mated fed female *A. persicus* on the 3rd daf with a single dose (10µl/female) of 50 ppm Lufox caused a significant decrease (% reduction) in the level of the total protein in the HL (43.04-55.57%) and ovaries (36.47-46.43%) during the period 4-7 daf (vitellogenesis and ovulation) and in the eggs (15.38%) freshly deposited by the treated female. This decrease in concentration of HL and ovary proteins is expected to have a direct impact on the ovary and may partially explain the observed reduction in fecundity, fertility and the delay of oocytes and embryonic development in the treated females as represented by egg number and % hatch and the latency of oviposition and egg hatching, respectively (Abdelhamid,2014). The reduction of the total protein in the HL and ovaries deprives the treated female *A. persicus* of enough and essential proteins required for deposition of yolk protein and egg maturation (Diehl *et al.,* 1982) and embryonic development (Kamel *et al.,* 1982). Vitellogenins (Vg), the main precursor of the yolk proteins constitutes a large proportion of the HL protein reaching about 85% of the total protein in the HL of *A.hermanni* during vitellogenesis (Helmy, 1988). Also Vgs concentration showed a 170-fold increase during the reproductive cycle of *Dermacentor variabilis* (Rosell and Coons, 1991).

In the present work, Lufox treatment altered the patterns of change in the concentration of HL and ovary total proteins during the reproductive cycle of the female *A. persicus*. Application of Lufox on the 3rd daf prevented any appreciable increase in the level of total proteins on the next days (4-7 daf) where the level remained constant in the ovary and even decreased in the HL. This suggests an impairment of protein production in both tissues which could be attributed to an interference with blood meal digestion, protein synthesis, and release into the HL and/or uptake in the ovary. Lufox treatment of female *A. persicus* on the 3rd daf excluded a probable interference of Lufox with early steps involved in protein production in the HL and ovary such as mating, blood meal ingestion and the engorgement. In female argasid ticks mating (Leahy and Galun, 1972) and acquisition of a blood meal are essential for vitellegenesis and completion of egg development (Diehl *et al.,* 1982, Chinzei,1986 & 1988, Chinzei *et al.,* 1991) by supplying nutrients and triggering synthesis and release of putative hormone (s) (Shanbaky and Khalil, 1975; Shanbaky *et al.,* 1990a) which stimulates digestion and initiates vitellogenesis (Tatchell, 1972, Aeschlimann and Grandjean, 1973, Chinzei *et al.,* 1992). In accordance with the present study, Kaufman *et al.*, (1986) and Lunke and Kaufman (1992) were able to show that the reduction of ovary maturation and vitellogenesis in

avermectins (CSI) treated female *Amblyomma hebraeum* was not a consequence of disruption of mating and feeding by direct injection of the compound into the hemocoele of mated, engorged female tick.

Generally, the reduction of the level of total protein in HL, ovary and freshly deposited eggs of Lufox treated female *A. persicus* might be attributed to a toxic action of the components of Lufox. In the peach fly *Bactrocera zonata*, Lufox was reported as the most potent contact toxicant among seven different insecticides tested against full grown larvae and pupae. The treatment reduced fecundity and % hatchability and caused high levels of sterility of emerged adult females in addition to a signficant reduction of the level of total protein and α-estrease enzymes (Halawa *et al.,* 2013).

Lufox is a mixture of JHA (fenoxycarb) and CSI (lufenuron). Regarding the effect (the role) of JH and JHA in ticks, old literature gave some proof to believe that JH has a role in vitellogenesis of ticks as evidenced by oocyte maturation and oviposition. Bassal and Roshdy (1974) demonstrated termination of reproductive diapause in *A.arboreus* after topical application of a JH mimic. Topical application of JH I and JH III induced maturation of oocytes and oviposition by fed virgin female *O. porcinus* (Obenchain and Mangos, 1980). Female A. *persicus, Ornithodoros coriaceus* and *Rhipicephalus sanguineus* were sterilized by the anti-allatotropin precocene 2 (Leahy and Booth, 1980). The sterility was partially reversed in the treated *O. parkeri* by application of JH III which restored oogenesis and oviposition in the tick (Pound and Oliver, 1979). Connat *et al.,* (1983) reported that natural JH and JH analogs stimulated vitellogenesis and oviposition in engorged virgin female of *O. moubata*.

In the present work, however, it is probably that the overloading of the treated tick by effects of the fenoxycarb (JHA) in Lufox in addition to effects of a natural putative hormone in female *A. persicus* as in other Argasids (Shanbaky and Khalil, 1975, Shanbaky *et al.,* 1990a) might have disturbed or interfered with the normal physiology of the treated tick leading to the observed reduction of the protein levels in the haemolymph and ovaries.

On the other hand Khalil *et al.,* (1984) found that topical application of JHI on adult *H. dromedarii* did not alter adult sexual behavior and caused only a small reduction in fertility of both sexes. The authors suggested that *H. dromedarii* systems did not respond to JHI because it may be very different from tick JH and/or the administration method was unsuitable. More recently, Chinzei *et al.,* (1991), showed that Vg synthesis in female *O. moubata* was not induced by application of JH or analogs to unengorged females. Schriefer (1991); Oliver and Datson (1993) mentioned that vitellogenesis in ticks may be controlled by interplay between juvenile hormone, ecdysteroids and/or synganglion factors.

Results obtained by Lui-Singize *et al.,* (1997) suggested that the synganglion may synthesize JH III and an unknown substance in *Haemaphysalis longicornis* (Ixodidae). Neurosecretory activity in general seems to increase shortly after a blood meal and then declines during egg production (Eisen *et al.,* 1973; Shanbaky *et al.,* 1990) suggesting that a neuropeptide may be part of the pathway controlling vitellogenesis (Chinzei *et al.,* 1992). Administration of synganglia homogenate from mated fed females induced oviposition (Aschliman, 1986) in *O. moubata* and ligation experiments in *A. arboreus* (Shanbaky and Khalil, 1975) and *A. hermanni* (Shanbaky *et al.,* 1990a) pointed to the synganglion as a source of gonadotropin hormone.

In *O. moubata*, Chinizei *et al.,* (1992) indicated that Vg-synthesis is initiated by a "vitellogenesis inducing factor (VIF); which is a neuropeptide that acts on some

unknown tissue to cause the release of a fat body stimulating factor (FSF) which is the vitellogenic hormone which in its turn stimulates Vg synthesis. The weight of the current evidence is in favor of an ecdysteroid being the vitellogenic hormone in ixodid ticks (Rees, 2004).

In the argasids *A. hermanni* 20-hydroxyecdysone also stimulates Vg synthesis (Friesen and Kaufman, 2002). Taylor *et al.,* (1991) concluded that, whereas JH appears not to stimulate Vg-synthesis in argasid ticks, it may have stimulated Vg uptake, ovulation and/or oviposition in those studies in which ticks were treated with exogenous JH (e.g., Pound and Oliver, 1979; Connat *et al.,* 1983; Connat, 2000). No known JH has been identified in hard (*Dermacentor variabilis*) and soft (*Ornithodoros parkeri*) ticks (Neese *et al.,* 2000). On the other hand extracts of *Boophilus microplus* have JH-like bioactivity (Connat, 2000). Moreover, Venkatesh *et al.,* (1990) and Roe *et al.,* (1993) showed JH esterase activity in *D. variabilis.* Sonenshine *et al.,* (1989) and Lomas *et al.,* (1996) described proteins in *D. variabilis* and *A.hebraeum*, respectively, that bound JH.

From the above mentioned discussion, the variation and contradiction of the different results concerning the role of JH in tick vitellogenesis may be due to intergeneric differences between the studied tick species, their physiological state and/or the chemical nature of the tested compounds and methods of treatment.

In the present study it is apparent that the chitin synthesis inhibitor (lufenuron) together with JHA (fenoxycarb) induce disturbance of protein synthesis in the treated females of *A. persicus*. This effect may be direct or indirect on protein synthesis during vitellogenesis. Kaufman *et al.,* (1986); Lunke and Kaufman (1991) found that injection of 100µg/kg low of the avermectin MK -243 into the hemocoele of fed *Amblyomma hebraeum* inhibited oviposition, ovary development, vitellogenesis and reduced the HL ecdysteroid concentration.

Similarly, Acheuck *et al.,* (2012) demonstrated that oral treatment of newly emerged female *Locusta migratoria* with Teflubenzuron (TFB) induced a drop in haemolymph protein and ecdysteroids level.

Soltani and Soltani-Mazouni (2006) demonstrated that the protein content and the number of oocytes per ovary recorded in newly emerged adults *Cydia pemonella (L.)* were significantly reduced after diflulenzruon topical treatment at pupal ecdysis. These results indicated that the reduction in fecundity and egg viability is probably due to interference of diflubenzuron with the vitellogenesis process.

Incorporation of Flucy-cloxuron, a benzoylphenyl urea derivative, into the diet of newly emerged female *Tenebrio molitor* reduced both oocytes number, ovary weight and size of the basal oocytes and ovarian ecdysteroids (Hami *et al.,* 2004). Generally, ecdysteroids and JH and their metabolism seemed to be targets for CSI including different benzoylphenyl urea (e.g., Diflubenzuran and Lufenuron) and reduction of ecdysteroid levels were reported in insects and other arthropods (Mayer *et al.,* 1990).

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ARABIC SUMMERY

تأثير اللوفكس على بروتينات هيموليمف ومبيض القراد اللين *ارجاس بيرسيكس*

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تمت دراسة التغيرات فى تركيز البروتين الكلى فى هيموليمف ومبايض اناث القراد المتزاوج والمغذى غير المعالج والمعالج باللوفكس *آرجاس بيرسيكس* خلال الدورة التناسلية وفى هيموليمف الذكور غير المعالجة. تبع امتلاء القراد انخفاض أولى فى تركيز البروتين الكلى للهيموليمف بعد التغذية مباشرة وحتى اليوم الثانى والثالث بعد التغذية فى كلا من الاناث والذكور. كما أزدادت مستويات البروتينات فى الهيموليمف تدريجيا خلال الايام التالية ليصل حده الاقصى خلال ٤-٧ أيام فى الاناث و ٦-٧ أيام فى الذكور. وجد ان مستوى البروتين فى هيموليمف الاناث خلال فترة تكون المح وبداية وضع البيض أعلى منه فى الذكور.كذلك أزداد مستوى البروتين الكلى فى مبايض الاناث غير المعالجة زيادة تدريجية مباشرة بعد التغذية وحتى اليوم الثانى والثالث بعد التغذية ليصل الحد الاقصى خلال ٥-٧ أيام بعد التغذية. كما انخفض مستوى البروتين فى الهيموليمف والمبايض بعد اكتمال وضع البيض (٢٠ يوم بعد التغذية).

تداخلت المعالجة الموضعية لاناث القراد المغذى والمتزاوج *آرجاس بيرسيكس* بجرعة واحدة من اللوفكس بتركيز ٥٠ جزء فى المليون خلال اليوم الثالث بعد التغذية مع انتاج البروتين، مسببة انخفاض ملحوظ فى مستوى البروتين الكلى فى الهيموليمف (٤٣,٠٤-٥٥,٥٧%) والمبايض (٤٧،٣٦-٤٣،٤٦%) خلال فترة تكون المح من ٤-٧ أيام بعد التغذية وفى البيض (١٥,٣٨%) الناتج من الاناث المعالجة. كما أدت المعالجة باللوفكس خلال اليوم الثالث بعد التغذية الى تغير النمط الطبيعى للتغير فى المستوى الكلى لبروتينات الهيموليمف والمبيض خلال الدورة التناسلية عن طريق منع اى ازدياد ملحوظ فى مستوى البروتين خلال الايام التالية (٤-٧ أيام بعد التغذية) حيث يظل ثابتا فى المبايض وينخفض فى الهيموليمف (٤-٥ ايام بعد التغذية).