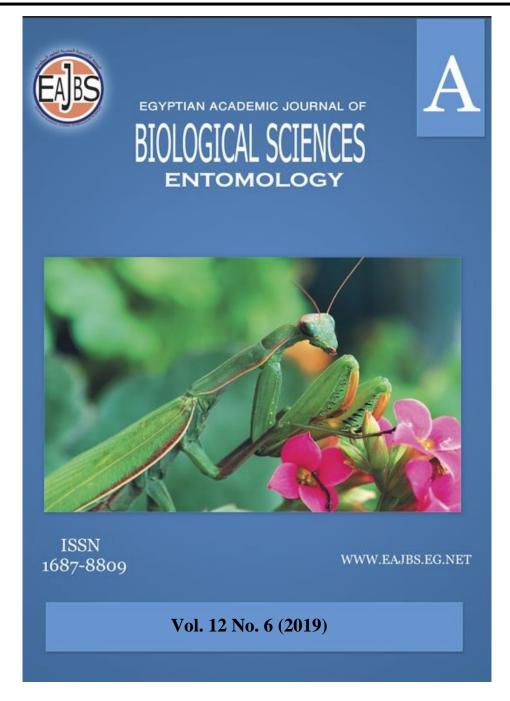
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Effect of Abamectin on Reproduction and Development of an Avian Tick, *Argas* (*Persicargas*) arboreus (Ixodoidea: Argasidae)

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#### **ABSTRACT**

Topical application of a single dose ( $10\mu$ I/tick or egg batch) of different concentrations of abamectin (an avermectin) has lethal and biological effects on the engorged mated adult female, immatures and egg of *Argas (Persicargas) arboreus* that induced 100% mortality at 25,15 and 10 ppm, respectively. Abamectin treatment at lower concentrations of 1-10 ppm interfered with the female reproduction, nymphal and egg development.

Abamectin topical (3-10 ppm) application on mated engorged adult female *A. (P.) arboreus* at different intervals of its reproductive cycle reduced fecundity and fertility of the female, prolonged its preoviposition, oviposition and the egg incubation periods. The effects were most prominent during vitellogenesis and the least at the start of oviposition.

Abamectin treatment of the newly moulted fed 2<sup>nd</sup> nymphal instar, delayed its moulting and of the resulted 3<sup>rd</sup> instar and reduced adult emergence. The treatment caused paralysis and various deformities (15.6-28.6%) as missing legs at the coxal level, changes in colour and failure to moult or to detach the old exuvia in the resulted 3<sup>rd</sup> nymphal instar.

Egg batch treatment by dipping the freshly deposited eggs for 20 seconds in an aqueous solution of 1-5 ppm abamectin was more effective in reducing normal hatching, increasing unhatching and total inhibition of egg development than topical treatment. However, both methods of treatment similarly prolonged the egg incubation periods and caused abnormal hatching, malformations, and anomalies in the eggs and embryos.

### **INTRODUCTION**

The tick, *Argas (Persicargas) arboreus* Kaiser, Hoogstraal, and Kohls (Khalil *et al.*, 1980) infests herons and other medium size wading birds in their heronries in many areas of Africa. In Egypt, *A. (P.) arboreus* commonly inhabits rookeries of the cattle egret or buff backed heron, *Bubulcus ibis ibis* (Linnaeus) in the Nile Valley, Delta, and nearby oases. The tick feeding, egg-laying, and development of immature stages coincide with the bird's nesting period during the spring-summer season (Taylor *et al.*, 1966; Guirgis 1971; and Khalil, 1974).

In Egypt, *Argas (P.) arboreus* is a host of the bacterium, *Salmonella typhimurium* (Floyd & Hoogstraal 1956), the rickettsia microorganism, *Wolbachia persica* (Suitor & Weiss 1961) and the spirochete *Borrelia anserina* (Hoogstraal, 1985). It is also naturally infected with Quaranfil and Nyamanini viruses (Taylor et al. 1966, Hoogstraal 1966). In

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addition to paralysis produced by *Argas* toxins, these agents of disease could be transmitted to host birds during tick feeding which may cause illness and death of the bird. Occasionally, dozens or hundreds of moribund immatures of the cattle egret were found on the ground under heronries infested with *A. (P.) arboreus* during the breeding season in Egypt (Hoogstraal, 1985). As this bird is beneficial to agriculture in Egypt, by feeding on harmful insects and small animals on field soil, it has been protected since 1912 when its numbers became very low (Kirkpatrick, 1925). Protection of the cattle egret *B. ibis* becomes more efficient by increasing our knowledge on the bird relationships with its environment and biological associates especially vectors of disease agents such as parasitic ticks and their control.

Avermectins (fermentation product of mycelia extracts of *Streptomyces avermitilis*) is a group of newly developed pesticides with activity against parasites and are most potent exhibiting noticeable selective toxicity for nematode and arthropod parasites over their vertebrate hosts (Wolstenholmes and Roger, 2005). Avermectins (AVMs) gave excellent control against insects and acarine pests of animals and man (Azambuja *et al.*, 1985; Drummond, 1985; Ash and Oliver, 1989; Tesh and Guzman, 1990; Mahmood *et al.*, 1991; Omura and Crump, 2004 and Davey *et al.*, 2010) and of agriculture (putter *et al.*, 1981; Kumar and Poehling, 2007).

Generally, when AVMs (ivermectin, abamectin, and doramectin) and their analogues (as MK-243 and milbemycin) are applied, the treated parasites such as nematodes or arthropods are unable to either move or feed (Ash and Oliver 1989; Martin,1997; Wolstenholmes and Roger, 2005), to digest blood meals as in mosquitoes (Mahmood *et al.*,1991) or to secrete vitellogenic hormones as in female ticks (Lunke and Kaufman,1992) which result in a reduction of oocytes development and egg production. The mode of action of AVMs is still unknown in ticks but it has been reported that AVMs selectively paralyze nematodes by increasing the opening of the Cl<sup>-</sup>ion channels at synapses and Cl<sup>-</sup>conduction (Martin, 1997) in the membrane of the muscle and most probably blocking γ-aminobutyric acid (Fritz *et al.*, 1979; Holden-Dye, 1988) and glutamate-activated Cl<sup>-</sup>ion channels (Wolstenholmes and Roger, 2005) leading to muscle paralysis. The latter Cl<sup>-</sup>ion channels are specifically expressed and occur in muscles and nerves of nematodes, insects and probably of some other arthropods but not in mammals (Narahashi *et al.*, 2010) which supports the selectivity and safety of the pesticide.

Mahmood *et al.* (1991) observed that ivermectin directly or indirectly affects at least the nervous, digestive and reproductive systems in the mosquito *Aedes aegypti*. Other effects of AVMs have been found in other arthropods when the chemicals inhibited chitin synthesis in the brine shrimp and interfered with DNA synthesis in some organisms (Calcott and Fatig, 1984; Mayer *et al.*, 1990).

In ticks, the AVM analogue MK-243 reduced egg production, markedly slowed down ovary development and inhibited vitellogenesis, increased oviposition latency or prevented oviposition and decreased concentration of haemolymph ecdysteroid in the engorged mated female *Amblyomma hebraeum* (Lunke and Kaufman, 1992). Ticks feeding on animal hosts treated with ivermectin took longer time to complete their blood meal and usually weighed less after detachment. Mating of AVM treated female *Rhipicephalus appendiculatus* was also disrupted (Jackson and Chesterman, 1989). Furthermore, AVMs reduced moulting success of nymphs of *A. hebraeum*, *R. evertsi*, *R. appendiculantus*, *Ornithodoros parkeri* and *O. moubata* (Centurier and Barth, 1980; Ash and Oliver, 1989; Soll *et al.*, 1989).

The present study is an attempt to investigate effects of abamectin (one of the main kinds of Avermectins) on female reproduction, immatures and egg development of the avian tick *Argas (Persicargas) arboreus* using different biological parameters during the

reproductive cycle of the female, nymphal development to adult emergence and egg development till hatching (incubation period).

#### MATERIALS AND METHODS

#### **Tick Rearing:**

Argas (Persicargas) arboreus was collected from trees supporting the rookeries of Bubulcus i. ibis at Al Mansoureya Canal, Giza governorate.

Ticks were held in the laboratory at  $28 \pm 1^{\circ}$  C and 75% relative humidity and subjected to normal changes of subdued daylight throughout the year.

The ticks were placed in plastic vials, with a bottom sealed with gypsum and a top securely screened with a muslin cloth. Ticks were fed on domestic pigeons *Columbia livia* as described by Kaiser (1966). Blood meal was required before moulting of larva and each nymphal instar and for the mated adult female before each oviposition period.

### **Preparation of the Avermectin Material:**

Abamectin is a macrocyclic lactone mixture containing a minimum of 80% avermectin B1a (i): 5-Odemethylavermectin B1a and a maximum of 20% avermectin B1b (ii): 5-Odemethyl-25-de (1- methylpropyl)-25-(1-methylethyl) avermectin B1b. The commercial product of abamectin (Biomectin 5% EC) was kindly supplied by Ministry of Agriculture, Egypt. It was dissolved in distilled water to prepare the different concentrations used in the present study.

## **Application of Abamectin:**

#### 1. Female Treatment:

For biological studies on reproduction, a single dose ( $10\mu$ I/tick) of three selected concentrations (3,5 and 10 ppm) of abamectin was used to apply, where preliminary experiment showed that higher doses either completely prevented egg production or hatching and caused very slow motion and mortality of the females to reach 100% at 25 ppm within 10-20 days after treatment.

An aqueous solution of abamectin was administered topically, with micro-syringe, on the ventral side of the posterior half of the female body. The abamectin was applied on mated fed female ticks at different periods of the reproductive cycle, including early previtellogenesis (first day after feeding, daf), early vitellogenesis (3 daf.) and at the start of oviposition (7 daf.) (Shanbaky and Khalil, 1975). Pairs of treated and untreated control females and normal males were kept separately in rearing tubes at  $28^{\circ}\text{C} \pm 1$  and 75% RH. The effects of the selected concentrations of abamectin on female reproduction were assessed using different biological parameters including preoviposition, oviposition period, mean daily and total eggs (fecundity) deposited per oviposition per female and egg viability(female fertility). Eggs were collected daily and counted, their hatchability and incubation period were recorded in treated and untreated females.

## 2. Nymph Treatment:

Newly moulted second instar nymphs were topically treated with four different concentrations of abamectin (3, 5, 10 and 15 ppm). Nymphs were placed in rearing tubes in the incubator and observed to determine percentage mortality, pre mounting period of the treated  $N_2$ , the resulted  $N_{3-4}$  instar, deformities, and percent adult emergence

## 3. Egg Treatment:

The freshly deposited eggs (1-8 hours old) were treated topically or by dipping using five different concentrations (1, 3, 4, 5 and 10 ppm) of abamectin on batches of 60 eggs. The abamectin solution was applied topically by a micro-pipette on each egg batch. In the dipping technique, the freshly deposited eggs (60 eggs/batch) were immersed in 100 ml aqueous solution of each of the selected five concentrations of abamectin for 20 seconds. In both

methods of application each experiment was replicated three times. Egg hatchability, incubation period and deformities were recorded at each concentration.

In all series of experiments, females, nymphs as well as eggs used as a control, were treated with appropriate amount of distilled water.

## **Statistical Analysis:**

The obtained data were manipulated statistically with **SPSS** version 16 while probabilities (p) were carried out using STATISTIC version 6 and ANOVA, using (p<0.05) Duncan multiple range test.

#### **RESULTS**

### Effect of Abamectin on Reproduction of Female Argas arboreus:

Effect of topical application of a selected dose ( $10\mu l/tick$ ) with different concentrations (3, 5 and 10ppm) of abamectin on fecundity, fertility, preoviposition and oviposition period, egg viability and incubation period of mated female *Argas arboreus*, were assessed at different periods of the reproductive cycle on the  $1^{st}$ ,  $3^{rd}$  and  $7^{th}$  day after feeding (daf).

# 1. Effect of Abamectin on the Fecundity of Female *Argas Arboreus* and Mean Daily Egg Output of the Deposited Eggs:

#### 1.1. Fecundity:

Results in table (1) show that female treatment with abamectin on the 1<sup>st</sup> daf caused an insignificant decrease (p> 0.05) in the mean total number of eggs laid/oviposition/female (fecundity) at all the three concentrations tested. Table (2) shows that female treatment with abamectin on the 3<sup>rd</sup> daf caused a significant decrease (p<0.05) in the fecundity of A. *arboreus* at all the three concentrations tested. The number of eggs laid by the females treated with abamectin on the 3<sup>rd</sup> daf decreased gradually (p<0.05) by increasing the concentration of abamectin. Table (3) shows that female treatment with abamectin on the 7<sup>th</sup> daf caused an insignificant decrease (p> 0.05) in the fecundity of A. *arboreus* at all the three concentrations tested.

Data in tables (1-3) show that there is a significant decrease (p<0.05) in fecundity of females treated with abamectin on the  $3^{rd}$  daf compared with the  $1^{st}$  and  $7^{th}$  daf treated females at each concentration studied. The effect was most prominent at 10 ppm where the average number of eggs laid was  $40\pm3.69$ ,  $59.1\pm6.7$  and  $67.9\pm2.2$  eggs/oviposition/female for the  $3^{rd}$ ,  $1^{st}$ , and  $7^{th}$  daf treated females, respectively.

## 1.2. The Mean Daily Output of Eggs of Female Argas arboreus:

Results in table (1) show that there is a significant decrease (p<0.05) in the mean daily output (mean number of eggs laid/day) of eggs laid by female A. *arboreus* treated with abamectin on the 1<sup>st</sup> daf at all concentrations tested. Table (2) shows that there is a significant decrease (p<0.05) in the daily output of eggs laid by female A. *arboreus* treated with abamectin on the 3<sup>rd</sup> daf at all concentrations tested. The mean daily output decreased gradually (p<0.05) by increasing concentration. Table (3) shows that there is a significant decrease (p<0.05) in the daily output of eggs laid by female A. *arboreus* treated with abamectin on the 7<sup>th</sup> daf at the highest concentration tested.

Data in tables (1-3) show that there is a significant decrease (p<0.05) in the mean daily output of eggs laid by females treated on the  $3^{rd}$  and  $1^{st}$  daf with 5 and 10 ppm when compared with the  $7^{th}$  daf treated females. The effect was most prominent in eggs of females treated with 10 ppm where daily output was decreased to reach  $3.99\pm0.57$ ,  $6.16\pm0.92$  and  $12.1\pm0.51$  eggs for the  $3^{rd}$ ,  $1^{st}$ , and  $7^{th}$  daf treated females, respectively.

# 2. Effect of Abamectin on Preoviposition and Oviposition Periods of Female Argas arboreus:

## 2.1. The Preoviposition and Oviposition Periods:

Results in table (1) show that the preoviposition and oviposition periods of females treated with abamectin on the 1<sup>st</sup> daf were significantly increased (p<0.05) at the two higher concentrations (5, 10 ppm) used. The preoviposition and oviposition increased gradually (p<0.05) by increasing concentration of abamectin. Table (2) shows that the preoviposition and oviposition periods of females treated with abamectin on the 3<sup>rd</sup> daf were significantly increased (p<0.05) at all the three concentrations tested (except at 3 ppm for oviposition). The preoviposition and oviposition increased gradually (p<0.05) by increasing concentration of abamectin. Table (3) shows that the preoviposition and oviposition periods of females treated with abamectin on the 7<sup>th</sup> daf were insignificantly changed (p>0.05) at all concentrations used. The preoviposition and oviposition periods show no significant change (p>0.05) by increasing concentration of abamectin.

Data in tables (1-3) show that there was a significant increase in the preoviposition period (p<0.05) of females treated with 10 ppm on the 3<sup>rd</sup> daf as compared with those of females treated on the 1<sup>st</sup> and 7<sup>th</sup> daf, where the preoviposition period reached 8.2±0.32, 7.3±0.36 and 5.2±0.24 days for the 3<sup>rd</sup>, 1<sup>st</sup> and 7<sup>th</sup> daf treated females, respectively. Also, there was a significant increase (p<0.05) in the oviposition periods of females treated with 10 ppm on the 3<sup>rd</sup> daf treated females compared with the 1<sup>st</sup> and 7<sup>th</sup> daf treated females, where the oviposition periods reached 11±1.1, 10.2±0.95 and 5.7±0.3 for the 3<sup>rd</sup>, 1<sup>st</sup>, and 7<sup>th</sup> daf treated females, respectively.

# 3. Effect of Abamectin on Viability and Incubation Period of Eggs Deposited by Female *A. arboreus:*

### 3.1. Egg Viability:

Results in table (1) show that female treatment with abamectin on the  $1^{st}$  daf caused a significant decrease (p<0.05) in the viability (hatching percent) of the deposited eggs at all the three concentrations used. Table (2) shows that female treatment with abamectin on the  $3^{rd}$  daf caused a significant decrease (p<0.05) in the viability (hatching percent) of the deposited eggs at all concentrations. The percentage of hatched eggs decreased gradually (p<0.05) by increasing the concentration of abamectin. Table (3) shows that female treatment with abamectin on the  $7^{th}$  daf caused a significant decrease (p<0.05) in the viability (hatching percent) of the deposited eggs at the highest concentration.

Data in tables (1-3) show that there is a significant decrease (p<0.05) in viability (hatching percent) of eggs deposited by females treated with abamectin on the 3<sup>rd</sup> daf compared with 1<sup>st</sup> and 7<sup>th</sup> daf treated females at each concentration studied. The effect was most prominent at 10 ppm where the percentages of hatching were56.6±4.3, 81.2±2.00 and 89.6±1.6% for 3<sup>rd</sup>, 1<sup>st</sup>, and 7<sup>th</sup> daf treated females, respectively.

### 3.2. Incubation Period of Eggs Deposited:

Results in table (1) show that there is a significant increase (p<0.05) in the incubation period (hatching period) of eggs laid by female A. *arboreus* treated with abamectin on the 1<sup>st</sup> daf at the highest concentration tested. Table (2) shows that there is a significant increase (p<0.05) in the incubation period (hatching period) of eggs laid by female A. *arboreus* treated with abamectin on the 3<sup>rd</sup> daf at the two higher concentrations tested. The incubation periods increased gradually (p<0.05) by increasing concentration. Table (3) shows that there is an insignificant increase (p>0.05) in the incubation period (hatching period) of eggs laid by female A. *arboreus* treated with abamectin on the 7<sup>th</sup> daf at all concentrations tested.

Data in tables (1-3) show that there is a significant increase (p<0.05) in the incubation periods of females treated on the  $3^{rd}$  and  $1^{st}$  daf with 5 and 10 ppm compared with  $7^{th}$  daf treated females. The effect was most prominent in eggs of females treated with 10 ppm where incubation period reached  $23.2\pm0.98$ ,  $20.2\pm1.09$  and  $14.5\pm0.4$  days for the  $3^{rd}$ ,  $1^{st}$  and  $7^{th}$  daf treated females, respectively.

**Table (1):** Effect of a single dose (10μl/female) of selected concentrations of abamectin on fecundity, fertility, preoviposition, oviposition, and egg incubation periods of *Argas arboreus* females treated topically on the 1<sup>st</sup> daf.

Conc. (ppm)	Preoviposition period (day)	Oviposition period	Mean daily egg output	Total no. of eggs	%hatched eggs	Eggs incubation
	mean±SE	(day)	mean±SE	laid/female	mean±SE	period (day)
	(range)	mean±SE	(range)	mean±SE	(range)	mean±SE
		(range)		(range)		(range)
Control	5±0.2ª	5.1±0.27a	15.87±1.2ª	78.1±2.56a*	94.85±0.91ª	15±0.6ª
	(4-6)	(4-6)	(10-22)	(60-88)	(90.4-97.56)	(12-18)
3	5.5±0.26ab	6.2±0.29ab	11.39±1.29b	68.2±5.8ª	88.3±0.99b	16.7±0.76ª
	(4-7)	(5-8)	(6.43-18)	(40-90)	(82.3-93.3)	(13-20)
5	6.2±0.29bc	8.4±0.6bc	8.2±0.52bc	67.5±4.5ª	86.5±2.1bc	18±0.77ab
	(5-8)	(5-11)	(5.64-10.75)	(39-86)	(70.1-94.4)	(13-21)
10	7.3±0.36c	10.2±0.95c	6.16±0.92c	59.1±6.7ª	81.2±2.00c	20.2±1.09b
	(6-9)	(6-15)	(2.65-13)	(23-87)	(67.5-91.8)	(16-25)

<sup>\*</sup>Means bearing different letters (within columns) are significantly different.

**Table (2)**: Effect of a single dose (10μl/female) of selected concentrations of abamectin on fecundity, fertility, preoviposition, oviposition, and egg incubation periods of *Argas arboreus* females treated topically on the 3<sup>rd</sup> daf.

Conc.	Preoviposition	Ovipositio	Mean daily	Total no. of	%hatched	Eggs
(ppm)	period (day)	n period	egg output	eggs	eggs	incubation
	mean±SE	(day)	mean±SE	laid/female	mean±SE	period (day)
	(range)	mean±SE	(range)	Mean±SE	(range)	mean±SE
		(range)		(range)		(range)
Control	5.1±0.23ª	5.2±0.24ª	15.2±1.04ª	77±2.9ª*	93.5±1.06ª	15±0.63ª
	(4-6)	(4-6)	(10.83-20)	(65-88)	(90-100)	(12-18)
3	6.6±0.54b	6.9±0.45ab	8.63±0.66b	57.6±3.08b	88.1±1.56b	17.6±0.52ab
	(5-10)	(5-10)	(4.9-12)	(41-72)	(78-94.2)	(15-20)
5	6.9±0.34bc	8.8±0.57bc	6.46±0.62bc	54.2±2.57b	83.1±1.43b	18.3±1.01b
	(6-9)	(6-11)	(3.91-11)	(43-66)	(73.4-89.2)	(14-24)
10	8.2±0.32c	11±1.1c	3.99±0.57c	40±3.69°	56.6±4.39°	23.2±0.98c
	(7-10)	(7-19)	(1.7-7.5)	(24-60)	(36.5-82.1)	(18-28)

<sup>\*</sup>Means bearing different letters (within columns) are significantly different.

**Table (3):** Effect of a single dose (10µl/female) of selected concentrations of abamectin on fecundity, fertility, preoviposition, oviposition and egg incubation periods of *Argas arboreus* females treated topically on the 7<sup>th</sup> daf.

	Preoviposition	Oviposition	Mean daily	Total no.	%hatched	Eggs
Conc.	period (day)	period	egg output	of eggs	eggs	incubation
(ppm)	mean±SE	(day)	mean±SE	laid/female	mean±SE	period (day)
	(range)	mean±SE	(range)	Mean±SE	(range)	mean±SE
		(range)		(range)		(range)
Control	5.1±0.23ª	4.9±0.23ª	15.89±0.63ª	77±2.9ª*	95.6±0.67ª	13.2±0.57ª
	(4-6)	(4-6)	(11-17.6)	(65-88)	(90.9-98.7)	(10-16)
3	5.4±0.22ª	5.1±0.23a	15.28±0.86ª	76.4±2.06ª	94.2±0.87ab	13.4±0.63ª
	(4-6)	(4-6)	(12.17-20)	(66-87)	(89.7-97.5)	(10-17)
5	5.2±0.24ª	4.9±0.27a	15.26±0.95ª	72.7±2.3ª	92.3±1.3ab	14.3±0.51ª
	(4-6)	(4-6)	(11-19.25)	(64-85)	(82.3-96.3)	(12-17)
10	5.2±0.24ª	5.7±0.3ª	12.1±0.51b	67.9±2.2ª	89.6±1.6b	14.5±0.4ª
	(4-6)	(4-7)	(9.14-15.5)	(60-79)	(76.8-95.8)	(12-16)

<sup>\*</sup>Means bearing different letters (within columns) are significantly different.

### 2. Effect of Abamectin on Immature Stages and Adult Emergence of Argas arboreus:

Results presented in table (4) show the effect of different concentrations of abamectin (3, 5, 10, and 15 ppm) on the newly moulted fed 2<sup>nd</sup> nymphal instar of *Argas arboreus* and subsequent stages.

The toxicity of abamectin caused a significant decrease (p<0.05) in the viability of the treated  $2^{nd}$  nymphal instar, where percentage mortality increased gradually (p<0.05) by increasing concentration to reach  $100\pm0.00\%$  at 15 ppm of abamectin compared with 0% for control nymphs. There was also a significant increase (p<0.05) in the duration of the premoulting period of the  $2^{nd}$  nymphal instar.

The  $3^{rd}$  nymphal instar resulted from the treated  $2^{nd}$  nymphal instar, showed no mortality. However, significant morphological deformities (p<0.05) were observed in these nymphs (table 4). The percentages of the malformed  $3^{rd}$  nymphal instar gradually increased by increasing abamectin concentration of the treated  $2^{nd}$  instar nymphs. Also, there was a significant increase (p<0.05) of the premoulting period of the  $3^{rd}$  nymphal instar at all tested concentrations which was most prominent at the highest dose (p<0.05). Adult emergence was completely prevented on treatment with 15 ppm of abamectin because of 100% mortality of the  $2^{nd}$  instar nymphs treated with this concentration.

#### 2.1. Malformations:

The treatment of the  $2^{nd}$  nymphal instar of *Argas arboreus* with a single dose  $(10\mu l/nymph)$  of 3, 5, and 10 ppm abamectin, induced malformations of development in the resulted  $3^{rd}$  nymphal instar.

Different deformities in the resulted 3<sup>rd</sup> nymphal instars included some individuals which were paralyzed and unable to complete the moulting process (plate I, fig.3) and some others had changed red color (plate I, fig.2). Many of the resulted 3<sup>rd</sup> nymphs were able to complete the moulting process but the old cuticle remained attached to their legs. A number of the malformed individuals had a missing leg or pair of legs at the coxal level (plate I, fig.4). However some of the resulted 3<sup>rd</sup> instar nymphs looked normal and were able to complete the moulting process to adult.

**Table** (4): Effect of a single dose (10μl/nymph) of different concentrations of abamectin on immature stages and emergence of adult of *Argas arboreus* resulted from topically treated 2<sup>nd</sup> nymphal instar.

Conc. (ppm)	2 <sup>nd</sup> nymphal instar		3 <sup>r</sup>	<sup>d</sup> nymphal instai	Adult stage		
фи	Mortality % mean±SE (range)	Premoulting period(day) mean±SE (range)	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±0.00a*	10.66±0.66 <sup>a</sup>	0±0.00 <sup>a</sup>	0±0.00a	12.33±0.88 <sup>a</sup>	100±0.00a	0±0.00 <sup>a</sup>
	(0)	(10-12)	(0)	(0)	(11-14)	(100-100)	(0)
3	9±2ª	20.66±1.76 <sup>b</sup>	0±0.00 <sup>a</sup>	15.6±5.9ab	24±1.15 <sup>b</sup>	75.7±5.9 <sup>b</sup>	24.3±5.9ab
	(7-13)	(18-24)	(0)	(7-27)	(22-26)	(67-87)	(13-33)
5	24.66±2.33b	21.3±1.76 <sup>b</sup>	0±0.00²	20±4.04 <sup>b</sup>	22±1.15 <sup>b</sup>	55.7±5.9 <sup>b</sup>	44.3±5.9 <sup>b</sup>
	(20-27)	(18-24)	(0)	(13-27)	(20-24)	(47-67)	(33-53)
10	40±4.04 <sup>c</sup>	20.66±2.66 <sup>b</sup>	0±0.00ª	28.6±4.3 <sup>b</sup>	29.33±0.66 <sup>c</sup>	28.7±4.3°	71.3±8.0 <sup>c</sup>
	(33-47)	(18-26)	(0)	(20-33)	(28-30)	(20-33)	(60-87)
15	100±0.00 <sup>d</sup> (100)	-	-	-	-	-	-

## 3. Effects of Direct Application of Abamectin on Eggs of Argas arboreus:

The freshly deposited eggs were treated topically or by dipping in five different concentrations (1, 3, 4, 5 and 10 ppm) of abamectin, within 1-8 hours of deposition. Sixty eggs were used as a batch for each concentration and the experiment was replicated 3 times.

## 3.1. Topical Application:

Results in table (5a) show that abamectin has a significant effect (p<0.05) on hatchability and incubation periods of topically treated eggs and the effect is concentration-dependent. The percentage of unhatched and plus abnormally hatched (i.e. percent total inhibition of egg development) increased (p<0.05) by increasing dose concentration to reach 100% unhatchability at 10 ppm. Also, the incubation periods were significantly prolonged (p<0.05) at the higher concentrations compared with the control.

On the other hand, the percentages of normally hatched eggs were gradually decreased (p<0.05) at the higher tested concentrations to reach 0 % at 10 ppm abamectin. Normally hatched eggs contained fully developed embryos which could be seen through a transparent eggshell and produced actively motile larvae on hatching.

## 3.2. Dipping Technique:

The freshly deposited eggs were immersed for 20 seconds in 100 ml of distilled water containing one of five different concentrations of abamectin (1, 3, 4, 5 and 10 ppm/egg batch). The effects of abamectin (Table 5b) were similar to those of topical application and followed the same pattern of response at the same concentration in each of the tested parameters.

Comparing the used parameters in the two methods of application (Tables 5 a&b) at each concentration showed that abamectin treatment by dipping caused more reduction of normal hatching (P<0.05) and increase of the percent unhatching and total percent inhibition of egg development (p<0.05) than in topical application.

However, there was no significant difference (P>0.05) between the incubation periods in the two methods of application at each concentration.

Table	(5a):	Hatchability	of	Argas	arboreus	eggs	treated	topically	with	different
concen	trations	s of abamectin	at d	lay of de	eposition					

Conc. (ppm)	No. of treated	Normal hatched%	Abnormal hatched%	Unhatched% Mean±SE	Total inhibition%	Incubation period(days)
(ррш)	eggs	Mean±SE	Mean±SE	Meanist	Mean±SE	Mean±SE
Control	60	97.77±0.55 <sup>a*</sup> (96.6 – 98.3)	0±0.00 <sup>a</sup> (0)	2.22±0.55 <sup>a</sup> (1.6 -3.3)	2.22±0.55a (1.6 – 3.3)	11.33±0.66 <sup>2</sup> (11 - 12)
1	60	79.44±2.94 <sup>b</sup> (75 - 85)	2.77±0.55ab (1.6 - 3.3)	18.11±2.76 <sup>b</sup> (12.6 – 21.6)	20.89±2.62 <sup>b</sup> (16 - 25)	14±0.57ab (13 – 15)
3	60	61.1±4.00° (53.3 – 66.6)	7.22±1.47 <sup>bc</sup> (5 - 10)	31.66±4.19° (26.6 - 40)	38.89±4.00° (33.3 – 46.6)	14.6±0.66 <sup>b</sup> (13 - 16)
4	60	54.4±1.47° (51.6 – 56.6)	6.11±1.47 <sup>bc</sup> (3.3 – 8.3)	39.44±2.77 <sup>cd</sup> (36.6 - 45)	45.55±1.47° (43.3 – 48.3)	14.3±0.33 <sup>b</sup> (13 – 16)
5	60	40±1.9 <sup>d</sup> (36.6 – 43.3)	10±0.96 <sup>c</sup> (8.3 – 11.6)	50±2.54 <sup>d</sup> (45 – 53.3)	60±1.92 <sup>d</sup> (56.6 – 63.3)	15±0.57 <sup>b</sup> (14 - 16)
10	60	0±00° (0)	0±00 <sup>d</sup> (0)	100±00° (100)	100±00° (100)	-

<sup>\*</sup>Means bearing different letters (within columns) are significantly different

Conc.	No. of	Normal	Abnormal	Unhatched%	Total	Incubation
(ppm)	treated	hatched%	hatched%	Mean±SE	inhibition%	period(days)
	eggs	Mean±SE	Mean±SE		Mean±SE	Mean±SE
Control	60	97.22±0.55ª*	0±0.00ª	2.77±0.55ª	2.77±0.55ª	12±0.57ª
		(96.6 - 98.3)	(0)	(1.6 - 3.3)	(1.6 - 3.3))	(11 - 13)
1	60	67.22±0.55b	4.44±1.11 <sup>b</sup>	32±1.01 <sup>b</sup>	36.44±1.7b	14±0.57ab
		(66.6 - 68.3)	(3.3 - 6.6)	(30 – 33.3)	(33.3 – 39.3)	(13 – 15)
3	60	53.33±2.5°	8.33±0.96 <sup>cd</sup>	25±5.0b	33.33±4.19b	14.3±0.33b
		(50 - 58.3)	(6.6 - 10)	(20 - 35)	(28.3 – 41.6)	(13 - 16)
4	60	34.44±4.8d	7.77±0.55bc	57.78±4.93°	65.5±4.8c	15±0.57b
		(26.6 - 43.3)	(6.6 - 8.3)	(48.3 - 65)	(56.6 – 73.3)	(14 - 16)
5	60	18.33±0.96e	11.66±0.96d	70±1.9°	81.6±0.96 <sup>d</sup>	15.33±0.33b
		(16.6 - 20)	(10 - 13.3)	(66.6 – 73.3)	(80 – 83.3)	(15 - 17)
10	60	0±00f	0±00e	100±00d	100±00°	
		(0)	(0)	(100)	(100)	

**Table (5b):** Hatchability of *Argas arboreus* eggs treated by dipping for 20 sec. in solution with different concentrations of abamectin at day of deposition.

# 3.3. Malformations and Anomalies in Eggs and Enclosed Embryos of *Argas arboreus* Treated With Abamectin:

Topical and dipping treatment of the freshly deposited eggs with five different concentrations of abamectin caused a wide range of anomalies and malformations in the treated eggs and their embryos (Plate II). Besides the brown smooth eggs, abamectin treatment has led to occurrence of brown, slightly wrinkled eggs on 1-3 days after deposition and treatment. These latter eggs became more wrinkled, hardened and brittle while some of the smooth brown eggs imploded and collapsed at the slightest touch or became flaccid on subsequent days (5-8 days) to darken, die and unhatch on next days of the egg incubation period and up to 21 days (Plate II, Figs 6-9). Also, fully developed embryos abnormally hatched or unhatched were quite visible and partially or totally entrapped inside the transparent eggshell (Plate II, Figs. 2-4). In abnormal hatching, only mouthparts and one or more leg(s) eclosed but not the idiosoma which remained entrapped inside the eggshell (Plate II, Fig. 3&4). In rare cases curled non-motile larvae hatched to die soon within few days (Plate II, Fig. 5). In the control, almost all eggs hatched normally (table 5 a&b) with eclosion of the fully developed embryos to give active motile normal larvae (Plate II, Fig.1). However, insignificantly low percentages (2.22-2.77%) of unhatched, non-viable, smooth brown eggs were found.

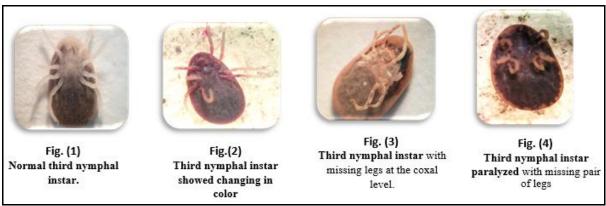
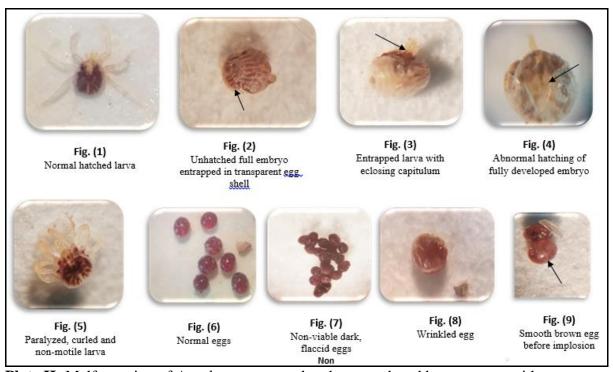


Plate I: Malformation of A. arboreus nymph produced by treatment with abamectin



**Plate II:** Malformation of *A. arboreus* egg and embryo produced by treatment with abamectin

#### **DISCUSSION**

Topical treatment of the engorged mated female, newly moulted fed 2<sup>nd</sup> nymphal instar and freshly deposited eggs of *Argas (Persicargas) arboreus* with a single dose (10µl/tick or egg batch) of different concentrations of abamectin (a main kind of AVMs) caused lethal and biological effects in the treated life stages. Abamectin application of relatively high concentrations caused slow locomotion, paralysis, prevented egg production and hatching and induced 100% mortality and unhatching of the treated adult female, 2<sup>nd</sup> stage nymphs and eggs at 25, 15 and10 ppm, respectively. Abamectin treatment at lower concentrations of 1-10ppm interfered with female reproduction, nymphal and egg development. Similarly, some effects have been reported for abamectin and other AVMs in other tick species (Campbell *et al.*, 1983; Ash and Oliver, 1989; Pereira *et al.*, 2009; Doan *et al.*, 2013). This

may contribute and explain the successful use of AVMs as acaricides (Drummond, 1985; Van Der Merwe *et al.*, 2005; Davey *et al.*, 2010; Montasser *et al.*, 2011).

Generally, the effects of abamectin on *A. arboreus* were dependent on the dose, increased by increasing concentration and dependent on the tick life stage and its physiological state. This is in agreement with effects of abamectin and other AVMs in studied ticks (Centurier and Barth, 1980; Ash and Oliver, 1989; Montasser *et al.*, 2011; Doan *et al.*, 2013).

### 1. Effect of Abamectin on Reproduction of Adult Female:

Topical treatment of mated fed adult female *A. arboreus* with a single dose (10µl/female)of abamectin at 3 different concentrations (3, 5 and 10 ppm) prolonged the preoviposition and oviposition period, reduced egg production by decreasing the mean daily and total number (fecundity) of deposited eggs/female/oviposition, decreased egg viability (% hatching) and prolonged their incubation period.

Prolongation of the preoviposition and oviposition periods suggested a delay and latency of oviposition due to probable slowing down of oocyte development, maturation (vitellogenesis) and ovulation within the ovary or the mechanical events (as muscles in the act of oviposition) involved in the oviposition of ticks. Lunke and Kaufman (1992) attributed the oviposition latency in *Amblyomma hebraeum* injected with the AVM analogue MK-243 to its probable acting on the neuro-muscular junctions of the inhibitory GABA nerve fibers (Fritz *et al.*, 1979; Holden-Dye *et al.*, 1988) supplying body muscles and in the central nervous system of this tick species (Gration *et al.*, 1986), which may result in paralyzing muscles of oviposition. However, a slowing down of oocyte development (Diehl *et al.*, 1982) and maturation (vitellogenesis) as in the AVMs treated *A. hebraeum* (Lunke and Kaufman, 1992) and most probably in *Argas* (*P.*) *arboreus* might have contributed to the observed prolongation of preoviposition and latency of oviposition.

In the present study, abamectin treatment of mated fed female Argas (P.) arboreus reduced the mean daily and total (fecundity) number of eggs/female/oviposition. The reduction of fecundity was significant (P<0.05) at all the three concentrations tested (3, 5 and 10ppm) reaching 48% at the highest concentration when the compound was applied to the female during its early vitellogenesis (3 daf.). The treatment showed the highest effect and the female was most sensitive during this period. The obtained reduction of fecundity conforms to findings on effects of other AVM (Lunke and Kaufman, 1992; Mayer et al., 1990), chitin synthesis inhibitors (CSI) and hormones in ticks (Solomon and Evans, 1978; Bakr et al., 2018). Several studies have reported the interference of abamectin and other AVMs with female fecundity and egg development and maturation (vitellogenesis) inside the ovaries of the treated ticks including Boophilus microplus (Campbell et al., 1983; Davey et al., 2010; Periera et al., 2009) and Haemaphsalis longicornis (Doan et al., 2013). However, no effect on fecundity and egg hatching of Ornithodoros parkeri were found when females had been treated with ivermectin (Ash and Oliver, 1989). Generally, inhibitory effects of AVMs on vitellogenesis caused the reduction of ovary and egg mass weight, oocyte size, and vitellin contents in addition to the reduction of the vitellogenic hormone (ecdysteroid) in the AVM treated females (Lunke and Kaufman, 1992; Doan et al., 2013). These effects need further studies on Argas arboreus.

In *Argas (P.) arboreus* and most of the aforementioned studies, the reducing effect of AVMs on tick fecundity was associated with a parallel reduction of percent hatching (viability) of the deposited eggs (i.e female fertility). However, none of the previous studies investigated effects on the eggs incubation period required for complete embryo development and hatching. In the present study, prolongation of the incubation period of eggs deposited by abamectin-treated female *A. arboreus* might point to slowing down or inhibition of embryo development or interference with its ability to hatch.

The aforementioned discussed results showed that all the biological parameters used for assessment of reproduction were decreased or increased in the abamectin treated female A. arboreus by application on the 1st (except fecundity) and 3rd daf. These effects occurred at all, higher or the highest concentration(s) of abamectin in each parameter. However, only two biological parameters including percent hatching and daily output of the deposited eggs of the treated female were affected at the highest concentration (10 ppm) by abamectin application on the 7<sup>th</sup> daf. The present findings suggested that the response of the abamectintreated female A. arboreus depended on its physiological state during application. The female was most sensitive and responding to the effects of abamectin application during early vitellogenesis on the 3<sup>rd</sup> daf (Shanbaky and Khalil, 1975), to less extent during the early previtellogenesis and the least sensitive and responding during the start of oviposition. During early oviposition most of oocytes have completed their development and did not respond. However, ovulating (Diehl et al., 1982) oocytes and some incompletely developing oocytes still were present in the ovaries due to the asynchronous nature of oocytes development in ticks (Balashov, 1972). These remaining oocytes may show response to abamectin effects at the highest concentration by reducing egg hatching and daily production. On the other hand, vitellogenesis started on the 3<sup>rd</sup> daf (Shanbaky and Khalil, 1975) is known as a period of high physiological activity of the tick ovary. During this period uptake of volk protein precursors (Helmy et al, 2018), vitellogenic hormones as ecdysteroids (Lunke and Kaufman, 1992) and other large molecules as chitin (Burgdorfer and Hayes, 1989) into the developing oocytes occur. This may facilitate entry of some other foreign materials as dyes (Diehl et al., 1982) and pathogens as Borrelia (Yousery, 2017).

## 2. Effect of Abamectin on Immature Stages of Argas arboreus:

Topical application of a single dose (10µl/tick) of abamectin induced mortality of the treated fed 2<sup>nd</sup> nymphal (N<sub>2</sub>) instar of *A. arboreus* to reach 100% at 15 ppm. Lower concentrations of 3-10 ppm abamectin interfered with immature development and adult emergence. The effects were dose-dependent, increased by increasing dose concentrations. The present results conform to findings on lethality and acaricidal effects of previously studied AVMs (Drummond, 1985; Ash and Oliver 1989 and Doan *et al.*, 2013).

In the present study, abamectin application slowed down development of *A. arboreus* by prolonging the premoulting periods of the treated N<sub>2</sub> and the resulted N<sub>3</sub> and consequently delayed adult emergence. At moulting, the abamectin treated N<sub>2</sub> produced malformations in the resulted N<sub>3</sub> which failed to give adults and contributed to the observed percent inhibition of adult emergences to reach 71.3% at 10 ppm as compared to zero % in control. Malformations of N<sub>3</sub> included paralysis, missing legs, color change and inability to moult or shed off exuvia. Paralysis, latency, and reduction of moulting success have been reported in AVMs-treated ticks as *Ornithodoros parkeri*, *O. moubata*, *Rhipicephalus evertsi*, *R. appendiculalus*, *Amblyomma herbraeum* and *Haemphysalis longicornis* (Centurier and Barth, 1980; Ash and Oliver, 1989; Soll *et al.*, 1989 and Doan *et al.*, 2013).

Generally, the inhibitory effects of AVMs on immature moulting point to interference with hormones involved in moulting. Ecdysteroids have been documented to control moulting in ticks (Diehl *et al.*, 1982; Khalil *et al.*, 1984) and their concentration was reduced in AVMs treated ticks (Centurier and Barth, 1980; Lunke and Kaufman, 1992). However, the paralyzing effect of abamectin on *A. arboreus* and other ticks might be attributed to a probable blocking of inhibitory neuromuscular junctions as was reported in other studied arthropods (Fritz *et al.*, 1979)

#### 3. Effect of Direct Application of Abamectin on Eggs of Argas arboreus:

Direct treatment of the freshly deposited eggs of A. arboreus by topical or dipping application of a single dose of different concentrations (1-10 ppm) of abamectin caused a wide range of effects and anomalies in the development of the treated eggs and their

embryos. The effects ranged from apparently early blocking of embryogenesis leading to unhatching of eggs to fully developed embryos hatched normally and abnormally or unhatched. These conform to ovicidal effects of pesticides, hormones, other compounds and adverse conditions studied in ticks (Buczek, 2000; Abdel-shafy and Zayed, 2002; Radwan et al., 2009) and insects (Slama, 1966; Shanbaky et al., 1993; Singh and Kumar, 2015). In the present study, the fully developed embryos had well defined mouthparts and segmented legs which are quite visible through the transparent eggshell as in the normal A. persicus (Mohammed, 2009), A. arboreus in the present study and Hyalomma dromedarii (El Kammah et al., 1982) and in insects (Singh and Kumar, 2015). On post-treatment of freshly deposited eggs of A. arboreus, the abamectin treated eggshell showed slight wrinkles in some brown eggs on the 1<sup>st</sup> day of deposition which has increased on subsequent days (2-3) and accompanied on next days by hardening of the egg leading to desiccation, brittleness, and unhatching. This suggested that a harmful effect of abamectin (probably on wax proofing of eggshell) started at early stage of embryonic development mostly at cleavage of nuclei and blastoderm formation during 1-3 days of egg deposition as was described in A. arboreus by Sayed (unpublished). Also, in the present study, some brown smooth eggs imploded and collapsed at the slightest touch and others became flaccid (5-8 days old) but darkened and died unhatched before full embryos were developed. Both effects suggested certain degrees of embryonic development (Sayed, unpublished) followed by death and decay but implosion might be attributed to weakness and improper chitinization of chorion (Diehl et al., 1982) where AVMs are considered as CSI in insects (Mayer et al., 1990) and other arthropods (Calcott and Fatig, 1984).

A high percentage of abamectin-treated eggs of *A. arboreus* contained fully developed embryos witch hatched normally and predominated at the lower concentrations to reach 79.44% and 67.22% at 1 ppm with the topical and dipping application, respectively. On the other hand, the percentage of the fully embryonated abnormally hatched treated eggs were much less (but significant) than normally hatched and increased at the higher concentration of treatment to reach 10 and 11.66% at 5 ppm of topical and dipping methods, respectively. Abnormal hatching was mostly partial (till death) and rarely complete eclosing non-motile curled paralyzed larvae which died soon after eclosion. As in normal, some abnormally hatched treated embryos split the eggshell along apparently weak hatching line as insects (Singh and Kumar, 2015) and eclosed their mouthparts or anterior leg but not the idiosoma. Other treated fully developed embryos remained unhatched and completely entrapped inside their transparent eggshell. The inability of the fully developed embryos to eclose might be attributed to their weakness probably due to their muscle paralysis (Martin, 1997) or poor chitinization of organs involved in hatching where AVMs have been reported as CSI in insects (Mayer *et al.*, 1990) and other arthropods (Calcott and Fatig, 1984).

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#### **ARABIC SUMMARY**

تاثير الابامكتين على تكاثر و تطور قراد الطيور ارجس (بيرسىكارجس) اربوريس (اكسوديدى:ارجسىدى)

خيرية سيد ابو طالب , نوال محمود شنبكى ,شيماء صلاح احمد , نادية حلمى قسم علم الحشرات- كلية العلوم-جامعة عين شمس- مصر

يكون للتطبيق الموضعي للجرعة المفردة(10 ميكروليتر\ للقراد او للبيض) بتركيزات مختلفة من الابامكتين (افرمكتين) اثار مميتة وبيولوجية على الاناث البالغة المتزاوجة , الاطوار غير الناضجة للارجاس (بيرسيكارجاس) اربوريس التي تسببت في وفيات 100% في جرعات 15,25,10 جزء في المليون , على التوالي . تتداخل المعالجة بمادة الابامكتين بتركيزات اقل من 10-1 جزء في المليون مع تكاثر الاناث , و تطور الحوريات و البيض . ادى تطبيق الابامكتين الموضعي(3-10 جزء في المليون) على الاناث البالغة المتزوجة في فترات مختلفة من دورتة الانجابية الي تقليل خصوبة الانثي و تقليل حيوية البيض , مما ادى الى اطالة فترة ما قبل الاباضة , و الاباضة و فترة حضانة البيض . علاج الابامكتين في مرحلة الطور الثاني للحوريات ادى الى تاخر في الانسلاخ للطور الثالث و تقليل ظهور الكبار. كما تسبب في الشلل و التشوهات المختلفة (6.15-8.28%) كارجل مفقودة , تغيرات في اللون و فشل في فصل جلد الانسلاخ مقديم في الطور الثالث الناتج. كان علاج البيض (60 بيضة) عن طريق الغمس البيض حديث الوضع لمدة 20 ثانية في محلول مائي من1-5 جزء في المليون من الابامكتين اكثر فاعلية في الحد من الفقس و زيادة التشوهان عن العلاج الموضعي . و مع ذلك , فان كلا طرق المعالجة تطيل فترات الحضانة للبيض.