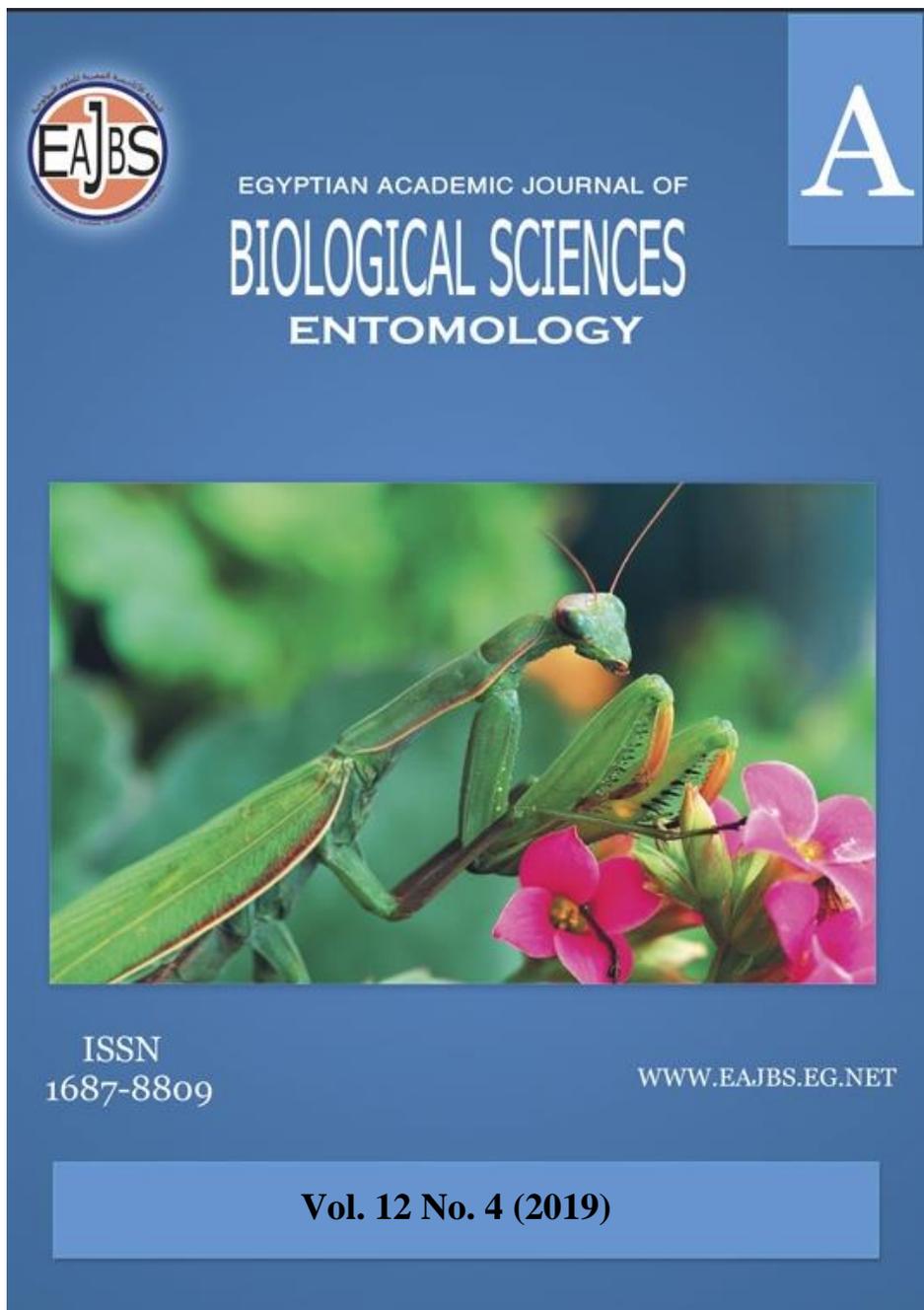


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**Deteriorated Adult Performance and Reproduction of the Greater Wax Moth, (*Galleria mellonella* (Lepidoptera: Pyralidae) by the Honey Bee Apitoxin.**

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**ARTICLE INFO**

**Article History**

Received:2/7/2019

Accepted:3/8/2019

**Keywords:**

incubation,  
emergence,  
fecundity, fertility,  
Melittin, mortality,  
oviposition, sterility,  
survival

**ABSTRACT**

The greater wax moth, *Galleria mellonella* is an important pest of wax combs of the honey bee in the world. The current investigation aimed to assess the effects of Apitoxin on the most important adult life parameters and reproduction of this pest. The freshly ecdysed 3<sup>rd</sup> instar larvae were treated with a series of Apitoxin concentrations (4000, 2000, 1000, 500, 250, 125 ppm) via the artificial diet. The present results can be summarized as follows. The adult emergence was slightly blocked only at the higher three concentration levels. Apitoxin failed to affect both adult survival and morphogenesis. The total longevity was significantly shortened, in a dose-dependent course. The ovarian maturation (pre-oviposition) period was prolonged, except the highest one at which this period was slightly shortened, as an odd datum. The reproductive life-time (oviposition period) was remarkably shortened, in no certain trend. Also, the post-oviposition period was considerably shortened. Apitoxin exerted a diverse action on the reproductive efficiency of adult females since the oviposition rate was enhanced or inhibited, depending on the concentration, but in no certain trend. Both fecundity and fertility had been significantly reduced, in a dose-dependent course. The incubation period was considerably prolonged in a dose-dependent manner.

**INTRODUCTION**

Some authors (Gulati and Kaushik, 2004; Sarwar, 2016; Rahman *et al.*, 2017) reported various aspects of the most common enemies of honeybee *Apis mellifera* (Hymenoptera: Apidae), described their nature of damage and provided a clear indication of control measures to protect hive and hive products. The greater wax moth *Galleria mellonella* L. (Lepidoptera: Pyralidae) is the most destructive pest of honey bees throughout the world (Nurullahoglu and Susurluk, 2001; Viraktamath, 2010). Larvae feed on the wax comb in weak colonies or during the storage of wax combs in winter (Charriere and Imdorf, 1997; Caron, 1999). In general, larvae of the greater wax moth cause considerable damage to beeswax combs left unattended by bees (Van Engelsdorp *et al.*, 2010) and cause economic loss to the beekeeping industry all over the world because they reduce the wax combs to a pile of debris, wax moth frass, and webbing (Shimanuki *et al.*, 1992; Ellis *et al.*, 2013). The voracious nature of the larva leads to the destruction of the honeycomb and then the death of

weak colonies (Elbehery *et al.*, 2016). Besides damaging wax combs and destroying frames and wooden parts in the hive, adult and larvae of *G. mellonella* can transfer pathogens of serious bee diseases, e.g. the bacterial disease foulbrood (Charrière and Imdorf, 1997; Owayss and Abd-Elgayed, 2007).

For the control of *G. mellonella*, various chemical and non-chemical methods have been adopted, including freezing, heating and CO<sub>2</sub> and sulphur fumigation against larvae and pupae (Ahmed *et al.*, 1993; Calderone, 2000; Owayss and Abd-Elgayed, 2007). Also, gamma irradiation has been investigated to control this pest *via* sterilization of the pharate adults (Carpenter *et al.*, 2005; Jafari *et al.*, 2010). In addition, several biological control agents, such as entomopathogenic nematodes, viruses and fungi, along with the natural enemies of predators and parasites, have been used (Dindo *et al.*, 2001; Armendariz *et al.*, 2002; Hussaini, 2003; Ellis *et al.*, 2013). However, the majority of physical, chemical and biological control measures seemed to be ineffective for satisfactory control of different stages of *G. mellonella* (Fraser, 1997; Coskun *et al.*, 2006; Owayss and Abd-Elgayed, 2007). In addition to these drawbacks, the extensive use of synthetic insecticides causes a biological imbalance in the ecosystem. Therefore, the natural products are an excellent alternative as a means to reduce negative impacts on human health and the environment (Ahn *et al.*, 1997; Koul *et al.*, 2008). Botanical pesticides are usually safer to humans and the environment than conventional pesticides, and have minimal residual effects (Pavela, 2009). Natural products of the plant origin have been widely assessed against *G. mellonella* (Swamy *et al.*, 2006; Izhar-ul-Haq *et al.*, 2008; Sankar *et al.*, 2009; Núñez, 2011; Basedow *et al.*, 2012; Ünsal and Güner, 2016; Elbehery *et al.*, 2016; Er *et al.*, 2017). Recently, natural products of the animal origin have been described as very good alternative agents for controlling *G. mellonella*, such as venomous insects (Dahlman *et al.*, 2003), scorpions (Froy *et al.*, 2000; Taniai *et al.*, 2002), spiders (Harrison and Bonning, 2000; Tedford *et al.*, 2004; Nicholson, 2006) and some marine animals (Olivera, 2002) as well as arthropod hormones and neuropeptides (Altstein *et al.*, 2000; Altstein, 2004).

Honey bee workers and queen produce the venom in a special branched acid gland at the end of their abdomen. This venom or toxin can be called Apitoxin (Molecular Formula: C<sub>129</sub>H<sub>224</sub>N<sub>38</sub>O<sub>31</sub>). The word was originated from the Latin *apis* (bee) and *toxikon* (venom) (Cruz-Landim and Abdalla, 2002). It is characterized as being clear, colorless, and highly soluble in water (Peiren *et al.*, 2008). Apitoxin contains a complex mixture of proteins, peptides, and low molecular components. The main active constituent is melittin, which has relatively low toxicity (Bogdanov, 2017). In a recent review, Azam *et al.* (2018) compiled information on the history, chemical composition and scientific evidence concerning the honey bee Apitoxin pharmaceutical research and different medical uses. Very recently, Ghoneim *et al.* (2019) examined the toxicity and disruptive effects of growth and development of *G. mellonella*. The objective of the current investigation was to assess the inhibitory impacts of Apitoxin on different parameters of the adult performance and reproduction of *G. mellonella*.

## MATERIALS AND METHODS

### Experimental Insect:

A culture of the greater wax moth, or honeycomb moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) was maintained in the laboratory of Entomology, Faculty of Science, Al-Azhar University, Cairo under controlled conditions (27±2°C, 65±5% R.H., photoperiod 14 h L and 10 h D). The culture was originated by a sample of larvae kindly obtained from the Plant Protection Unit, Desert Research Centre, Cairo, Egypt. Larvae were transferred into glass containers, tightly covered with muslin cloth secured with rubber bands.

After reviewing different techniques of the artificial diet described by some authors (Metwally *et al.*, 2012; Nitin *et al.*, 2012), *G. mellonella* larvae in the present culture had been provided with an artificial diet as described by Bhatnagar and Bareth, (2004). It contained maize flour (400 g), wheat flour, wheat bran and milk powder, 200 g of each. Also, the diet was provided with glycerol (400g), bee honey (400g), yeast (100g). The resulting pupae were then collected and transferred into clean jars provided with a layer of moistened saw dust on the bottom. The emerged adult moths were kept in glass containers provided with white paper scraps, as oviposition sites. After mating, female moths were allowed to lay eggs. The egg patches were collected daily and transferred into Petri dishes containing a layer of an artificial diet for feeding of the hatching larvae.

### **Collection of Apitoxin from Honey Bee Workers:**

The electric shock method was used to collect the bee venom from six honey bee hives. According to Dantas *et al.* (2013), bee venom was extracted using a collector composed of plates and a pulse generator, which induces the bees to sting the electric collector plate resting on a glass plate. Volatile phase of the venom evaporates onto the glass plate, from where the Apitoxin is then collected by scraping.

### **Preparation of Concentrations And Larval Treatment:**

A series of concentration levels of Apitoxin was prepared by diluting with distilled water in volumetric flasks as follows: 4000, 2000, 1000, 500, 250, 125 ppm. Bioassay test was carried out using the newly moulted 3<sup>rd</sup> instar larvae. Ten grams of diet were mixed with 2ml of each concentration of Apitoxin before introduction to larvae, as a food. Control larvae were provided with a water-treated diet. Ten replicates of treated and control larvae (one larva/replicate) were kept separately in glass vials under the aforementioned laboratory conditions. The larvae were allowed to feed on this treated diet along the larval stage. All biological criteria were recorded daily after the first 24 hrs feeding.

### **Criteria of Study:**

#### **1. Parameters of Adult Performance:**

**Adult Emergence:** Number of successfully metamorphosed adults was expressed in % according to Jimenez-Peydro *et al.* (1995) as follows:

$[\text{No. of completely emerged adults} / \text{No. of pupae}] \times 100$

**Adulticidal Activity:** The adulticidal activity of Apitoxin was determined by observed adult mortality.

**Adult Longevity:** The most important compartments of the total longevity adult females were measured in days $\pm$ SD: pre-oviposition (ovarian maturation period), oviposition period (reproductive life-time) and post-oviposition period.

#### **2. Reproductive Potential Criteria:**

The emerged adult moths were kept separately in glass jars (3 L) provided with white paper scraps, as oviposition sites. Each adult female was coupled with normal adult males (1:2) of the same age obtained from the main culture. After mating, female moths were allowed to lay eggs. The egg patches were collected daily and carefully transferred into Petri dishes to count eggs.

#### **Oviposition Efficiency:**

Oviposition efficiency would be indicated by the oviposition rate which was calculated as follows:

Number of laid eggs per ♀/reproductive lifetime (in days).

#### **Reproductive Capacity:**

**Fecundity:** the laid eggs were counted for calculating the number of eggs per female.

**Fertility:** the hatchability was usually expressed in the hatching percentage of laid eggs.

**Sterility index:** It was calculated according to Topozada *et al.* (1966) as follows:

$$\text{Sterility Index} = 100 - [(a/b / A/B) \times 100]$$

Where: a: mean number of eggs laid per female in the treatment. b: percentage of hatching in the treatment. A: mean number of eggs laid per female in the controls. B: percentage of hatching in the controls.

### **Embryonic Development.**

The embryonic developmental rate can be indicated by the incubation period of eggs. The laid eggs were kept in Petri dishes under the same controlled laboratory conditions as previously mentioned. Just after the oviposition, eggs were observed until hatching to estimate the incubation period (in days $\pm$ SD).

### **Statistical Analysis of Data:**

Data obtained were analyzed by the Student's *t*-distribution, and refined by Bessel correction (Moroney, 1956) for the test significance of the difference between means.

## **RESULTS**

### **Effects of Apitoxin on the Adult Performance of *G. mellonella*:**

After treatment of 3<sup>rd</sup> instar larvae of *G. mellonella* with six concentration levels of Apitoxin (4000, 2000, 1000, 500, 250 & 125 ppm), *via* the artificial diet, data of the most important parameters of adult female performance had been arranged in Table (1). Depending on these data, the adult emergence was slightly blocked only at the higher three concentration levels (83.3, 75.0 & 75.0% emergence, at 1000, 2000 & 4000 ppm, respectively, *vs.* 100% emergence of control adults). Thus, Apitoxin failed to hinder this vital metamorphosis process at other concentration levels.

Also, Apitoxin failed to affect the adult survival because no adult mortality was observed. In addition, the tested product failed to impair the adult morphogenesis because no malformed adult had been produced.

With regard to the adult longevity and its main compartments (pre-oviposition period, oviposition period and post-oviposition period), data assorted in the same table revealed that the total longevity was significantly shortened, in a dose-dependent course (11.05 $\pm$ 0.6, 11.00 $\pm$ 1.2, 10.91 $\pm$ 0.5, 10.91 $\pm$ 1.1, 10.90 $\pm$ 1.1 and 08.82 $\pm$ 1.0 days, at 125, 250, 500, 1000, 2000 and 4000 ppm, respectively, *vs.* 11.50 $\pm$ 0.1 days of control adult females).

The ovarian maturation (pre-oviposition) period was significantly or insignificantly prolonged at all concentration levels of Apitoxin, except the highest one at which this period was slightly shortened, as an odd datum. This delaying action of Apitoxin on the ovarian maturation was reversely correlated with its concentration (4.00 $\pm$ 0.1, 4.00 $\pm$ 0.2, 3.33 $\pm$ 0.5, 3.10 $\pm$ 0.1 and 2.60 $\pm$ 0.1 days, at 125, 250, 500, 1000 and 2000 ppm, respectively, *vs.* 2.50 $\pm$ 0.8 days of control females).

As clearly shown in the previously mentioned table, the reproductive life-time (oviposition period) was remarkably shortened, in no certain trend (5.20 $\pm$ 1.0, 6.11 $\pm$ 0.8, 5.40 $\pm$ 1.3, 5.50 $\pm$ 0.8, 5.60 $\pm$ 0.9 and 4.55 $\pm$ 1.1 days, at 125, 250, 500, 1000, 2000 and 4000 ppm, respectively, compared to 6.65 $\pm$ 1.0 days of the control congeners). Also, the post-oviposition period was considerably shortened (for detail, see table 1).

### **Effects of Apitoxin on the Reproductive Potential of *G. mellonella*:**

After treatment of 3<sup>rd</sup> instar larvae of *G. mellonella* with Apitoxin, data of the most important reproductive criteria had been assorted in Table (2). Depending on these data, Apitoxin exerted a diverse action on the oviposition efficiency of adult females, since the oviposition rate was enhanced or inhibited, depending on the concentration, but in no certain trend.

With regard to the reproductive capacity, fecundity (mean number of eggs/ $\text{♀}$ ) was significantly reduced, proportional to the concentration level (286.8 $\pm$ 35.5, 277.6 $\pm$ 66.3,

271.1±72.2, 260.6±52.0, 255.5±43.1 and 251.2±55.2 eggs/♀, at 125, 250, 500, 1000, 2000 and 4000 ppm, respectively, vs. 305.2±32.1 eggs/ control females). Another informative parameter of the reproductive capacity is fertility (hatching % of laid eggs). As easily seen in Table (2), fertility was dramatically reduced, in a dose-dependent course. In other words, the sterility considerably increased as the Apitoxin concentration was increased (35.27, 40.47, 41.45, 48.05, 53.99 and 54.99%, at 125, 250, 500, 1000, 2000 and 4000 ppm, respectively). In insects, the incubation period of the laid eggs is usually used as a good indicator of the embryonic developmental rate, i.e., the shorter period denotes a faster rate and *vice versa*. Depending on data of Table (2), the embryonic development was severely retarded as an effect of Apitoxin, since the incubation period was considerably prolonged in a dose-dependent manner (09.10±0.3, 09.18±0.5, 11.11±1.1, 13.00±5.2, 15.38±1.2 and 15.43±0.5 days, at 125, 250, 500, 1000, 2000 and 4000 ppm, respectively, vs. 08.20±1.0 days of eggs laid by control females).

**Table (1): Effects of the *A. mellifera* Apitoxin on adult performance parameters of *G. mellonella***

Conc. (ppm)	Adult emergence (%)	Adult mortality (%)	Duration (Mean days±SD)			
			Ovarian maturation period	Reproductive life-time	Post-oviposition period	Total Longevity
4000	75.0	0	1.99±0.11 a	4.55±1.1 c	2.00±1.2 a	08.82±1.0 d
2000	75.0	0	2.6±0.10 a	5.6±0.9 a	2.3±0.6 a	10.91±1.1 b
1000	83.3	0	3.1±0.11 a	5.5±0.8 b	1.99±0.2 b	10.93±0.5 c
500	100	0	3.33±0.5 b	5.40±1.3 b	2.00±0.8 a	10.91±0.8 b
250	100	0	4.00±0.2 d	6.11±0.8 a	2.00±0.8 a	11.00±1.2 a
125	100	0	4.00±0.1 d	5.2±1.0 c	1.5±0.5 d	11.05±0.6 b
control	100	0	2.5±0.84	6.66±1.0	2.50±0.4	11.50±0.1

Conc.: concentration level, Mean±SD followed with (a): insignificantly different ( $P > 0.05$ ). (b): significantly different ( $P < 0.05$ ). (c): highly significantly different ( $P < 0.01$ ). (d): very highly significantly different ( $P < 0.001$ ).

**Table (2): Effects of the *A. mellifera* Apitoxin on oviposition efficiency and reproductive capacity of *G. mellonella*.**

Conc. (ppm)	Oviposition rate (Mean±SD)	Reproductive capacity			Incubation period (Mean days ±SD)
		Fecundity (Mean eggs ±SD)	Fertility (%)	Sterility index (%)	
4000	55.21±1.3 d	251.2±55.2 c	50.33±0.7 d	54.99	15.43±0.5 d
2000	45.63±2.5 a	255.5±43.1 b	50.58±1.2 d	53.99	15.38±1.2 d
1000	47.38±5.11 a	260.6±52.0 a	55.99±0.6 d	48.05	13.00±5.2 b
500	50.20±3.8 b	271.1±72.2 a	60.66±2.5 d	41.45	11.11±1.1 d
250	45.43±0.29 d	277.6±66.3 a	60.23±0.8 d	40.47	09.18±0.5 d
125	55.15±0.63 d	286.8±35.5 a	63.39±0.3 d	35.27	09.10±0.3 b
control	46.69±0.4	305.2±32.1	90.31±0.2	---	08.20±1.0

Conc.: concentration level, a, b, c, d: See footnote of Table (1).

## DISCUSSION

### Disturbance of the Adult Performance of *G. mellonella* by Apitoxin:

#### 1. Blocked Adult Emergence:

It is important to emphasize that the adult emergence in insects is a crucial physiological process and regulated by the eclosion hormone. Disturbance of this hormone partially or completely arrest the adults to emerge (Josephraj Kumar *et al.*, 1999). After treatment of 3<sup>rd</sup> instar larvae of *G. mellonella* with Apitoxin, in the present study, the adult emergence was slightly blocked at the higher three concentrations. This result can be interpreted by the interference of Apitoxin with some aspects of the hormonal regulation of adult emergence, such as disturbance of release of adult eclosion hormone and/or inhibition of the neurosecretion (prothoracicotropic hormone)(Al-Sharook *et al.*, 1991). On the molecular basis, Apitoxin may cause misexpression of certain genes, particularly the *brood* complex (*br-C*) transcription factor gene, leading to blocking of the adult emergence (Wilson, 2004; Nandi and Chakravarty, 2011).

#### 2. Unaffected Adult Survival and Morphogenesis:

The honey bee venom was assessed against adults of the granary weevil *Sitophilus granaries* by Nassar (2013). He observed higher and lower mortalities (94.3 and 20.2%) after 72 hr of adult treatment with the doses 6.3 and 1.1µg/insect, respectively. The current investigation was inconsistent with this reported result since Apitoxin failed to exhibit an insecticidal activity against the adults of *G. mellonella*. Moreover, it failed to impair their morphogenesis.

#### 3. Disturbed Adult Longevity:

##### *Shortened Total Adult Longevity:*

After the attainment of sexual maturity, insects often show degenerative changes in some tissues and organs which can be called 'senility' or 'aging'. In insects, the affected adult longevity can be considered as an informative indicator for the adult aging, i.e., shortening of adult longevity may denote an acceleration of aging and *vice versa*, although the death is usually the destiny of all creatures (Ghoneim and Al-keridis, 2019). After treatment of 3<sup>rd</sup> instar larvae of *G. mellonella* with Apitoxin, in the current study, the successfully emerged adult females had significantly shortened total longevity, in a dose-dependent course. This may indicate a general accelerating action of Apitoxin on the adult senility ending in death. In some detail, this shortened longevity of *G. mellonella* adult females may be attributed to the effect of Apitoxin on a hormonal activity because there is a close relation between certain hormones, such as peptide hormone, lipophilic hormones and bioactive amines, and adult longevity, as reported for *Drosophila* (Simon *et al.*, 2003; Broughton *et al.*, 2005; Carbone *et al.*, 2006). In that fly, at least one of the insulin-linked peptides expressed in the median neurosecretory cells (which produce prothoracicotropic hormone) is likely to contribute to the endocrine regulation of longevity (Toivonen and Partridge, 2009). As reported by Yamamoto *et al.* (2013), JH controls aging, to some extent, because it directly affects mechanisms of somatic survival. Therefore, Apitoxin might affect the JH level and/or functions leading to the shortening of adult longevity of *G. mellonella*, in the present study. Also, shortened longevity of *G. mellonella* in the current investigation can be interpreted by the accumulation of xenobiotics (Apitoxin residues) in the adult body which upsets a complicated balance of factors such as absorption, excretion and detoxification (Abdel-Aal, 1996). In insects, the fat body serves many vital functions (Arrese and Soulages, 2010) and it is therefore not surprising that longevity mechanisms occur within the fat body (Hwangbo *et al.* 2004). Thus, Apitoxin might adversely affect the fat bodies resulting in shortened longevity of *G. mellonella* adults, in the current investigation. However, the exact mode of

action of Apitoxin on the biochemical sites in adults of *G. mellonella* is unknown until now!

**Retarded Ovarian Maturation:**

In the present study, the pre-oviposition period of the successfully emerged adult females of *G. mellonella* was prolonged after larval treatment with Apitoxin. This indicated a delaying action of Apitoxin on the ovarian maturation but in a reverse correlation with its concentration. In some detail, many lepidopterous species have a relatively short, non-feeding adult stage, such as the present insect *G. mellonella*, which requires the adult female to emerge with most of her eggs ready to be fertilized and oviposited within hours. This lifestyle constrains these insects to a program of ovarian organogenesis and follicle development that must occur at stages earlier than in other insects (Ghoneim and Al-keridis, 2019). In the light of this information, the retarding effect of Apitoxin on the ovarian maturation in *G. mellonella*, in the present study, may be understood by influenced germ band or the number of germ cells formed in the embryo (Hodin and Riddiford, 1998). However, the exact mode of retarding action is unfortunately available right now but the interference of the tested product with the hormonal regulation needs further investigation in the future.

**Reduced Reproductive Life-Time:**

As recorded in the present study, treatment of 3<sup>rd</sup> instar larvae of *G. mellonella* with Apitoxin resulted in successfully emerged adult females which laid their eggs during remarkably shortened oviposition period (or reproductive life-time), in no certain trend. On the basis of this result, the present natural product exerted tremendously enforcing actions on the ovipositing females to lay eggs quickly. This accelerated oviposition may be attributed to a physiological behaviour of the ovipositing adult females to avoid a long time interval under stress of the tested product as a xenobiotic factor (Tanani and Ghoneim, 2018).

**Prolonged Post-Oviposition Period:**

After treatment of 3<sup>rd</sup> instar larvae of *G. mellonella* with Apitoxin, in the present study, the post-oviposition period of the successfully emerged adult females was considerably shortened. Unfortunately, there is no acceptable interpretation for this result right now!!

**4. Disrupted Reproductive Potential of *G. mellonella* by Apitoxin:**

Reproduction in insects is mainly controlled by corpus allatum hormone (juvenile hormone, JH), which is also responsible for protein metabolism, and is specifically needed for egg maturation (Ghoneim *et al.*, 2014). On the other hand, ecdysteroids have essential functions in controlling the processes involved in insect reproduction, i.e., vitellogenesis, ovulation of matured eggs and spermatocyte growth (Wigglesworth, 1984; Hagedorn, 1985).

**4.1. Influenced Oviposition Efficiency:**

After treatment of 3<sup>rd</sup> instar larvae of *G. mellonella* with Apitoxin, in the current investigation, this bee product exerted a diverse action on the oviposition efficiency of adult females, since the oviposition rate was enhanced or prohibited, depending on the concentration, but in no certain trend. However, the prohibited oviposition efficiency, in the current study, may be explained as a result of inhibition of ovarian DNA synthesis or the interference of Apitoxin with vitellogenesis in *G. mellonella* via certain biochemical processes. In addition, Apitoxin might exert a reverse action to those exerted by the ecdysteroids which stimulate the neurosecretory cells to release a myotropic ovulation hormone (Parween *et al.*, 2001).

**4.2. Perturbation of Reproductive Capacity:**

The reproductive capacity of an insect can be detected by two major parameters: fecundity (mean number of eggs/female) and fertility (egg hatching % or egg viability).

**Fecundity:**

After treatment of 3<sup>rd</sup> instar larvae of *G. mellonella* with Apitoxin, in the current study, fecundity of the successfully emerged and oviposited adult females was drastically

prohibited, proportional to the concentration level. This prohibition of fecundity might be due to the interference of this bee product with one or more processes, from the ovarian follicle development to egg maturation. In some detail, Apitoxin might cause some disorders in the ovaries, including cell death in the germarium, resorption of oocytes in the pre-vitellarium and vitellarium, formation of vitellin envelopes and undue proliferation of follicle cells sometimes resulting in malformation of the whole ovary (Lucantoni *et al.*, 2006; Khan *et al.*, 2007). It might inhibit the development of some ovarioles and/or synthesis and metabolism of proteinaceous constituents during the oogenesis (Salem *et al.*, 1997). It might exert an inhibitory action on the ecdysone activity, the threshold of which is essential for the normal oogenesis (Terashima *et al.*, 2005). On the basis of hormonal regulation of insect reproduction, Apitoxin might disturb the production and/or function of the gonadotropic hormone (JH) responsible for the synthesis of vitellogenins (yolk precursors) and vitellogenesis (Di Ilio *et al.*, 1999). Eggs might develop normally in ovaries, but they could not be lay, owing to the adversely deformed ovipositor of adult females or to the reduced mechanical strength (Moreno *et al.*, 1994) or their reabsorption before oviposition (Zhou *et al.*, 2016).

#### **Fertility:**

After treatment of 3<sup>rd</sup> instar larvae of *G. mellonella* with Apitoxin, in the current investigation, the fertility of the successfully emerged and mated adult females was dramatically reduced, in a dose-dependent course. In other words, the sterility considerably increased as the Apitoxin concentration was increased. This result may be in agreement with that result reported by Mahgoub *et al.* (2018) who treated the newly deposited eggs of the lesser wax moth *Achroia grisella* with the honey bee venom and recorded a significant reduction of the egg hatchability.

For explicating the fertility reduction in *G. mellonella* by Apitoxin, in the present study, some suggestions can be provided herein. Maturation of the insect eggs depends basically on the vitellogenins, precursor materials of vitellins, including proteins, lipids and carbohydrates, all of which are necessarily required for the embryonic development (Soltani and Mazouni, 1992; Chapman, 1998). These materials are synthesized primarily by fat body during the immature stages (Telfer, 2009) or by the ovary *in situ* (Indrasith *et al.*, 1988). The tested bee product might disturb the production and/or accumulation of these materials in adult females of *G. mellonella* leading to a reduction of fertility. Apitoxin might indirectly affect the fertility *via* the disruption of opening of the intracellular spaces in follicular epithelium or generally inhibition the role of JH (gonadotropic hormone) responsible for the regulation of vitellogenesis (Davey and Gordon, 1996). The fertility reduction might be due to the penetration of residual amounts of Apitoxin into *G. mellonella* eggs and disturbance of embryonic cuticle synthesis. So, the fully mature embryos had weakened chitinous mouth parts incapable to perforate the surrounding vitellin membrane for the hatching process (Sallam, 1999; Sammour *et al.*, 2008).

#### **Affected Embryonic Development:**

In insects, the incubation period of the laid eggs is usually used as a good indicator of the embryonic developmental rate, i.e., the shorter period denotes a faster rate and *vice versa*. After treatment of 3<sup>rd</sup> instar larvae of *G. mellonella* with Apitoxin, in the current investigation, the embryonic development in eggs laid by successfully emerged and mated adult females was severely retarded since the incubation period was considerably prolonged in a dose-dependent manner. This retarded embryonic development in *G. mellonella* might be due to their effects of Apitoxin on ecdysteroids responsible for the regulation of embryogenesis at certain stages, especially those originating from the ovary *in situ* (Chapman, 1998).

Finally, it is important to mention that Apitoxin contains a complex mixture of proteins,

peptides, and low molecular components. The chief components are apamin, melittin and phospholipase A2 (Quistad *et al.*, 1988) while some authors (Mazdak *et al.*, 2004; Bogdanov, 2017) reported that the main active constituent is melittin. Therefore, all effects of Apitoxin, in the present study should be attributed to melittin.

### Conclusion:

In view of the present results for *G. mellonella*, Apitoxin blocked the adult emergence. Total longevity and oviposition period had been significantly shortened, ovarian maturation period and post-oviposition period had been prolonged. Also, the oviposition rate was affected and both fecundity and fertility had been significantly reduced. The embryonic developmental rate was considerably retarded. Therefore, Apitoxin can be used as an effective control agent in the management program against the present pest.

### Acknowledgement:

The authors would like to acknowledge Dr. Saber Riad, Faculty of Science, Al-Azhar University, Cairo, Egypt, for the technical support in order to extract Apitoxin from honey bee workers.

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