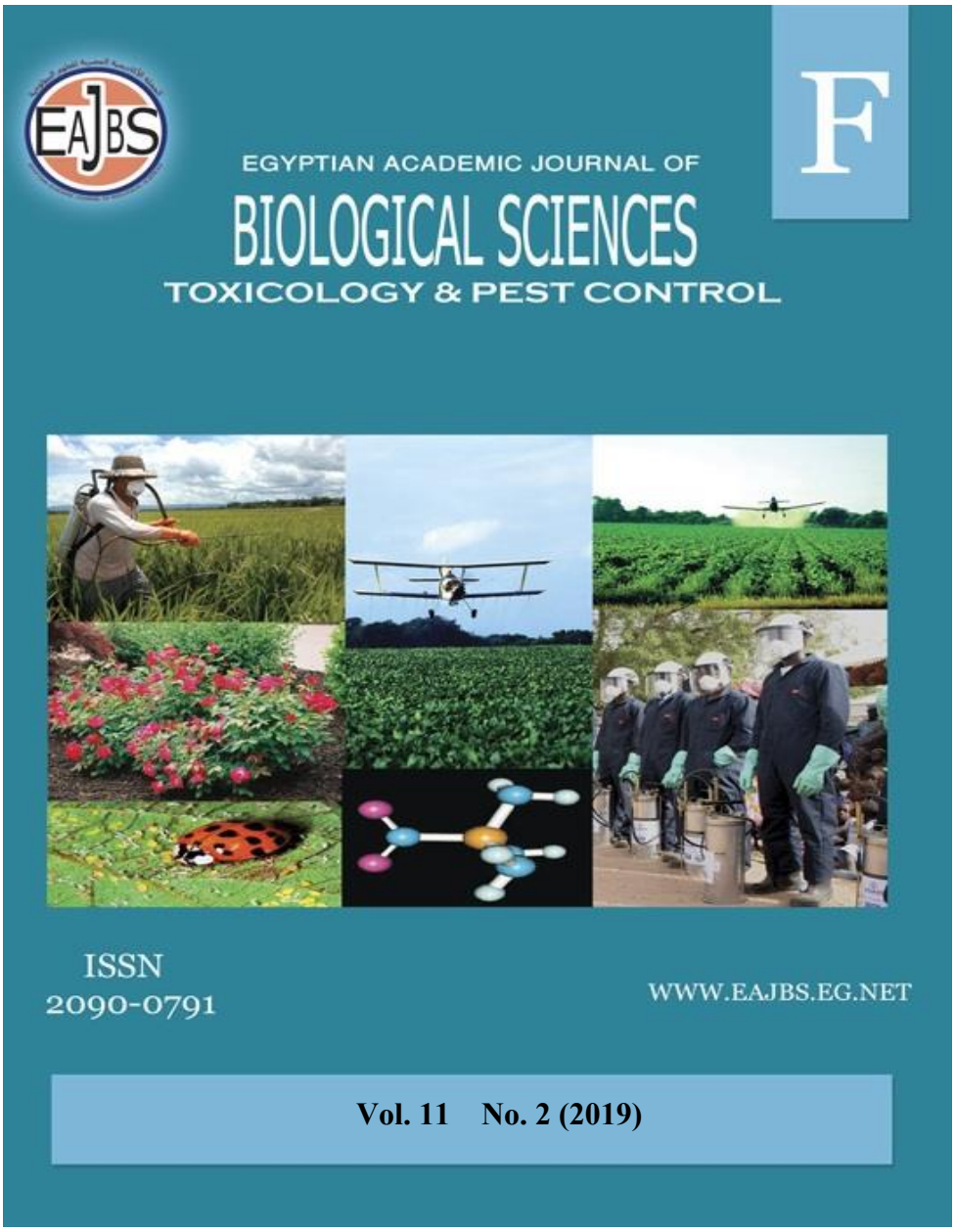

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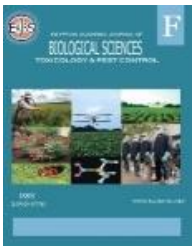

Toxicity and Disruptive Impacts of the Honeybee Apitoxin on Growth and Development of The Greater Wax Moth, *Galleria mellonella* (Lepidoptera: Pyralidae).

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ABSTRACT

The greater wax moth, or honeycomb moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) is widely distributed throughout the world. It is an economically important pest of wax combs of the honey bee. The present study was conducted aiming at the evaluation of toxicity and disruptive impacts of Apitoxin on growth, development and metamorphosis of this pest. The newly moulted 3rd instar larvae were fed on artificial diet treated with a series of Apitoxin concentrations (4000, 2000, 1000, 500, 250, 125 ppm). The present results can be summarized as follows. Apitoxin exhibited a dose-dependent toxicity on larvae except at the lowest concentration. The developed pupae suffered a toxic action of the tested product, in a dose-dependent course, except at the lower two concentrations. LC_{50} value was calculated in 956.16 ppm. The somatic weight gain and growth rate had been slightly reduced while the larval duration was considerably shortened, in a dose-dependent course. The developmental rate of larvae indicated an enhancing action of Apitoxin, since the treated larvae developed in a faster rate than control congeners. The pupal duration was slightly or remarkably shortened, depending on the concentration. The developed pupae from treated larvae lost more body water than control pupae. The tested product failed to affect metamorphosis and morphogenesis programs, but the pupation rate was regressed in a dose-dependent course.

INTRODUCTION

The honey bee *Apis mellifica* has been attacked by many pests, such as *Varroa* mites, wax moths (greater and lesser), small hive beetles, *Vespa* hornets and parasitic flies. These enemies cause serious damages in colonies and hives (Core *et al*., 2012; Dietemann *et al*., 2013; Monceau *et al.*, 2014; and Neumann *et al*., 2016). The greater wax moth, or honeycomb moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) is widely distributed throughout the world. Its presumed native range includes Europe and adjacent Eurasia, and was introduced into other continents, including North America and Australia (Savela, 2009). The biology of this moth has been well studied, and it can complete their development on different types of food (Coskun *et al*., 2006; Abou-Shaara, 2017a).

G. mellonella is an economically important pest of wax comb of honey bee because of the feeding habits of larvae and tunnelling through the combs in temperate, tropical and subtropical beekeeping regions, since the warm temperature enhances the rapid development of this moth (Jackman and Drees 1998; Chandel *et al*., 2003; Mohamed *et al*., 2014). Although the adults do not feed, because they have atrophied mouth parts, the voracious nature of larval feeding leads to the destruction of the honeycomb, and subsequent to the death of weak colonies (Awasthi and Sharma, 2013; Ellis *et al*., 2013; Kwadha *et al*., 2017).

There are different mechanical and chemical methods to control *G. mellonella*, outside the hives (Ellis *et al*., 2013), but inside the hives, the control measures are very limited and mainly depend on boosting the strength of colonies, or using specific traps (Abou-Shaara, 2017b). To protect hive products in different countries, different chemical agents have been used, such as sulphurous gas, acetic acid, methyl bromide, ethylene dibromide, calcium cyanide and phosphine (Burges, 1981; Ben Hamida, 1999). Also, biological control agents have been used, such as predators, parasitoids, pathogens, and genetic manipulations (releasing sterile insects)(Cantwell and Shieh, 1981; Dougherty *et al*., 1982). In addition, hormone analogues, insect growth regulators, plant growth regulators and oviposition attractants have been used inside the hive (Hussein, 1983). Recently, an important interest of investigation by agrochemical companies in the development of highly selective bio-pesticides derived from animals, 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 long and thin branched acid gland at the end of their abdomen. This venom or toxin can be called Apitoxin (Molecular Formula: $C_{129}H_{224}N_{38}O_{31}$). The word was linguistically originated from the Latin *apis* (bee) and *toxikon* (venom) (Cruz-Landim and Abdalla, 2002). It is characterized as being clear, colourless, and highly soluble in water (Peiren *et al*., 2008). Apitoxin is a complex mixture of proteins, peptides, and low molecular components. The main active constituent is melittin (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 pharmaceutic research and different medical uses.

Piek (1987) suggested that the toxins of *A. mellifera* venom attracted a great interest of research aiming at the development of new models of bio-insecticides. This venom had been studied for its chemical features as well as its action on mammals although little is known about its action on insects (Quistad *et al*., 1988). The present study was conducted aiming at the evaluation of toxicity and disruptive impacts of Apitoxin on growth, development and metamorphosis 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, Egypt under controlled conditions (27±2^{o}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 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 sawdust 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 3rd instar larvae. Ten grams of the 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. Toxicity and Lethal Effects:

All mortalities of treated and control (larvae, pupae and adults) were recorded every day and corrected according to Abbott's formula (Abbott, 1925) as follows:

$$\% \text{ corrected mortality} = \frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \text{ X } 100$$

The LC_{50} was calculated for general mortality by Microsoft® office Excel (2007), according to Finny (1971).

2. Growth, Development and Metamorphosis::

Weight gain: Each individual larva (treated and control) was carefully weighed every day using a digital balance for calculating the body weight gain as follows:

Initial weight (before the beginning of the experiment) - final weight (at the end of the experiment).

Growth Rate: Growth rate was calculated according to (Waldbauer, **1968**) as follows:

GR = fresh weight gain during feeding period/feeding period X mean fresh bodyweight of larvae during the feeding period.

Developmental Rate: Dempster's equation (1957) was applied for calculating the developmental duration, and Richard's equation (1957) was used for calculating the developmental rate.

Pupation Rate: The pupation rate was expressed in % of the successfully developed pupae.

***Pupal Water Loss*:** Pupal water loss was calculated as follows:

Water loss % = [initial weight – final weight /initial Weight] × 100

Statistical Data Analysis:

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

RESULTS

Toxicity and Lethal Effects of Apitoxin on *G. mellonella*:

After treatment of the newly moulted 3rd instar larvae of *G. mellonella* with six concentration levels of Apitoxin (4000, 2000, 1000, 500, 250 and 125 ppm), *via* the artificial diet, the tested product exhibited dose-dependent toxicity on larvae except at the lowest concentration. The successfully developed pupae suffered a toxic action of the tested product, in a dose-dependent course, except at the lower two concentration levels since no mortality was observed. The corrected mortality was found in a dose-dependent manner (10, 50, 60, 70 and 70% mortality, at 250, 500, 1000, 2000 and 4000 ppm, respectively). The LC_{50} value was calculated in 956.16 ppm (Table 1).

Table (1): Lethal effects (%) of honey bee *A. mellifera* Apitoxin on the developmental stages of *G. mellonella*.

Conc. (ppm)	Larval mortality	Pupal mortality	Adult mortality	Total mortality	Corrected mortality	LC_{50} (ppm)
4000	60	25.00	0.00	70	70	
2000	60	25.00	0.00	70	70	
1000	50	20.00	0.00	60	60	
500	40	16.67	0.00	50	50	956.16
250	10	00.00	0.00	10	10	
125	00	00.00	0.00	00	00	
Control	00	00.00	0.00	00	---	

Conc.: concentration levels

Effect of Apitoxin on Growth and Development of *G. mellonella*:

The most important criteria of growth, development and metamorphosis of *G. mellonella*, after treatment of 3rd instar larvae with Apitoxin concentration levels, were summarized in Table (**2**). According to data of this table, the somatic weight gain of larvae was insignificantly reduced by the increasing concentration of Apitoxin. A similar result was recorded for the growth rate. In contrast, the larval duration was considerably shortened, in a dose-dependent fashion (29.4±0.9, 28.00±1.2, 27.00±2.1, 27.00±2.7, 26.43±1.6 and 24.86±1.3 days, at 125, 250, 500, 1000, 2000 and 4000 ppm, respectively, compared to 31.00±1.2 days of control larvae).

Depending on the data arranged in the same sable, the developmental rate of larvae indicated an enhancing action of Apitoxin, since the treated larvae developed in a faster rate than control congeners. The pupal duration was slightly shortened, depending on the Apitoxin concentration, denoting a faster developmental rate of treated pupae.

Because the pupal death may be due to the desiccation caused by Apitoxin, loss of body water was estimated in %. The successfully developed pupae from treated larvae lost more body water than control pupae, in a dose-dependent course (34.09, 34.19, 34.61, 34.66, 34.81 and 35.00%, at 125, 250, 500, 1000, 2000 and 4000 ppm, respectively, in comparison with 34.09% water loss in control pupae).

With regard to the activity of Apitoxin against metamorphosis and morphogenesis of

G. mellonella, data of table (2) revealed that the tested product failed to affect these two programs since neither larval-pupal intermediates nor malformed pupae had been produced. On the other hand, the pupation rate was regressed in a dose-dependent course, i.e. the pupation was increasingly blocked as the concentration was increased (90, 60, 50, 40 and 40% pupation, at 250, 500, 1000, 2000 and 4000 ppm of Apitoxin, respectively). No effect was exhibited at the lowest concentration level of Apitoxin.

Table (2): Effects of the *A. mellifera* Apitoxin on growth and development of *G. mellonella*.

Conc. (ppm)	Larval stage					Pupal stage	
	Weight gain (Mean mg ±SD)	Growth rate (Mean ±SD)	Duration (Mean days ±SD)	Develop. rate	Pupation %	Duration (Mean days ±SD)	Water loss (%)
4000	0.1912±0.04 a	0.0018±0.007 a	24.86±1.3 d	4.02	40	8.63±2.1 b	35.00
2000	0.2021±0.02 a	0.0018±0.005 a	26.43±1.6 d	3.78	40	8.55±2.6 a	34.81
1000	0.2081±0.05 a	0.0017±0.005 a	27.00±2.7 c	3.70	50	8.59±1.1 a	34.66
500	0.2099±0.02 a	0.0018±0.005 a	27.00±2.1 d	3.70	60	8.81±1.5 a	34.61
250	0.2118±0.02 a	0.0017±0.005 a	28.00±1.2 d	3.57	90	8.88±1.5 a	34.19
125	0.2200±0.04 a	0.0018±0.004 a	29.14±0.9 d	3.43	100	9.00±1.3 a	34.09
control	0.2516±0.04	0.0024±0.007	31.00±1.2	3.23	100	9.00±1.2	34.09

Conc.: concentration level, Develop: Developmental. 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 ($P < 0.001$).

DISCUSSION

Insecticidal Activity of Apitoxin against *G. mellonella*:

Bee venom had insecticidal activities against cricket nymphs (Jerome *et al*., 2001). Bee venom can challenge the larvae of *Senotainia tricupis*, *Mermis* sp. and the parasitic mites *Acarapis* sp. and *Varroa jacobsoni* (Hider, 1988; Hoffman, 1996; Glinski and Jarosz, 2001; Charles, 2005). In the present study, Apitoxin exhibited a dose-dependent toxicity on larvae and pupae after the treatment of the 3rd instar larvae of *G. mellonella*. This result was in agreement with the reported results of honey bee venom against some insects, such as the corn earworm *Heliothis zea* (Ross *et al*., 1987), the tobacco hornworm *Manduca sexta* (Quistad *et al*., 1988) and the lesser wax moth *Achroia grisella* (Mahgoub *et al*., 2018).

To explicate the toxicity and lethal effects of Apitoxin on *G. mellonella*, in the current study, it may be important to mention that Apitoxin is a complex mixture of proteins, peptides, and low molecular components. The toxicity reaction of bee venom was due to these biologically active components (Hoffman, 1996; Charles, 2005). Mazdak *et al*. (2004) reported that melittin is responsible for venom toxicity. Melittin has a relatively low toxicity (Bogdanov, 2017). Therefore, the lethal effect of honey bee venom may have resulted primarily from the synergic interaction of the venom components, mainly apamin, melittin and phospholipase A2 (Quistad *et al*., 1988).

On the other hand, the larval deaths of *G. mellonella* by Apitoxin, in the current work, may be attributed to the failure of larvae to moult owing to the inhibition of chitin formation (Abdel Rahman *et al*., 2007; Adel, 2012) or to the inability to shed their exocuticle during ecdysis (Linton *et al*., 1997). Also, the larval deaths may be due to an antifeedant activity of Apitoxin and continuous starvation of larvae (Ghoneim *et al*.,

2000). The pupal deaths in *G. mellonella*, in the present investigation, could be directly or indirectly relate to activities of Apitoxin against some vital processes, such as suffocation, bleeding and desiccation owing to imperfect exuvation, failure of vital homeostatic mechanisms, *etc*. (Smagghe and Degheele, 1994). This suggestion can easily be substantiated since the tested product exerted a general desiccating action on pupae after treatment of 3rd instar larvae of *G. mellonella*, in the present study.

In the current investigation, LC_{50} value of Apitoxin against *G. mellonella* was calculated in 956.16 ppm while it was estimated in 76µg/g for honey bee venom against *M. sexta* (Quistad *et al*., 1988) and 38.27 µg /µl against *A. grisella* (Mahgoub *et al*., 2018). However, LC_{50} values depend on several factors, such as susceptibility of the insect and its treated stage or instar, lethal potency of the tested compound or product and its concentration levels, method and time of treatment, as well as the experimental conditions (Ghoneim *et al*., 2017).

Disrupted Growth and Development of *G. mellonella* by Apitoxin:

In the present study, both larval weight gain and growth rate had been slightly reduced after treatment of *G. mellonella* 3rd instar larvae with different concentrations of Apitoxin. The present result of larval weight gain reduction corroborated with the recorded reduction of larval bodyweight of the corn earworm *Heliothis zea* after injection of 3rd instar larvae with honey bee venom (Ross *et al*., 1987). The inhibited growth of *G. mellonella* by Apitoxin, in the current study, might be a result of the blocked release of certain peptides, causing alteration in the ecdysteroid and juvenoid titers (Barnby and Klocke, 1990). Also, some constituents of Apitoxin, such as apamin, melittin (Charles, 2005; Mazdak *et al*., 2004), might affect the tissues and cells undergoing mitosis (Nasiruddin and Mordue, 1994).

In the present study, the treatment of 3rd instar larvae of G. mellonella with Apitoxin resulted in considerable shortening of the larval duration, in a dose-dependent course. Also, pupal duration was slightly or remarkably shortened, depending on the concentration. The developmental rate of treated larvae indicated an enhancing action of Apitoxin, since the treated larvae developed in a faster rate than control congeners. The present shortened durations of larvae and pupae might be due to their avoiding Apitoxin adverse action, as a xenobiotic agent. Apitoxin might prevent nuclear receptors formation of the cells, caused disturbance in developmental durations (Riddiford and Truman, 1993) In the present study, Apitoxin failed to affect the metamorphosis and morphogenesis programs in *G. mellonella*, but the pupation rate regressed in a dose-dependent course. For interpretation of the regression of pupation rate, the tested product might exert a suppressive action on the chitin synthesis and prevented the normal deposition of the new cuticle during apolysis (Retnakaran *et al*., 1985).

Conclusion:

Depending on the results of the present study on *G. mellonella*, Apitoxin exhibited a high toxicity on larvae and pupae, at the majority of concentrations. Also, it significantly reduced the somatic weight gain, larval growth rate and blocked the pupation as well as it affected the larval and pupal durations. Therefore, Apitoxin should be taken into account among other efficient components of the management program against the greater wax moth.

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