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**ABSTRACT**

The greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) is the most destructive pest of the honey bee, *Apis mellifera*, throughout the world. The current study was carried out to evaluate the toxicity and disruptive effects of the venom of deathstalker scorpion, *Leiurus quinquestriatus* (Buthidae: Scorpiones) on the growth, development, and metamorphosis of this pest. The newly moulted 3rd instar larvae were fed on an artificial diet treated with a series of venom concentrations (250, 500, 1000, 2000, 4000, and 8000 ppm). The present results could be summarized as follows. The venom exhibited considerable toxicity against larvae. Also, the pupae suffered a lethal action of the venom, in a dose-dependent course. In contrast, adult survival was not affected by the tested venom. The LC50 was calculated in 3428.9 ppm. With regard to growth and development, the somatic weight gain of larvae was slightly reduced proportionally to the concentration. The larval growth rate increasingly regressed by the ascending concentration. The larval duration was remarkably shortened, in a dose-dependent manner. The pupation rate of treated larvae was significantly regressed, in a dose-dependent course. Also, the pupal duration was shortened. Pupae lost more body water than control pupae. The venom failed to affect the metamorphosis program.

**INTRODUCTION**

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). Although the adults do not feed, because they have atrophied mouth parts, the voracious nature of larval feeding and tunneling lead to the destruction of the honeycomb, and subsequent to the death of weak colonies (Awasthi and Sharma, 2013; Kwadha *et al.*, 2017), especially in temperate, tropical and subtropical beekeeping regions, since the warm temperature enhances a rapid development of this moth (Chandel *et al.*, 2003; Ellis *et al.*, 2013; Mohamed *et al.*, 2014).

For the control of *G. mellonella*, various physical methods have been adopted,
including freezing, heating (high temperature), CO₂, and sulphur fumigation against larvae and pupae (Ahmed et al., 1993; Calderone, 2000; Owayss and Abd-Elgayed, 2007). Akyol et al. (2009) determined the effectiveness of CO₂ against the eggs, larvae and pupal period of G. mellonella in stored honeycombs. For more information, see Ramos-Rodriguez et al. (2007) and Christen et al. (2008). Ozone gas was assessed as a control measure for G. mellonella and honey bee Apis mellifera pathogens (James, 2011). Synthetic insecticides of different categories had been used for controlling G. mellonella (Durmuş and Büyükgüzel, 2008; Sak and Uckan, 2009). Several biological control agents, such as the natural enemies of predators and parasitoids, along with entomopathogenic nematodes, viruses and fungi, had been assessed on G. mellonella (Dindo et al., 2001; Armendariz et al., 2002; Hussaini, 2003; Ellis et al., 2013; George et al., 2019). Also, many studies had been conducted on the sterile insect technique (or inherited sterility) for controlling G. mellonella (Khalil et al., 1993; Ebadi et al., 2001; Carpenter et al., 2005; El-Kholy and Mikhaiel, 2008). In addition, insect hormone analogues, insect growth regulators had been assessed against G. mellonella (Willems et al., 2002; Izzetoglu and Karacali, 2003; Unsal et al., 2004; Awasthi and Sharma, 2012; Pamita and Priyanka, 2013). Natural compounds from the plant origins could be efficient alternatives to conventional fumigants against G. mellonella (Rajendran and Sriranjini, 2008; Abbasipour et al., 2009; Mahmoudvand et al., 2011; Basedow et al., 2012; Elbehery et al., 2016; El et al., 2017).

In the last two decades, an important interest of investigation by agrochemical companies in the development of highly selective biopesticides derived from animals. Also, natural products of animal origin have been described as very good alternative agents for controlling G. mellonella. One source of these selective animal-derived biopesticides leads is venom-derived peptides from different sources including the venomous predatory/parasitoid arthropods, such as spiders (Harrison and Bonning, 2000; Tedford et al., 2004; Nicholson, 2006), scorpions (Froy et al., 2000; Taniai et al., 2002), wasps (Dahlman et al., 2003), cone snails (Olivera, 2002) and some marine animals (Whetstone and Hammock, 2007; Windley et al., 2012; Nakasu et al., 2014), as well as arthropod hormones and neuropeptides (Altstein et al., 2000; Altstein, 2004).

Scorpion is a mysterious creature in the animal world. It has poisonous venom (Possani et al., 2000) and can be fluorescent (Frost et al., 2001) that unique features increasingly attracted scientists’ attention and interests throughout the world (Cao et al., 2013; Ma and Shi, 2014). The deathstalker scorpion or yellow scorpion Leiurus quinquestriatus Hemprich & Ehrenberg (Buthidae: Scorpiones: Arachnida) can be found in desert and scrubland habitats ranging from North Africa through to the Middle East. In Egypt, Saleh et al. (2017) reported that two species of scorpions (Androctonus amoreuxi and L. quinquestriatus) have been recorded from six eco-geographical regions. The toxicity of venom solutions from some scorpion species was assessed against the mealworm Tenebrio molitor. The most potent toxicity was exhibited by the venom of L. quinquestriatus (Valk and Meijden, 2014).

As reported by some authors (Gurevitz, 2010; Leng et al., 2011), the scorpion toxins contain toxins active against insects and are valuable as leads for the development and synthesis of eco-friendly insecticides, since they exhibited no effect on beneficial insects or mammals (Fabiano et al., 2008; Gurevitz, 2010). However, Joseph and George (2012) reviewed the insecticidal activities of scorpion toxins on a broad range of insect pests and concluded that the scorpion toxins provide safe biopesticides. The objective of the current study was to evaluate the toxicity and disruptive effects of L. quinquestriatus venom on the growth, development, and metamorphosis of G. mellonella.
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MATERIALS AND METHODS

Experimental Insect:
A culture of the greater wax moth *Galleria mellonella* (Lepidoptera: Pyralidae) was maintained in the laboratory of Entomology, Faculty of Science, Al-Azhar University, Cairo, Egypt under controlled conditions (27±2°C, 65±5% R.H., photoperiod 14 h L, and 10 h D). This culture was originated from a sample of larvae kindly obtained from a culture of susceptible strain maintained for several generations in Plant Protection Unit, Desert Research Center, 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 (400 g), bee honey (400 g), yeast (100 g). 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.

Scorpion Collection, Identification, and Obtaining of Venom:
Sixty-five adult individuals of the deathstalker scorpion, *Leiurus quinquestriatus* (Buthidae: Scorpiones), were collected from Garf Hessin at 23.289024N32.776828E, west of Nasser Lake, Aswan, Egypt. Scorpions were collected in the daytime by random searching their hiding places, mostly under rocks and other favorable shelters (Williams, 1968). The collected specimens were kept individually in plastic containers at 25-28°C. The specimens were examined with a stereoscopic binocular microscope and taxonomically identified to the species using the morphological description keys (Vachon, 1966; El-Hennawy, 1987; Badry *et al*., 2018). Venom was obtained by electric stimulation (20 Volt) in the articulation of the telson according to Sarhan *et al*., (2012). Milking of scorpion had been carried out as venom drops collected into an Eppendorf tube. Then, the collected drops were centrifuged at 14000 r.p.m for 15 minutes at 4°C. The supernatant was pooled, freeze-dried, and stored at 20°C. The lyophilized samples were dissolved in distilled water and centrifuged at 15000 r.p.m for 15 minutes at 4°C.

Preparation of Concentrations and Larval Treatment:
A series of concentration levels of the scorpion *L. quinquestriatus* venom was prepared by diluting with distilled water in volumetric flasks as follows: 8000, 4000, 2000, 1000, 500 & 250 ppm. Bioassay tests were carried out using the newly moulted 3rd instar larvae. Ten grams of the diet were mixed with 2ml of each concentration of the animal product 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 Test:
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:
% corrected mortality = \left( \frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \right) \times 100

The LC50 values were calculated for general mortality 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 (GR) can be calculated according to (Waldbauer, 1968) as follows:
\[ \text{GR} = \text{fresh weight gain during feeding period} / \text{feeding period} \times \text{mean fresh bodyweight of larvae during the feeding period}. \]

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

Pupal water loss: Pupal water loss was calculated depending on the date of the initial and final weights of the pupae, as follows:
\[ \text{Water loss} \% = \left( \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \right) \times 100 \]

Statistical Data Analysis:
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

Toxicity and Lethal Effects of \textit{L. quinquestriatus} venom:
The newly moulted 3\textsuperscript{rd} instar larvae of \textit{G. mellonella} were treated with six concentration levels of scorpion venom (250, 500, 1000, 2000, 4000, and 8000 ppm), via the artificial diet. Data of toxicity and lethal effects were assorted in Table (1). Depending on these data, scorpion venom exhibited toxicity against larvae, except at the lowest concentration level. At the higher three concentration levels, scorpion venom treatment caused 40\% larval mortality and then decreased with the decreasing concentration. No mortality was observed among control larvae. The successfully developed pupae suffered a lethal action of scorpion venom, in a dose-dependent course, except at the two lower concentration levels, since no mortality was observed (33.33, 16.67, 16.67 and 12.50\% mortality, at 8000, 4000, 2000 and 1000 ppm, respectively, compared to 00\% mortality of control pupae). With regard to the successfully emerged adults, scorpion venom failed to affect their survival. The corrected mortality was found in a dose-dependent manner (20, 30, 50, 50 and 60\%, at 500, 1000, 2000, 4000 and 8000 ppm, respectively). The LC50 value was calculated in 3428.9 ppm (Table 1).

Effects of \textit{L. quinquestriatus} Venom on Growth and Development:
The most important criteria of growth, development, and metamorphosis of \textit{G. mellonella}, after treatment of 3\textsuperscript{rd} instar larvae with six concentration levels of scorpion venom, were summarized in Table (2). Depending on these data, the somatic weight gain of larvae was slightly reduced proportionally to the concentration level. A similar result was recorded for the growth rate. In contrast, the larval duration was considerably shortened, in a dose-dependent manner (29.00±1.3, 27.00±2.0, 27.00±2.7, 27.00±1.4, 26.00±1.3 and 24.67±1.6 days, at 250, 500, 1000, 2000, 4000 and 8000 ppm, respectively, compared to 30.86±1.6 days of control larvae). In addition, the developmental rate of treated larvae was higher than that of control larvae. Depending on the data arranged in the same table, the pupation rate of treated larvae was not affected at
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the lowest concentration level of scorpion venom but it regressed parallel to other concentration levels. In other words, scorpion venom hindered the pupation process in a dose-dependent course. Also, the pupal duration was slightly shortened, depending on the venom concentration (8.88±2.0, 8.78±2.1, 8.31±2.0, 7.99±2.2 and 7.92±2.1 days of treated pupae, at 500, 1000, 2000, 4000, and 8000 ppm, respectively, vs. 9.00±1.2 days of control pupae).

Because the pupal death may be due to the desiccation caused by the scorpion venom, loss of body water was estimated in %. The successfully developed pupae lost more body water than the control pupae but in a reverse correlation with the venom concentration (see Table 2). However, the tested scorpion venom failed to affect the metamorphosis program, since no larval-pupal or malformed pupae had been produced.

Table 1: Lethal effects (%) of scorpion L. quinquestriatus venom on the developmental stages of G. mellonella.

<table>
<thead>
<tr>
<th>Conc. (ppm)</th>
<th>Larval mortality</th>
<th>Pupal mortality</th>
<th>Adult mortality</th>
<th>Total mortality</th>
<th>Corrected mortality</th>
<th>LC₅₀ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8000</td>
<td>40.00</td>
<td>33.33</td>
<td>0.00</td>
<td>60.00</td>
<td>60.00</td>
<td></td>
</tr>
<tr>
<td>4000</td>
<td>40.00</td>
<td>16.67</td>
<td>0.00</td>
<td>50.00</td>
<td>50.00</td>
<td>3428.9</td>
</tr>
<tr>
<td>2000</td>
<td>20.00</td>
<td>12.50</td>
<td>0.00</td>
<td>30.00</td>
<td>30.00</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>10.00</td>
<td>0.00</td>
<td>0.00</td>
<td>20.00</td>
<td>20.00</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
</tr>
</tbody>
</table>

Conc.: concentration level.

Table 2: Effects of the scorpion L. quinquestriatus venom on the growth and development of G. mellonella.

<table>
<thead>
<tr>
<th>Conc. (ppm)</th>
<th>Weight gain (Mean mg ±SD)</th>
<th>Growth rate (Mean ±SD)</th>
<th>Duration (Mean days ±SD)</th>
<th>Develop. rate</th>
<th>Pupation (%)</th>
<th>Duration (Mean days ±SD)</th>
<th>Water loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8000</td>
<td>0.2208±0.01 b</td>
<td>0.0025±0.007 b</td>
<td>24.67±1.6 d</td>
<td>4.05</td>
<td>50</td>
<td>7.92±2.1 b</td>
<td>33.9</td>
</tr>
<tr>
<td>4000</td>
<td>0.2231±0.01 a</td>
<td>0.0025±0.005 a</td>
<td>26.00±1.3 d</td>
<td>3.85</td>
<td>60</td>
<td>7.99±2.2 a</td>
<td>34.22</td>
</tr>
<tr>
<td>2000</td>
<td>0.2252±0.02 a</td>
<td>0.0024±0.005 a</td>
<td>27.00±1.4 d</td>
<td>3.70</td>
<td>60</td>
<td>8.31±2.0 a</td>
<td>34.71</td>
</tr>
<tr>
<td>1000</td>
<td>0.2261±0.01 a</td>
<td>0.0025±0.002 a</td>
<td>27.00±2.7 e</td>
<td>3.66</td>
<td>80</td>
<td>8.78±2.1 a</td>
<td>34.82</td>
</tr>
<tr>
<td>500</td>
<td>0.2301±0.01 a</td>
<td>0.0025±0.004 a</td>
<td>27.33±2.0 d</td>
<td>3.45</td>
<td>90</td>
<td>8.88±2.0 a</td>
<td>34.82</td>
</tr>
<tr>
<td>250</td>
<td>0.2377±0.02 a</td>
<td>0.0025±0.005 a</td>
<td>29.00±1.3 b</td>
<td>3.24</td>
<td>100</td>
<td>9.00±1.3 a</td>
<td>34.91</td>
</tr>
<tr>
<td>Control</td>
<td>0.2387±0.03</td>
<td>0.0029±0.00 a</td>
<td>30.86±1.6</td>
<td>3.24</td>
<td>100</td>
<td>9.00±1.2</td>
<td>33.02</td>
</tr>
</tbody>
</table>

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 L. quinquestriatus Venom Against G. mellonella:

Many researchers reported the effectiveness of venoms extracted from different
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scorpions against insect pests, since scorpion toxins exhibited high toxicity against some insects, such as leaf-eating moths, locusts, flies and beetles (Gurevitz, 2010). For example, the venom of the scorpion Mesobuthus tamulus was effective against the corn worm moth Heliothis virescens (Wudayagiri et al., 2001). The scorpion insectotoxin "AaIT" was tested on larvae of the diamondback moth Plutella xylostella. Within the first 24 h, more than 60% of larvae were killed (Ji et al., 2002). The scorpion insectotoxin "AaIT" was tested on larvae of the diamondback moth Plutella xylostella. Within the first 24 h, more than 60% of larvae were killed (Ji et al., 2002). The scorpion insectotoxin "AaIT" was tested on larvae of the diamondback moth Plutella xylostella. Within the first 24 h, more than 60% of larvae were killed (Ji et al., 2002).

The scorpion venom exhibited toxicity against three insects from different orders, viz., Apis mellifera, Gryllus assimilis, and Diatraea saccharalis (Manzoli-Palma et al., 2003). The venom of the scorpion Liocheles australasiae was toxic for some insects (Miyashita et al., 2007). The toxicity of venom solutions from some scorpion species was assessed against the mealworm Tenebrio molitor. The most potent toxicity was exhibited by the venom of L. quinquestriatus (Valk and Meijden, 2014).

Results of the present study were, to a great extent, in agreement with the previously reported results, since the treatment of newly moulted 3rd instar larvae of the greater wax moth, Galleria mellonella, with six concentrations of the venom milked from the scorpion L. quinquestriatus exhibited toxicity against larvae. The pupae suffered a lethal action of the venom, in a dose-dependent course. In contrast, this venom failed to affect the adult survival. To explain the lethal effect of the L. quinquestriatus venom on larvae and pupae of G. mellonella, it might be due to certain chemical constituents of the venom, such as salts, small molecules, ions, neurotransmitters, peptides, and proteins (Moskowitz et al., 1998; Possani et al., 1999). This venom also contains enzymes, such as phospholipase, hyaluronidase, lipase, alkaline phosphotases, and proteolytic enzymes (Park et al., 2007). Also, L. quinquestriatus venom was found to contain different compounds similar to polypeptide neurotoxin, especially (Lqh7) and (lqh6) (Hamon et al., 2002). It contains, also, 5 toxic compounds of basic proteins with low molecular weight bound to disulphide bridges (Kopygan et al., 2006). However, different active toxins had been isolated from other scorpion species, such as "Pil" from Pandinus imperator (Rogowski et al., 1996), noxiustoxin, and margatoxin from Centruroides limpidus (Kharrat et al., 1996) and maurotoxin from Scorpio maurusalmatus (Carlier et al., 2000).

In general, the larval deaths of G. mellonella by the tested arthropod products, 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 ecysis (Linton et al., 1997). Also, the larval deaths might be due to the antifeedant activities of the present venoms and secretion causing continuous starvation of larvae (Ghoneim et al., 2000). The pupal deaths in G. mellonella could be directly or indirectly relate to activities of the tested venom against some vital processes, such as suffocation, bleeding and desiccation owing to imperfect exuviation, failure of vital homeostatic mechanisms, etc. (Smagghe and Degheele, 1994). This suggestion can easily be substantiated since the tested L. quinquestriatus venom exerted a general desiccating action on pupae after treatment of 3rd instar larvae of G. mellonella, in the present study.

With regard to the LC50 of L. quinquestriatus venom against G. mellonella, in the present study, it was calculated in 3428.9 ppm. Thus, the toxicity of the L. quinquestriatus venom was weak in comparison with other scorpion venoms, such as scorpion Odontobuthus odonturus against the aphid Rhopalosiphum erysimi, since Tahir et al. (2015) calculated it as 0.44 µl. However, LC50 values depend on several factors, such as susceptibility of the insect and its treated stage or instar, lethal potency of the tested compound or venom 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 *L. quinquestriatus* Venom:

On the basis of the available literature, different products and compounds of animal origin showed disruptive effects on the development of various insect pests. In the present investigation, the somatic weight gain of *G. mellonella* larvae was reduced proportionally to the concentration level of *L. quinquestriatus* venom. Also, the larval growth rate is increasingly regressed by the ascending concentration.

The inhibited growth of *G. mellonella*, in the current study, might be a result of the blocked release of certain peptides, causing an alteration in the ecdysteroid and juvenoid titer (Barnby and Klocke, 1990), since the scorpion *L. quinquestriatus* venom contains different biologically active compounds similar to polypeptide neurotoxin (Tan et al., 2006; Ortiz and Possani, 2015). Two types of venoms were identified in *L. quinquestriatus* venom (Lqh7) and (lqh6) (Hamon et al., 2002). Some of these constituents might affect the tissues and cells undergoing mitosis (Nasiruddin and Mordue, 1994).

The larval duration and pupal duration of *G. mellonella*, in the current study, had been remarkably shortened, in a venom dose-dependent manner. The present shortened durations of larvae and pupae might be due to their avoiding the adverse actions of the tested venom, as a xenobiotic agent. Also, the *L. quinquestriatus* venom might prevent the formation of the nuclear receptor of the cells, causing a disturbance in the developmental durations (Riddiford and Truman, 1993).

In the present study, the pupation rate of treated *G. mellonella* larvae was regressed after larval treatment with the majority of concentration levels of *L. quinquestriatus* venom. In other words, scorpion venom hindered the pupation process in a dose-dependent course. For interpretation of the regression of pupation rate, the tested venom might exert a suppressive action on the chitin synthesis to prevent the normal deposition of the new cuticle during pupation (Retnakaran et al., 1985). On the other hand, the tested venom failed to affect the metamorphosis program of *G. mellonella*, since no larval-pupal or malformed pupae had been produced. Unfortunately, we have no conceivable explication of this failure!!

**Conclusion:**

Depending on the results of the present study on *G. mellonella, L. quinquestriatus* venom exhibited 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 affected the larval and pupal durations. Therefore, this venom should be taken into account among other efficient components of the integrated management program against *G. mellonella*. However, novel delivery systems should be explored in order for the scorpion toxins can find their way into commercial applications in foreseeable future.

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http://dx.doi.org/10.1016/S0300-9084(00)01167-6


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