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Comparative impairing effects of selected arthropod venoms on the main body metabolites of *Galleria mellonella* (Lepidoptera: Pyralidae)

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Abstract

The greater wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae) is widely distributed in the world. It is the most destructive pest of honey bee, *Apis mellifera* (Hymenoptera: Apidae), throughout the world. The present study was conducted to estimate the disturbance of main body metabolites in the tissues of 5th and 7th instar larvae, as well as early-, mid- and late-aged pupae of *G. mellonella*, by certain arthropod venoms. The 3rd instar larvae were treated, *via* an artificial diet, with LC_{50} of death stalker scorpion *Leiurus quinquestriatus* venom, oriental Hornet (wasp) *Vespa orientalis* venom, or Apitoxin of honey bee *Apis mellifera* (3428.9, 2412.6, and 956.16 ppm, respectively). The important results could be summarized as follows. The total protein content in larvae was drastically reduced in larvae, regardless the tested venom or the larval instar. Also, all venoms predominantly prevented the successfully developed pupae to attain normal protein level. All venoms exhibited prevalent inducing effects on larvae to gain more lipids than control congeners of both 5th and 7th instars. Also, all tested venoms unexceptionally enhanced the pupae to gain more lipids than the control congeners. Treatment of the 3rd instar larvae with each of the tested venoms resulted in a dramatic reduction of carbohydrate content in 5th and 7th instar larvae. The carbohydrate content in treated pupae had been drastically reduced, regardless the tested venom or the pupal age.

Keywords: *Apitoxin, Carbohydrate, Hornet, Larva, Lipid, Protein, Pupa, Scorpion*

1. Introduction

The greater wax moth *Galleria mellonella* L. (Lepidoptera: Pyralidae) is widely distributed throughout the world. 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 subsequently to the death of weak colonies (Chandel *et al*., 2003; Awasthi and Sharma, 2013; Mohamed *et al*., 2014; and Kwadha *et al*., 2017). For the control of *G. mellonella*, various physical methods have been adopted; including freezing, heating, CO_2, Ozone gas and sulphur fumigation against larvae and pupae (Owayss and Abd-Elgayed, 2007; Christen *et al*., 2008;

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Akyol *et al*., 2009; and James, 2011). Conventional insecticides of different categories had been used for controlling *G. mellonella* (Durmş and Büyükgüzel, 2008; and Sak and Uckan, 2009). Several biological control agents, such as the natural enemies, predators and parasitoids, along with entomopathogenic nematodes, viruses and fungi, had been assessed for controlling this pest (Dindo *et al*., 2001; Armendariz *et al*., 2002; Hussaini, 2003; Ellis *et al*., 2013; and George *et al*., 2019). The sterile insect technique (or inherited sterility) has been assessed against this pest (Ebadi *et al*., 2001; Carpenter *et al*., 2005; and El-Kholy and Mikhaiel, 2008). Also, insect hormone analogues, insect growth regulators had been assessed against it (Izzetoglu and Karacali, 2003; Unsal *et al*., 2004; Awasthi and Sharma, 2012; and Pamita and Priyanka, 2013). Natural compounds of the plant origin may be efficient alternatives to conventional fumigants against *G. mellonella* (Abbasipour *et al*., 2009; Mahmoudvand *et al*., 2011; Basedow *et al*., 2012; Elbehery *et al*., 2016; and Er *et al*., 2017).

In the last few decades, a great interest of investigation by agrochemical companies is the development of highly selective biopesticides derived from animals. Natural products of the animal origin have been described as very good alternative agents to the conventional insecticides for controlling some insect pests. These animal-derived biopesticides include the venom-derived peptides from different sources including the venomous arthropods, such as spiders (Harrison and Bonning, 2000; Tedford *et al*., 2004; and Nicholson, 2006), scorpions (Froy *et al*., 2000; and Taniai *et al*., 2002), wasps (Dahlman *et al*., 2003), as well as cone snails (Olivera, 2002) and some marine animals (Whetstone and Hammock, 2007; Windley *et al*., 2012; and Nakasu *et al*., 2014). In addition, the arthropod hormones and neuropeptides may be effective control agents against various insect pests (Altstein *et al*., 2000; and Altstein, 2004).

Scorpion is a mysterious creature in the animal world. It has poisonous venom (Possani *et al*., 2000) which has increasingly attracted the scientists' attention throughout the world (Cao *et al*., 2013; and Ma and Shi, 2014). The death stalker scorpion, or yellow scorpion, *Leiurus quinquestriatus* (Buthidae: 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 the occurrence of this scorpion species in six eco-geographical regions. Among different scorpion venoms, venom of *L. quinquestriatus* exhibited the most potent toxicity against the meal worm *Tenebrio molitor* (Valk and Meijden, 2014). As reported by some authors (Gurevitz, 2010; and Leng *et al*., 2011), the scorpion toxins contain active toxins 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; and 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.

Workers and queen of the honey bee *Apis mellifera* (Apidae: Hymenoptera) produce the venom in a special long and thin branched acid gland at the end of their abdomens. This venom or toxin can be called Apitoxin; since the word was originated from the Latin *apis* (bee) and *toxikon* (venom) (Cruz-Landim and Abdalla, 2002). In a recent review, Azam *et al*. (2019) compiled information on the history, chemical composition and scientific evidence concerning the Apitoxin pharmaceutic research and different medical uses. The honey bee venom had been studied for its action on mammals although little is known about its action on insects (Piek, 1987; and Quistad *et al*., 1988**)**. This venom exhibited toxic effects on 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). Recently, Ghoneim *et al*. (2019a) recorded a dose-dependent toxicity of Apitoxin on larvae and pupae of *G. mellonella*, as well as the reduction of larval weight gain and growth rate. In another recent study, Apitoxin blocked the adult emergence, prohibited the fecundity and fertility of *G. mellonella* (Ghoneim *et al*., 2019b).

The oriental hornet *Vespa orientalis* (Hymenoptera: Vespidae), is a social wasp in the Middle East (Khodairy and Awad, 2013; and Abdelaal and El-defrawy, 2014). The world distribution of this wasp species comprises, also, North Africa, southeastern Europe, Southwest Asia across Turkey and Arabian Peninsula to India and Nepal (Carpenter and Kojima, 1997; and Archer, 1998). In addition, it was accidentally introduced into Madagascar and China (Carpenter and Kojima, 1997; and Archer, 1998) and recorded in Mexico (Dvorak, 2006). Many studies had been conducted for examining the toxicity of wasp venoms on insects (Libersat *et al*., 1999; Coudron *et al*., 2000; Haspel and Libersat, 2003; and Nadolski, 2013). In Egypt, many studies had been conducted on *V. orientalis* focusing on ecology, biology, control and its dangerous effect on apiculture (Khater *et al*., 2001; AbdAl-Fattah and Ibrahim, 2009; and Taha, 2014).

In insects, the main body metabolites, *viz*., Carbohydrates, protein and lipids, have an essential role in different biological and physiological activities, such as body size, growth rate, fecundity and fertility (Fagan *et al*., 2002). Therefore, the contents of these macromolecules are good indicators of the level of metabolism in insects after treatment with chemicals or other materials (Zhu *et al*., 2012).

As reported by many authors (Hassan, 2002; Chapman, 2012; Cohen, 2010; and Sugumaran, 2010), proteins perform many functions within the living organisms, such as catalyzing metabolic reactions, replicating DNA, synthesis of ATP, the cell division, enzymes and hormones controlling many chemical reactions in the cell metabolism. In insects, protein metabolism plays a key role in rebuilding adult structures during the transformation of larvae / pupae into adults (Resmitha *et al*., 2014) as well as energy production and insect reproduction (Taşkin and Aksoylar, 2011).

Lipids represent an important source of energy, hormone precursors and structural members for insects and are transported from their synthesis site of storage *via* the haemolymph towards the user organs, in particular the vitellogenesis (Zhou and Miesfeld, 2009) and cuticular synthesis (Dapporto *et al*., 2008). Also lipids located in the egg play an important role in meeting the energy needs for developing embryo (Boz and Gülel, 2012). Quantity of lipids available for the reserves seems to be the result of a balance between the catch of food and the requests for reserves by processes, such as maintenance, growth and reproduction, and this balance is disturbed by any toxic product (Canavoso *et al*., 2001).

Carbohydrates play an important role in the structure and function of all tissues during metamorphosis as well as for the normal functioning of the male and female reproductive organs and embryonic development (*cf*. Chippendale, 1978). They increase during the rest periods, like metamorphosis, and decrease during the growth periods, like the stages of maturation of the gonads in insects (Bouaziz *et al*., 2011). On the other hand, the carbohydrate content in the haemolymph is an important indicator of the level of metabolism in insects, and a dynamic balance of the absorption, metabolism, and utilization by different tissues (Zhu *et al*., 2012). According to the currently available literature, many studies had been conducted for examining the toxicity of arthropod venoms against insects, but very little research attention had been paid to their effects on the physiological criteria. Therefore, the present study was conducted aiming at the estimation of disturbing effects of *L. quinquestriatus* venom, *V. orientalis* venom and Apitoxin of *A. mellifera* on the main body metabolites in larvae and pupae of *G. mellonella*.

2. Materials and methods

2.1. Experimental insect

A culture of the greater wax moth *G. mellonella* L. (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 by 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; and 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 full grown larvae metamorphosed into pupae. The resulting pupae were collected and transferred into clean jars provided with a layer of moistened saw dust on the bottom. Then, 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 artificial diet for feeding of the hatching larvae.

2.2. Collection and preparation of arthropod venoms

2.2.1. Scorpion collection and obtaining of venom

Sixty five adult individuals of the Death stalker scorpion *Leiurus quinquestriatus* Hemprich and Ehrenberg (Buthidae: Scorpiones: Arachnida) were collected from Garf Hessin at 23.289024N32.776828E, west of Nasser Lake, Aswan, Egypt, during October 2014. Scorpions were collected at 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 ºC. 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; and Badry *et al*., 2018).

Scorpion 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 min at 4 ºC. The supernatant was pooled, freeze dried and stored at 20 ºC. The lyophilized samples were dissolved in distilled water and centrifuged at 15000 r.p.m for 15 min at 4 ºC.

2.2.2. Wasp collection and obtaining of venom

Adults of the oriental hornet (wasp) *Vespa orientalis* L. (Vespidae: Hymenoptera: Insecta) were collected during summer seasons by the wasp traps which settled among the honeybee nests at the Department of honey bee researches, Institute of plant protection, Doqqi, Giza, Egypt. Wasp individuals were refrigerated at –20 °C to keep them immobilized and thereby enhance ease of handling and dissection.

The preparation of venom sac extract was carried out according to Friedman and Ishay (1987) with some improvements. After defreezing, the wasp specimens had been manipulated at room temperature. The sting apparatus, at the abdomen tip, was gently pulled out using fine forceps. Along with string, a small white colored venom sac was obtained in a tube containing the extraction solvent. Each 200 venom sacs were equal to one gram. Each venom sac yielded approximately 0.5 mg venom extract (Neuman *et al*., 1987). Each 0.5g (100 venom sacs) was homogenized in 2ml solvent using the ultra homogenizer for 10 min. Then, it was centrifuged at 10000 r.p.m for 15 min at –4 °C using cooling centrifuge. The supernatant was left to evaporate at room temperature (about 27 °C).

2.2.3. Collection of Apitoxin from honey bee workers

Using six bee hives, the electric shock technique was applied for the collection of venom from the honey bee *Apis mellifera* (Apidae: Hymenoptera: Insecta) workers. 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.

2.3. Larval treatment and tissue preparation

According to preliminary tests on 3rd instar larvae of *G. mellonella*, LC_{50} values of the aforementioned venoms had been estimated as 956.16 ppm, 2412.6 ppm and 3428.91 ppm for honey bee *A. mellifera* venom, oriental wasp *V. orientalis* venom and Deathstalker scorpion L. *quinquestriatus* venom, respectively.

After treatment of 3rd instar larvae with LC_{50} values of these venoms, healthy treated and control larvae of 5th and 7th instars, as well as healthy treated and control pupae (of different ages: 0-, 4-, and 7-day old, i.e., early, mid and late, respectively) were weighed and then homogenized in a saline solution (one pupa / 1 ml saline solution 0.7 %) using a fine electric homogenizer, tissue grinder for 2 min. Homogenates were centrifuged at 4000 r.p.m. for 15 min. The supernatant was used directly or frozen until use. Seven replicates were used and homogenates of two individuals were never mixed.

2.4. Determination of the main body metabolites

Quantitative determination of the total protein content (mg / g) was conducted in the larval tissues and pupal homogenate according to the method of Weichselbaum (1946) and using the kit of Biodiagnostics. The method depends on the protein forms a violet complex with cupric ions in alkaline medium, and then measured the absorbance at 550 nm using a spectrophotometer.

Quantitative determination of the total lipid content (mg / g) was conducted in the larval tissues and pupal homogenate according to the technique of Folch *et al*. (1957) and lipid estimation was taken place by phosphovanillin reagent depending on Knight *et al*. (1972) and using the Spectrophotometer at 520 nm.

Quantitative determination of the total carbohydrate (as glycogen) content (mg / g) was conducted in the larval tissues and pupal homogenate using the anthrone reagent according to Singh and Sinha (1977) and utilizing the Spectrophotometer at 620 nm.

2.5. 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 difference between means.

3. Results

3.1. Effects of arthropod venoms on the total protein content of *G. mellonella*

Depending on the data assorted in Table 1, the protein level in body tissues of control larvae gradually elevated along the larval stage (2.90 ± 0.50 and 5.35 ± 0.40 mg/g in 5th instar and 7th instar larvae, respectively). On the other hand, the total protein content was drastically reduced in the treated larvae, regardless the tested venom or the larval instar. The strongest reducing potency was exhibited by Apitoxin in the 5th instar larvae (19.80% reduction) and exhibited by wasp venom in the 7th instar larvae (34.77% reduction). The least reducing potency was exhibited by scorpion venom (17.45 and 15.7% reductions, in 5th and 7th instar larvae, respectively).

Table 1: Influenced total protein content (mg/g) in the larval tissues after treatment of the 3rd instar larvae of *G. mellonella* with LC_{50} values of certain arthropod venoms

Venom		Larval instar	
		5th	7th
A. mellifera	Mean ± SD	2.39 ± 1.33 d	3.77 ± 2.19 d
	Change (%)	–19.80	–29.53
V. orientalis	Mean ± SD	2.47 ± 0.24 d	3.49 ± 1.48 d
	Change (%)	–17.11	–34.77
L. quinquestriatus	Mean ± SD	2.64 ± 0.03 d	4.51 ± 1.16 c
	Change (%)	–17.45	–15.7
Control	Mean ± SD	2.98 ± 0.50	5.35 ± 0.40

Note: Mean ± SD followed with c: highly significantly different ($p < 0.01$). d: very highly significantly different ($p < 0.001$).

With regard to the disturbance of protein content in the body tissues of the successfully developed pupae, data of Table 2 obviously revealed that the protein content as gradually declined throughout the pupal stage of controls (1.96 ± 0.19, 1.42 ± 1.41 and 0.99 ± 0.31 mg/g in early-, mid- and late-aged pupae, respectively). In general, all arthropod venoms predominantly prevented the pupae to attain normal protein level. In the early-aged pupae, the most powerful suppressing action on proteins was exerted by wasp venom while scorpion venom exhibited the least reducing potency. In the mid-aged pupae, the strongest reducing action was exhibited by the scorpion venom while the weakest reducing action was exerted by Apitoxin. In the late-aged pupae, Apitoxin had the strongest reducing potency on proteins but scorpion venom had the least reducing potency (46.46 and 4.04% reductions, respectively).

3.2. Effects of arthropod venoms on the total lipid content of *G. mellonella*

Depending on the data arranged in Table 3, the lipid content gradually decreased with instar of normal larvae (265.77 ± 18.0 and 247.75 mg/g, in 5th and 7th instars, respectively). Also, these data revealed a prevalent inducing effect of all arthropod venoms on larvae to gain more lipids than control congeners. In the 5th instar larvae, Apitoxin achieved the least enhancing action. In the 7th instar larvae, the least enhancing effect was achieved by scorpion venom on lipids.

In connection with the lipid content in the pupae, data listed in Table 4 revealed a gradual decreasing content with the age (151.65 ± 65.0, 149.44 ± 25.2 and 67.57 ± 11.9 mg/g, in early-, mid- and late-aged pupae, respectively). The lipid content in pupal tissues significantly increased after treatment of 3rd instar larvae with

Table 2: Influenced total protein content (mg/g) in the pupal tissues after treatment of the 3rd instar larvae of *G. mellonella* with LC_{50} values of certain arthropod venoms

Venom		Pupal age		
		Early	Mid	Late
A. mellifera	Mean ± SD	1.64 ± 0.75 d	1.34 ± 1.21 a	0.53 ± 0.84 c
	Change (%)	–16.33	–5.63	–46.46
V. orientalis	Mean ± SD	1.49 ± 0.36 d	1.35 ± 0.44 a	0.91 ± 1.08 c
	Change (%)	–23.98	–4.93	–8.08
L. quinquestriatus	Mean ± SD	1.74 ± 1.43 d	1.31 ± 0.94 a	1.03 ± 1.55 c
	Change (%)	–11.22	–7.75	4.04
Control	Mean ± SD	1.96 ± 0.19	1.42 ± 1.49	0.99 ± 0.31

Note: Mean ± SD followed with a: insignificantly different ($p > 0.05$). c, d: see footnote of Table (1).

Table 3: Influenced total lipid content (mg/g) in the larval tissues after treatment of the 3rd instar larvae of *G. mellonella* with LC_{50} values of certain arthropod venoms

Venom		Larval instar	
		5th	7th
A. mellifera	Mean ± SD	345.35 ± 101.6 d	618.62 ± 148.3 c
	Change (%)	29.94	149.70
V. orientalis	Mean ± SD	358.86 ± 93.8 d	528.53 ± 131.8 c
	Change (%)	35.03	113.33
L. quinquestriatus	Mean ± SD	363.36 ± 71.6 d	414.41 ± 240.3 c
	Change (%)	36.72	67.27
Control	Mean ± SD	265.77 ± 18.0	247.75 ± 99.6

Note: c, d: see footnote of Table (1).

each of the tested venoms, regardless the pupal age. In other words, the tested venoms unexceptionally enhanced the pupae to gain more lipids than the control congeners. For some detail, Apitoxin exhibited the most potent inducing action but scorpion venom exhibited the least enhancing action on the early-aged pupae to gain more lipids (31.68 and 20.79% increments, respectively). With regard to mid-aged pupae, Apitoxin exhibited the strongest enhancing effect while the scorpion venom exhibited the least one (15.54 and 0.48% increments, respectively). The wasp venom had the highest promoting potency against lipids of late-aged pupae but scorpion venom had the least potency (102.22 and 51.1% increments, respectively).

3.3. Effects of arthropod venoms on the total carbohydrate content of G. mellonella

According to the data distributed in Table 5, treatment of the 3rd instar larvae of *G. mellonella* with LC_{50} value of each venom resulted in a dramatic reduction of carbohydrate content in body tissues of both 5th and 7th instar larvae. In regard of the normal larvae, carbohydrate content decreased with the instar (0.0366 ± 0.007 and 0.0314 ± 0.006 mg/g, in 5th and 7th instars, respectively). For some detail, the least reducing action on

carbohydrates in the 5th larval instar was exerted by the wasp venom while a similar action on carbohydrates in 7th larval instar was exerted by the scorpion venom (21.04 and 11.78% reductions, respectively).

Table 4: Influenced total lipid content (mg/g) in the pupal tissues after treatment of the 3rd instar larvae of *G. mellonella* with LC_{50} values of certain arthropod venoms

Venom		Pupal age		
		Early	Mid	Late
A. mellifera	Mean ± SD	199.70 ± 2.6 c	172.67 ± 34.1 d	93.09 ± 13.0 d
	Change (%)	+31.68	+15.54	+37.77
V. orientalis	Mean ± SD	198.19 ± 11.9 c	162.16 ± 11.9 d	136.64 ± 60.0 d
	Change (%)	+30.69	+8.51	+102.22
L. quinquestriatus	Mean ± SD	183.18 ± 15.8 c	150.15 ± 6.9 d	102.10 ± 5.2 d
	Change (%)	+20.79	+0.48	+51.1
Control	Mean ± SD	151.65 ± 65	149.44 ± 25.2	67.57 ± 11.9

Note: c, d: see footnote of Table (1).

Table 5: Influenced total carbohydrate content (mg/g) in the larval tissues after treatment of the 3rd instar larvae of *G. mellonella* with LC_{50} values of certain arthropod venoms

Venom		Larval instar	
		5th	7th
A. mellifera	Mean ± SD	0.0262 ± 0.004 d	0.0237 ± 0.006 d
	Change (%)	–28.42	–24.52
V. orientalis	Mean ± SD	0.0289 ± 0.007 d	0.0253 ± 0.004 d
	Change (%)	–21.04	–19.43
L. quinquestriatus	Mean ± SD	0.0284 ± 0.009 d	0.0277 ± 0.003 d
	Change (%)	–22.40	–11.78
Control	Mean ± SD	0.0366 ± 0.007	0.0314 ± 0.006

Note: d: see footnote of Table (1).

Data of the determined carbohydrate content in the body tissues of pupae were assorted in Table 6. In the light of these data, the carbohydrate content gradually decreased in normal pupae with the age (0.0125 ± 0.003, 0.0101 ± 0.003 and 0.0063 ± 0.002 mg/g in the early-, mid- and late-aged pupae, respectively). As clearly seen in the same table, the carbohydrate content in treated pupae had been drastically reduced, regardless the tested venom. In respect of the early-aged pupae, the strongest reducing effect was exhibited by wasp venom (27.20% carbohydrate reduction) while the least reducing effect was exhibited by Apitoxin (19.20% carbohydrate reduction). In the mid-aged pupae, wasp venom had the most potent reducing potency (49.50% carbohydrate reduction) but scorpion venom had the least reducing potency (6.93% carbohydrate reduction). Apitoxin could not affect the carbohydrate content in the body tissues of late-aged pupae while the wasp venom exhibited the most powerful reducing effect (46.30% carbohydrate reduction).

Table 6: Influenced total carbohydrate content (mg/g) in the pupal tissues after treatment of the 3rd instar larvae of *G. mellonella* with LC_{50} values of certain arthropod venoms

Venom		Pupal age		
		Early	Mid	Late
A. mellifera	Mean ± SD	0.0101 ± 0.001 d	0.0057 ± 0.002 c	0.0063 ± 0.008 c
	Change (%)	–19.20	–43.56	0.00
V. orientalis	Mean ± SD	0.0091 ± 0.001 d	0.0051 ± 0.007 c	0.0034 ± 0.001 c
	Change (%)	–27.20	–49.50	–46.30
L. quinquestriatus	Mean ± SD	0.0093 ± 0.001 d	0.0060 ± 0.001 c	0.0047 ± 0.002 c
	Change (%)	–25.60	–6.93	–25.40
Control	Mean ± SD	0.0125 ± 0.003	0.0101 ± 0.003	0.0063 ± 0.002

Note: c, d: see footnote of Table (1).

4. Discussion

4.1. Disturbed protein content in G. mellonella by arthropod venoms

As reported by some authors (Cohen, 2010; and Sugumaran, 2010), proteins perform many functions within the living organisms, such as catalyzing metabolic reactions, replicating DNA, synthesis of ATP, enzymes and hormones controlling many chemical reactions in the cell metabolism. Also, protein metabolism plays a key role in rebuilding adult structures during the transformation of larvae/pupae into adults in insects, (Resmitha *et al*., 2014) as well as energy production and insect reproduction (Taþkýn and Aksoylar, 2011). Proteins regulate and integrate several physiological and metabolic processes in the insect body through hormones, enzymes and nucleoproteins (Chapman, 2012).

After treatment of the 3rd instar larvae of *G. mellonella* with venoms of *L. quinquestriatus* venom, *V. orientalis* venom or Apitoxin of *A. mellifera*, the total protein contents in both 5th and 7th instar larvae had been drastically reduced. Also, all venoms predominantly prevented the successfully developed pupae to attain normal protein level, since remarkably reduced protein content was estimated. This reduction of protein content in larvae and pupae of *G. mellonella*, in the present study, after larval treatment with the tested arthropod venoms can be interpreted in the light of some conceivable suggestions, as follows. (1) It was suggested that the protein plays a major role in synthesis of the microsomal detoxifying enzymes against toxicants (foreign compounds) entering into the insect body (Kyung and Kim, 1990; and Hassan, 2002). In the present study, reduction of proteins might be due to their binding with the tested arthropod venoms and therefore might reflect the depressed activity of the detoxifying enzymes. (2) The toxins' stress can inhibit the total proteins owing to the breakdown of protein into amino acids, so with the entrance of these amino acids to TCA cycle as a keto acid (Schoonhoven, 1982), they will help to supply energy for the insect (Etebari and Matindoost, 2004). Therefore, protein reduction in the tissues of *G. mellonella* might play a role in compensatory mechanisms under the present toxins' stresses, to provide intermediates to the Krebs cycle (Nath *et al*., 1997). (3) The protein reduction in the current study might, also, be due to the interference of the tested arthropod venoms with the insect endocrine system causing a hormonal imbalance (Sugumaran, 2010) and affecting the general metabolism (De Mark and Bennett, 1989) or protein synthesis, in particular (Padmaja and Rao, 2000). The tested *L. quinquestriatus* venom, *V. orientalis* venom or Apitoxin of *A. mellifera* might either act on the hormonal regulation of the protein synthesis, degradation and inhibition or act on the neurosecretory cells which control endocrine organs (Bouaziz *et al*., 2011; and Djeghader *et al*., 2014). Further investigation should be conducted in the foreseeable future for good understanding the modes of action of some chemical constituents of the tested venoms, in the present investigation.

4.2. Disturbed lipid content in G. mellonella by arthropod venoms

Lipids represent an important source of energy, hormone precursors and structural members for insects and are transported from their synthesis site of storage *via* the haemolymph towards the user organs (Dapporto *et al*., 2008; and Zhou and Miesfeld, 2009). In the current study, all venoms of *L. quinquestriatus, V. orientalis* and *A. mellifera* exhibited prevalent enhancing effects on larvae to gain more lipids than control larvae of both 5th and 7th instars. Also, all tested venoms unexceptionally induced the pupae to gain more lipids than the control congeners. This result of enhanced lipids disagreed with many results of reduced lipid content in different insect species after treatment with various toxic compounds. On the other hand, the present result was, in some extent, in agreement with the reported results of induced lipids in various insect species after larval treatment with some insect growth regulators (Ghoneim, 1994; Soltani-Mazouni *et al*., 1999; and Bouaziz *et al*., 2011).

To interpret the enhancement of lipids in larvae and pupae of *G. mellonella*, in the current work, it is important to point out that the influenced synthesis of lipids in insects has been resulted in disruptively affected physiology and subsequently deranged vital functions of growth and reproduction. The balance between quantity of lipids available for the reserves and the requests for these reserves can be disturbed by any toxic material (Canavoso *et al*., 2001). Therefore, induction of lipids in larvae and pupae of *G. mellonella* by the tested arthropod venoms might be due to their interference with not only the lipid synthesis but also the lipid mobilization as promoted to convert into other metabolites or fatty acids. Also, the increase of lipids might be due to the accumulation of carbohydrates which might lead to an inverse in their conversion rate to lipids as a reverse material. However, the exact interpretation of lipid induction in larvae and pupae of *G. mellonella*, in the present study, remains speculative.

4.3. Disturbed carbohydrate content in G. mellonella by arthropod venoms

Carbohydrates play an important role in the structure and function of all tissues during metamorphosis in insects. Also, carbohydrates are prerequisite metabolites for the normal functioning of male and female reproductive organs and embryonic development (cf. Chippendale, 1978). In general, carbohydrates play a key role in the physiology of those insects subjected to foreign toxins (Kaufmann and Brown, 2008). In the present investigation, treatment of the 3rd instar larvae with each of the tested venoms, *viz*., scorpion *L. quinquestriatus*, wasp *V. orientalis* and *A. mellifera*, resulted in dramatic reduction of carbohydrate content in tissues of 5th and 7th instar larvae. Also, carbohydrate content in treated pupae had been drastically reduced, regardless the tested venom or the pupal age.

The production or utilization of the main body metabolites, such as carbohydrates, is controlled by juvenile hormone or related to various hormonal organs and neurosecretion (Gade *et al*., 1997; Gade, 2004; and Sugumaran, 2010). Thus, the prevalent reduction of carbohydrate content in larvae and pupae of *G. mellonella*, in the present study, might be due to interference of the tested arthropod venoms with the hormonal regulation of carbohydrate metabolism (Imboden and Luscher, 1976) or to their effects on the carboxylase activity (Mukherjee and Sharma, 1996). Also, it is suggested that this carbohydrate depletion might be due to utilization of the reserved glucose sources of the larval tissues as a result of venoms' stresses (Sharma *et al*., 2011). In addition, the present result of carbohydrate reduction in *G. mellonella* might be due to stresses of the tested arthropod venoms on proteins to be degraded into amino acids to take part in the TCA cycle of acetic acid. Subsequently, the carbohydrate metabolic functions to make up for the lower energy were altered under the stress of present toxic materials in the body of *G. mellonella* (Nath *et al*., 1997; Nath, 2000). In general, detoxification in the larvae required a larger portion of consumed substances to be transformed into energy after arthropod venom treatments, in the current investigation, which might be another reason for the reduction in both proteins and carbohydrates in *G. mellonella* larvae (Xu *et al*., 2016).

Finally, it is important to mention that the disturbing effects of the tested arthropod venoms on the main metabolites in *G. mellonella*, in the present study, should be achieved by certain constituent (s) in each of these venoms; since venom of the scorpion *L. quinquestriatus* contains different compounds, such as polypeptide neurotoxins and basic proteins with low molecular weight bound to disulphide bridges especially (Hamon *et al*., 2002; and Kopygan *et al*., 2006); venom of the wasp *V. orientalis* contains a mixture of proteins and chemical constituents, such as serotonin, adrenaline, nor-adrenaline, dopamine, kinins and histidine (Biggs *et al*., 2007; and Klochkov *et al*., 2008); and Apitoxin of *A. mellifera* contains a complex mixture of proteins, peptides, and

low molecular components. The chief components are apamin, melittin and phospholipase A_2 (Khajehpour *et al*., 2004; and Bogdanov, 2012).

5. Conclusion

Depending on the present results, scorpion *L. quinquestriatus* venom, wasp *V. orientalis* venom and Apitoxin of *A. mellifera* caused reduction in proteins and carbohydrates but induced lipids, in the tissues of larvae and pupae of *G. mellonella*. Reduction of proteins might reflect the depressed activity of the detoxifying enzymes in this insect. The accumulated lipids in these tissues indicated a disruption of the balance between quantity of available lipids for the reserves and the requests for these reserves by processes, which caused by the tested venoms. Thus, these venoms seriously disturbed the main body metabolism. Therefore, the tested arthropod venoms may be good control agents, in an integrated management program against the present pest, *G. mellonella*. However, novel delivery systems should be explored in order to the insectotoxins can find their way into commercial applications in foreseeable future.

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