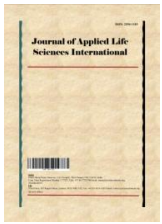

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# Quantitative and Qualitative Impacts of Selected Arthropod Venoms on the Larval Haemogram of the Greater Wax Moth, *Galleria mellonella* (Linnaeus) (Lepidoptera: Pyralidae)

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author KG designed the study and formulated the manuscript. Author KH tabulated the obtained data. Author MT carried out the statistical analysis of data. Author DE performed the practical work. All authors read and approved the final manuscript*

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## ABSTRACT

The greater wax moth, *Galleria mellonella* (Linnaeus) (Lepidoptera: Pyralidae) is the most destructive pest of honey bee, *Apis mellifera* Linnaeus (Hymenoptera: Apidae), throughout the world. The present study was conducted to determine the quantitative and qualitative impairing effects of the arthropod venoms, *viz*., death stalker scorpion *Leiurus quinquestriatus* (Hemprich & Ehrenberg) venom (SV), oriental Hornet (wasp) *Vespa orientalis* Linnaeus venom (WV) and Apitoxin of *A. mellifera* (AP) on the larval haemogram. For this purpose, the 3rd instar larvae were treated with $LC_{50}$ of each of these venoms (3428.9, 2412.6, and 956.16 ppm, respectively). The haematological investigation was conducted in haemolymph of the 5th and 7th (last) instar larvae. The important results could be summarized as follows. Five basic types of the freely circulating haemocytes in the haemolymph of last instar (7th) larvae of *G. mellonella* had been identified: Prohemocytes (PRs), Plasmatocytes (PLs), Granulocytes (GRs), Spherulocytes (SPs) and Oenocytoids (OEs). All venoms unexceptionally prohibited the larvae to produce normal hemocyte population (count). No certain trend of disturbance in the differential hemocyte counts of circulating

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hemocytes in larvae of *G. mellonella* after treatment with the arthropod venoms. Increasing or decreasing population of the circulating hemocytes seemed to depend on the potency of the venom, hemocyte type and the larval instar. In PRs of last instar larvae, some cytopathological features had been observed after treatment with AP or WV, but SV failed to cause cytopathological features. With regard to PLs, some cytopathological features had been observed after treatment with AP while both SV and WV failed to cause cytopathological features in this hemocyte type. No venom exhibited cytopathological effects on GRs, SPs or OEs.

*Keywords: Apitoxin; granulocytes; hornet; larva; oenocytoids; plasmatocytes; prohemocytes; scorpion; spherulocytes.*

## 1. INTRODUCTION

The greater wax moth *Galleria mellonella* (Linnaeus) (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 [1-4]. 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 [5-8]. Conventional insecticides of different categories had been used for controlling *G. mellonella* [9,10]. 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 [11-15]. The sterile insect technique (or inherited sterility) has been assessed against this pest [16-18]. Also, insect hormone analogues, insect growth regulators had been assessed against it [19-22]. Natural compounds of the plant origin may be efficient alternatives to conventional fumigants against *G. mellonella* [23-27].

In the last few decades, a great interest of investigation by agrochemical companies is the development of highly selective biopesticides derived from animals. Among many animal taxes, venomous arthropods are most successful in utilizing their venoms against predators and paralyzing their prey [28]. 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 [29-31], scorpions [32,33], wasps [34], as well as cone snails [35] and some marine animals [36-38]. In addition, the arthropod hormones and neuropeptides may be effective control agents against various insect pests [39, 40].

Scorpion is a mysterious creature in the animal world. It has poisonous venom [41] which has increasingly attracted the scientists' attention throughout the world [37,42,43]. The death stalker scorpion, or yellow scorpion, *Leiurus quinquestriatus* (Hemprich & Ehrenberg) (Buthidae: Arachnida) can be found in desert and scrubland habitats ranging from North Africa through to the Middle East. In Egypt, Saleh et al. [44] 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* Linnaeus [45]. As reported by some authors [46, 47], 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 [48,49]. However, Joseph and George [50] 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* Linnaeus (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) [51]. In a recent review, Azam et al. [52] 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 [53,54]. This venom exhibited toxic effects on some insects, such as the corn earworm *Heliothis zea* (Boddie) [55], the tobacco hornworm *Manduca sexta*

(Linnaeus) [54] and the lesser wax moth *Achroia grisella* (Fabricius) [56]. Recently, Ghoneim et al. [57] 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* [58].

The oriental hornet *Vespa orientalis* Linnaeus (Hymenoptera: Vespidae), is a social wasp in the Middle East [59,60]. The world distribution of this wasp species comprises, also, North Africa, southeastern Europe, Southwest Asia across Turkey and Arabian Peninsula to India and Nepal [61,62]. In addition, it was accidentally introduced into Madagascar and China [61,62] and recorded in Mexico [63]. Many studies had been conducted for examining the toxicity of wasp venoms on insects [64-67]. In Egypt, many studies had been conducted on *V. orientalis* focusing on ecology, biology, control and its dangerous effect on apiculture [68-70].

In insects, there is an open circulatory system containing various types of haemocytes. Haematological studies are very important in insect physiology because the haemocyte performs various physiological functions in the body [71-73]. The primary functions of haemocytes are: coagulation to prevent loss of blood, phagocytosis, encapsulation of foreign bodies in the insect body cavity, nodule formation, detoxification of metabolites and biological active materials and distribution of nutritive materials to various tissues and stored them also and may be hormones (for more detail, see: Ribeiro and Brehelin [74], Siddiqui and Al-Khalifa [75], Chavan et al. [76]. The insect pests may be controlled by disturbing their physiological activities, *viz*. feeding, moulting, reproduction and immune system [77]. Insects lack an acquired immune system like of the higher animals but have a well-developed innate response. The cellular defense of insects refers to haemocyte-mediated immune responses [78, 79].

Due to economical and ethical problems with the use of vertebrates in biomedical studies, insects have been suggested as alternative biomodels for toxicological preclinical studies [80,81]. In addition, insects have been widely used in other fields of biomedical research, such as neuroscience [82,83]. In general, knowledge of the haemogram of an insect is necessary to physiologists, toxicologists and biochemists, as alterations in hemocyte structure, types and number reflects changes in different physiological and biochemical processes [84, 85]. Therefore, the current study was conducted to investigate the disruptive effects of venoms of *L. quinquestriatus*, *A. mellifera* and *V. orientalis* on the most important parameters of larval haemogram of *G. mellonella*.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Insect

A culture of the greater wax moth *Galleria mellonella* (Linnaeus)(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). 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 [86,87], *G. mellonella* larvae in the present culture had been provided with an artificial diet as described by Bhatnagar and Bareth [88]. 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 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 & 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 [89]. The collected specimens were kept individually in plastic containers at 25-28$^{o}$C. The specimens were examined with a stereoscopic binocular microscope and taxonomically identified to the species using the morphological description keys [90-92].

Scorpion venom was obtained by electric stimulation (20 Volt) in the articulation of the telson according to Sarhan et al. [93]. 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 4ºC. The supernatant was pooled, freeze dried and stored at 20ºC. The lyophilized samples were dissolved in distilled water and centrifuged at 15000 r.p.m for 15 minutes at 4 ºC.

#### 2.2.2 Wasp collection and obtaining of venom

Adults of the oriental hornet (wasp) *Vespa orientalis* Linnaeus (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 (VSE) was carried out according to Friedman and Ishay [94] 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 [95]. Each 0.5g (100 venom sacs) was homogenized in 2ml solvent using the ultra homogenizer for 10 minutes. Then, it was centrifuged at 10000 r.p.m for 15 minutes 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* Linnaeus (Apidae: Hymenoptera: Insecta) workers. According to Dantas et al. [96], 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 Haematology Investigation

For the evaluation of disruptive effects of the present arthropod venoms on different haematological parameters, the 3$^{rd}$ instar larvae of *G. mellonella* were treated with $LC_{50}$ values of Apitoxin of *A. mellifera* (956.16 ppm), *V. orientalis* venom (2412.6 ppm), and *L. quinquestriatus* venom (3428.91 ppm). The successfully moulted 5$^{th}$ and 7$^{th}$ (last) instar larvae were used to examine the influenced hematological parameters.

#### 2.3.1 Collection of haemolymph

For conducting the hematological investigation, haemolymph was collected from the treated and control, 5$^{th}$ and 7$^{th}$ instar larvae. The haemolymph was obtained by amputation of one or two prothoracic legs, before coxa of the larva using fine scissors. Gentle pressure was done on the thorax for obtaining haemolymph drops by non-heparinized capillary tube. Seven replicates were used and the haemolymph from two individuals was never mixed.

#### 2.3.2 Hemocyte identification and influenced hematological parameters

Depending on the cell morphology, cytoplasmic ratio, cytoplasmic inclusions, shape of nucleus and dye-staining properties, the freely circulating haemocytes in the haemolymph of 5$^{th}$ and 7$^{th}$ (last) instar larvae of *G. mellonella* had been identified and distinguished basing on the technique described by some researchers [97-99]. Also, the influenced hematological criteria, after treatment of 3$^{rd}$ instar larvae with $LC_{50}$ values of in the arthropod venoms, had been examined in the previously mentioned later instars.

#### 2.3.3 Total haemocyte count

The haemolymph was collected into thoma-white blood cell diluting pipette to the mark (0.5). Diluting solution (Na Cl 4.65 gm, K Cl 0.15 gm,

$CaCl_2$ 0.11 gm, Crystal violet 0.05 gm and acetic acid 1.25 ml / liter distilled water) was taken up to the mark (11) on the pipette (dilution is 20 times). The first three drops were discharged to avoid errors. The mixture was dispended to the chamber of counting slide. After three minutes, the total numbers of cells recognized in 64 squares of the four corners were counted. If the cells clumped or uneven distributed, the preparation was discarded. The number of haemocytes per cubic millimeter was calculated according to the formula of Jones [100] as follows:

> Number of haemocyte counted per champer X dilution X depth factor / Number of 1 mm squares counted

Where the depth factor is usually 10.

#### 2.3.4 Differential haemocyte counts

Stained haemolymph preparations were carried out, according to Arnold and Hinks [101]. The haemolymph was smeared on clean glass slides, allowed to dry for 1 minute, and fixed for 2 minutes with drops of absolute methyl alcohol. Fixed cells were stained with Giemsa's solution (diluted 1 : 20 in distilled water) for 20 minutes, washed several times with tap water, and dipped in distilled water. The stained smears were air-dried and mounted in DPX with slip cover. The haemocytes were viewed under light microscope at a magnification 10 X 40 **=** 400 and 100 cells per slide were examined. The cell shape, cytoplasmic ratio, cytoplasmic inclusions and shape of nucleus were used for classification of haemocytes using the classification scheme of Brehelin and Zachary [102]. The percentages of haemocyte types were calculated by the formula:

$$\frac{\text{Number of each haemocyte type}}{\text{Total number of haemocytes examined}} \times 100$$

#### 2.3.5 Characterization of cytopathological features

For recording of the haemocyte deformities caused by the arthropod venoms, photomicrographs were obtained by using a light microscope provided with a camera at a magnification 10 X 40 = 400.

### 2.4 Statistical Data Analysis

Data obtained were analyzed by the Student's *t*-distribution, and refined by Bessel correction [103] for the test significance of difference between means.

## 3. RESULTS

### 3.1 Identification and Description of Normal Circulating Haemocytes in Larvae of *G. mellonella*

Depending on the cell shape, cytoplasmic ratio, cytoplasmic inclusions and shape of nucleus, the freely circulating haemocytes in the haemolymph of last instar ($7^{th}$) larvae of *G. mellonella*, in the present study, had been identified and distinguished into five basic types, *viz.*, Prohemocytes (PRs), Plasmatocytes (PLs), Granulocytes (GRs), Spherulocytes (SPs) and Oenocytoids (OEs). The most important diagnostic characteristics of each main type could be given as follows.

#### 3.1.1 PRs

PRs could be described as variable in size (3-7 µm wide and 6-8 µm long). They were observed as ovoid cells but nearly round or spherical in shape. It had a large centrally located nucleus and a prominent nucleolus. This nucleus occupied most of the cell volume. Abundant cytoplasm was deeply stained containing few organelles, such as sparse rough endoplasmic reticulum. Some vesiculation of the plasma membrane was evidently observed in few cases (see Fig. 1).

#### 3.1.2 PLs

PLs were observed as spindle-shaped cells and measured about 16x 4 µm. A large nucleus (occupying 40-50% of the cell volume) was observed as elongate, round or spherical and centric or eccentric in position with a distinct nucleolus. Cytoplasm was basophilic (faintly stained) and rich in organelles, such as a moderate amount of rough endoplasmic reticulum, many pinocytotic vesicles, scattered chromatin masses and several tapering projections (see Fig. 3).

#### 3.1.3 GRs

GRs appeared as spherical to ovoid cells of 10-12 µm in diameter. Nucleus was centrally located

and might be centric or eccentric occupying 45-55% of the cell volume. Nucleus had a number of scattered chromatin masses and nucleolus. Cytoplasm was basophilic (deeply stained) and contained few types of granules, endoplasmic reticulum and an occasional lipid droplet. A progressive accumulation of lipid droplets in this type of hemocytes might be give indication to misidentify it as ADs. Some GRs appeared with extrusion of granules (see Fig. 5).

#### 3.1.4 SPs

SPs were distinguished as basophilic or acidophilic cells of variable size (8-20 µm wide and 7-24 µm long). They were observed in a round or ovoid shape and characterized by several cytoplasmic inclusions as well as intracytoplasmic spherules occupying almost all the cytoplasm. These spherules contained either granular, fine-textured filaments or flocculent material. Some cells liberated the entire content of their spherules, leaving on the enclosing membranes. Nucleus appeared small, centric or eccentric in position, mostly deformed by the spherules (see Fig. 6).

#### 3.1.5 OEs

OEs were the largest hemocytes observed in the haemolymph of full grown larvae of *G. mellonella*. They were observed as spherical (22-35.5 µm in diameter) or ovoid (18.7-25 µm long and 26.5-35.6 µm wide) cells. When stained with Geimsa stain, cytoplasm was seen homogenous basophilic showing clusters of fibrous structures interspersed with scarce groups of some organelles, including round adipophilic granules. Nucleus was small, slightly eccentric and darkly stained (see Fig. 7).

### 3.2 Effects of Arthropod Venoms on the Total Hemocyte Count

In a preliminary experiment on *G. mellonella*, $LC_{50}$ values of the arthropod products, *viz*., death stalker scorpion, *Leiurus quinquestriatus*, oriental (Hornet) wasp, *Vespa orientalis* and Apitoxin of honey bee *Apis mellifera* were found 3428.9, 2412.6 and 956.16 ppm, respectively. After treatment of the 3rd instar larvae with $LC_{50}$ of each of these venoms, the newly moulted 5th and 7th instar larvae were used to investigate their effects on some important hematological parameters.

Data of the total hemocyte count (THC) in the haemolymph of 5th and 7th instar larvae were arranged in Table (1). Depending on these data, THC in normal larvae increased with the larval age (27400±38.6 and 28900±28.7 cells/$mm^3$ in 5th instar and 7th instar, respectively). Data of the same table revealed that all venoms unexceptionally prohibited the larvae to produce normal hemocyte population. According to the inhibitory potency, the tested arthropod venoms could be arranged as Apitoxin, *V. orientalis* venom and *L. quinquestriatus* venom (14.25, 11.68 and 08.03% THC reductions, respectively), in the case of 5th instar larvae. A similar trend could easily be seen in the previously mentioned table for 7th instar larvae (14.19, 10.38 and 05.54% THC reductions, by Apitoxin**,** *V. orientalis* venom and *L. quinquestriatus* venom, respectively).

### 3.3 Effects of Arthropod Venoms on the Differential Hemocyte Counts

#### 3.3.1 Fluctuated PRs population

As clearly seen in Table (2), the PRs population gradually decreased with the age of control larvae (38.0±2.8 and 37.7±3.3 cells/$mm^3$ in haemolymph of 5th instar and 7th instar, respectively). After treatment of 3rd instar larvae with $LC_{50}$ values of the tested arthropod venoms, data of differential hemocyte count (DHC) of PRs were assorted in the same table. Depending on these data, *L. quinquestriatus* venom was only the venom enhancing the 5th instar larvae to produce high PRs population (1.39% increment) while other venoms suppressed the larvae to produce normal PRs population. The strongest suppressing action was exerted by Apitoxin (7.4% PRs reduction), followed with *V. orientalis* venom (5.3% PRs reduction).

With regard to PRs population in 7th instar larvae, data of the same revealed that all tested venoms prevented them to produce normal PRs population. For comparative purpose, the reducing potencies of these venoms could be arranged as follows: Apitoxin**,** *V. orientalis* venom and *L. quinquestriatus* venom (14.3, 13.3 and 1.06% PRs reductions, respectively).

#### 3.3.2 Fluctuated PLs population

Data listed in Table (3) clearly revealed that the PLs population gradually decreased in haemolymph with the larval instar (9.6±0.5 and 8.8±0.4 cells/$mm^3$, in 5th and 7th instars,

respectively). After treatment of 3rd instar larvae with $LC_{50}$ of each of the venoms, data of disturbance in PLs population had been assorted in the same table. In the light of these data, the tested venoms prohibited these larvae to produce normal population of PLs. The strongest hindering effect was exhibited by *V. orientalis* venom, followed with Apitoxin and *L. quinquestriatus* venom (14.6, 8.3 and 6.25% PLs reductions, respectively). With regard to the 7th instar larvae, data of the same table revealed a contradictory action on PLs population, since 13.6 and 10.2% PLs increments were gained by the inducing effects of Apitoxin and *V. orientalis* venom, respectively. Only *L. quinquestriatus* venom exhibited reducing effect on PLs count in haemolymph of these last instar larvae (6.82% PLs reduction).

### 3.3.3 Fluctuated GRs population

In normal larvae of *G. mellonella*, data presented in Table (4) displayed a slight decrease of GRs population with the instar (14.4±0.8 and 14.3±2.5 cells/$mm^3$, in 5th instar and 7th instar, respectively). After treatment of 3rd instar larvae with the arthropod venoms, data of the same table revealed that Apitoxin suppressed the 5th instar larvae to produce normal GRs population (7.6% GRs reduction). In contrast, the larvae were stimulated to produce more GRs population by *V. orientalis* venom and *L. quinquestriatus* venom (18.1 and 4.86% GRs increments, respectively). In respect of GRs in haemolymph of 7th instar larvae, Apitoxin and *L. quinquestriatus* venom treatments resulted in reduced population of GRs (22.4 and 1.4% reductions, respectively) while *V. orientalis* venom enhanced the larvae to gain more GRs population.

### 3.3.4 Fluctuated SPs population

According to data of Table (5), SPs population gradually increased in normal larvae with age (18.9±0.8 and 20.2±0.3 cells/$mm^3$, in 5th instar and 7th instar larvae, respectively). For investigating the fluctuation of SPs in haemolymph after treatment of 3rd instar larvae with $LC_{50}$ values of the tested venoms, the same table indicated diverse effects of these products, as follows. In the 5th instar larvae, SPs population significantly increased after treatment with Apitoxin (17.5 % increased SPs population) while *L. quinquestriatus* venom and *V. orientalis* venom treatments resulted in decreased SPs population (6.35 and 0.5% reduction, respectively). In connection with the 7th instar larvae, only Apitoxin stimulated these larvae to produce increasing population of SPs (23.8% increment) while other products prevented the larvae to attain the normal SPs population (12.9 and 0.99% SPs reductions, by *V. orientalis* venom and *L. quinquestriatus* venom, respectively).

### 3.3.5 Fluctuated OEs population

Data assorted in Table (6) clearly revealed a slight increase of OEs population in normal larvae with the age (19.2±1.1 and 19.4±0.7 cells/$mm^3$, in 5th instar and 7th instar larvae, respectively). As exiguously shown in the same table, 5th instar larvae had been enhanced to produce more OEs in haemolymph after treatment of 3rd instar larvae with Apitoxin and *L. quinquestriatus* venom (12.0 and 6.77% OEs increasing population, respectively). On the contrary, *V. orientalis* venom exhibited an inhibitory effect on OEs population in larvae. With regard to 7th instar larvae, *V. orientalis* venom and Apitoxin enhanced them to produce an increasing OEs population while *L. quinquestriatus* venom reduced it (for detail, see Table 6).

In conclusion, data distributed in Tables 2-6 revealed no certain trend of the disturbance in different hemocyte populations because increasing or decreasing population of these circulating hemocytes depended on the potency of the tested arthropod venoms, hemocyte type and the larval instar. In other words, the tested venoms exerted diverse actions on the differentiated hemocyte counts.

## 3.4 Qualitative Effects of Arthropod Venoms on the Hemocyte Profile

Depending on the available technique, the last (7th) instar larvae were used for this parameter of the present haematological investigation, because of enough haemolymph samples and cytopathological features were elaborately photographed.

### 3.4.1 Impaired profile of PRs

To shed some light on the cytopathological impacts of the tested arthropod venoms on PRs in haemolymph of last instar larvae of *G. mellonella*, photomicrographs in Fig. (2) clearly demonstrated some deformations after treatment of 3rd instar larvae with $LC_{50}$ of Apitoxin, such as

darkly stained cells with degenerated nucleus, destroyed membrane and extruded cytoplasmic contents. *V. orientalis* venom caused different features of deranged PRs, such as degenerated nuclei, destroyed membranes and extruded cytoplasmic contents. *L. quinquestriatus* venom failed to cause cytopathological features in this hemocyte type.

### 3.4.2 Impaired profile of PLs

Fig. 4 contains photomicrographs of cytopathological features in PLs after treatment with the present venoms. Apitoxin caused darkly stained degenerated nuclei and vacuolated cytoplasm. No cytopathological features could be observed after treatment with *V. orientalis* venom or *L. quinquestriatus* venom.

### 3.4.3 Impaired profiles of GRs, SPs and OEs

After treatment of 3rd instar larvae of *G. mellonella* with $LC_{50}$ values of Apitoxin, wasp venom or scorpion venom, no venom could exhibit any cytopathological effect on GRs, SPs or OEs.

**Table 1. Total haemocyte counts (cell/mm$^3$) in the *G. mellonella* larvae as affected by $LC_{50}$ values of selected arthropod venoms**

| Venom | | Larval instar | |
|---|---|---|---|
| | | 5th | 7th |
| Apitoxin of *A. mellifera* | Mean±SD | 23500±65.5 d | 24800±98.5 d |
| | Change (%) | -14.23 | -14.19 |
| Venom of wasp *V. orientalis* | Mean±SD | 24200±105.7 c | 25900±108.4 d |
| | Change (%) | -11.68 | -10.38 |
| Venom of scorpion *L. quinquestriatus* | Mean±SD | 25200±101.1 c | 27300±122.2 b |
| | Change (%) | -8.03 | -5.54 |
| **Control** | Mean±SD | 27400±38.6 | 28900±28.7 |

*Mean±SD followed with c: highly significantly different (P<0.01). d: very highly significantly different (P<0.001).*

**Table 2. Differential Prohemocyte count (Mean±SD) in *G. mellonella* larvae as disturbed by selected arthropod venoms**

| Larval instar | | Venom | | |
|---|---|---|---|---|
| | | **Apitoxin of *A. mellifera*** | **Venom of wasp *V. orientalis*** | **Venom of scorpion *L. quinquestriatus*** |
| 5th | Treated | 35.2±3.0 b | 36.0±3.8 b | 36.6±3.1 b |
| | Control | 38.0±2.8 | 38.0±2.8 | 38.0±2.8 |
| | Change (%) | -7.4 | -5.3 | +1.39 |
| 7th | Treated | 32.3±1.4 d | 32.7±1.9 b | 37.3±2.5 c |
| | Control | 37.7±3.3 | 37.7±3.3 | 37.7±3.3 |
| | Change (%) | -14.3 | -13.3 | -1.06 |

*Mean±SD followed with b: significantly different (P<0.05), c, d: see footnote of Table 1.*

**Table 3. Differential Plasmatocyte count (Mean±SD) in *G. mellonella* larvae as disturbed by selected arthropod venoms**

| Larval instar | | Venom | | |
|---|---|---|---|---|
| | | **Apitoxin of *A. mellifera*** | **Venom of wasp *V. orientalis*** | **Venom of scorpion *L. quinquestriatus*** |
| 5th | Treated | 8.8±1.1 c | 8.2±2.2 b | 9.0±1.8 a |
| | Control | 9.6±0.5 | 9.6±0.5 | 9.6±0.5 |
| | Change (%) | -8.3 | -14.6 | -6.25 |
| 7th | Treated | 10.0±0.5 b | 7.9±1.5 c | 8.2±1.0 b |
| | Control | 8.8±0.4 | 8.8±0.4 | 8.8±0.4 |
| | Change (%) | +13.6 | +10.2 | -6.82 |

*Mean±SD followed with a: insignificantly different (P>0.05). b: see footnote of Table 2. (c): see footnote of Table 1*

**Table 4. Differential Granulocyte count (Mean±SD) in *G. mellonella* larvae as disturbed by selected arthropod venoms**

| Larval instar | | Venom | | |
|---|---|---|---|---|
| | | **Apitoxin of *A. mellifera*** | **Venom of wasp *V. orientalis*** | **Venom of scorpion *L. quinquestriatus*** |
| **5th** | Treated | 13.3±0.7 b | 17.0±0.9 a | 15.1±0.9 a |
| | Control | 14.4±0.8 | 14.4±0.8 | 14.4±0.8 |
| | Change (%) | -7.6 | +18.1 | +4.86 |
| **7th** | Treated | 11.1±1.1 c | 16.6±3.1 b | 14.1±1.3 a |
| | Control | 14.3±2.5 | 14.3±2.5 | 14.3±2.5 |
| | Change (%) | -22.4 | +16.1 | -1.40 |

*a: see footnote of Table-3. b: see footnote of Table 2. c: see footnote of Table 1.*

**Table 5. Differential Spherulocyte count (Mean±SD) in *G. mellonella* larvae as disturbed by selected arthropod venoms**

| Larval instar | | Venom | | |
|---|---|---|---|---|
| | | **Apitoxin of *A. mellifera*** | **Venom of wasp *V. orientalis*** | **Venom of scorpion *L. quinquestriatus*** |
| **5th** | Treated | 22.2±1.2 c | 18.8±2.1 a | 17.7±3.2 a |
| | Control | 18.9±0.8 | 18.9±0.8 | 18.9±0.8 |
| | Change (%) | +17.5 | -0.5 | -6.35 |
| **7th** | Treated | 25.0±3.0 a | 17.6±1.1 a | 20±2.2 a |
| | Control | 20.2±0.3 | 20.2±0.3 | 20.2±0.3 |
| | Change (%) | +23.8 | -12.9 | -0.99 |

*a: see footnote of Table 3. c: see footnote of Table 1.*

**Table 6. Differential Oenocytoid count (Mean±SD) in *G. mellonella* larvae as disturbed by selected arthropod venoms**

| Larval instar | | Venom | | |
|---|---|---|---|---|
| | | **Apitoxin of *A. mellifera*** | **Venom of wasp *V. orientalis*** | **Venom of scorpion *L. quinquestriatus*** |
| **5th** | Treated | 21.5±2.0 a | 19.0±1.9 a | 20.5±2.2 a |
| | Control | 19.2±1.1 | 19.2±1.1 | 19.2±1.1 |
| | Change (%) | +12.0 | -1.0 | +6.77 |
| **7th** | Treated | 20.6±1.4 a | 25.1±2.0 b | 19.0±0.8 a |
| | Control | 19.4±0.7 | 19.4±0.7 | 19.4±0.7 |
| | Change (%) | +6.2 | +29.4 | -2.06 |

*a: see footnote of Table 3. b: see footnote of Table 2.*

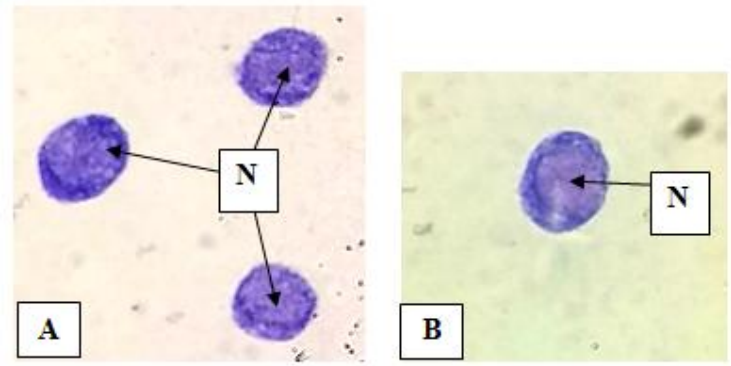


**Fig. 1. Photomicrographs of Prohemocytes (PRs) in the haemolymph of last (7th) instar larvae of *G. mellonella* (Geimsa stain, 1000x). [A] & [B]: Typical normal cells. N: nucleus**

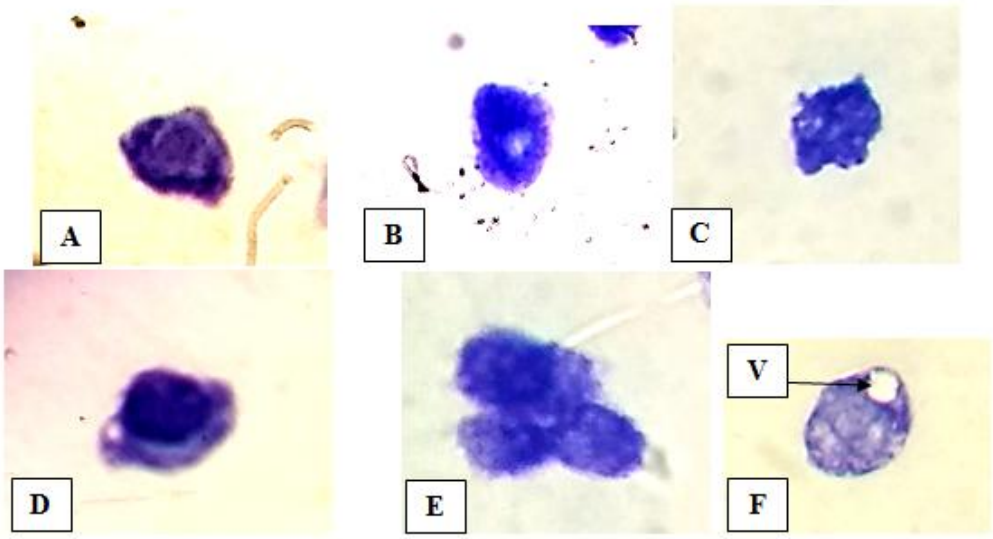


**Fig. 2. Photomicrographs of Prohemocytes (PRs) in the haemolymph of last (7th) instar larvae of G. mellonella (Geimsa stain, 1000x). [C] & [D]: PRs deformations by $LC_{50}$ of Apitoxin: darkly stained cells with degenerated nucleus, destroyed membrane and extruded cytoplasmic contents. [E] & [F]: PRs deformations by $LC_{50}$ of the wasp venom: degenerated nuclei, destroyed membranes and extruded cytoplasmic contents. Degenerated nucleus and vacuolated cytoplasm, V: vacuole [F]**

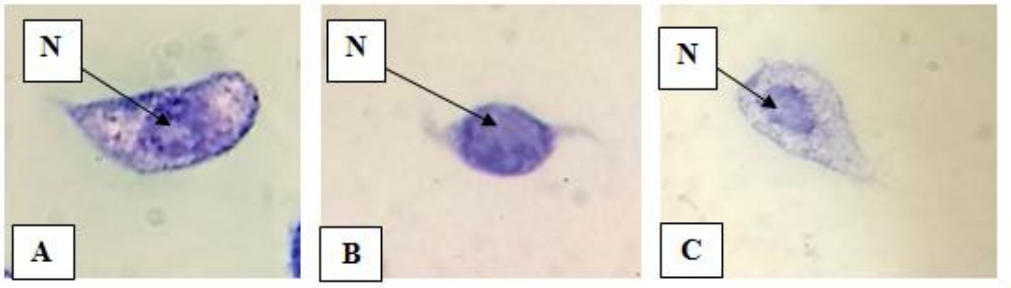


**Fig. 3. Photomicrographs of Plasmatocytes (PLs) in the haemolymph of last ($7^{th}$) instar larvae of *G. mellonella* (Geimsa stain, 1000x). [A], [B] & [C]: Typical normal cells. N: nucleus**

## 4. DISCUSSION

Haematological studies are very important in insect physiology because the haemocyte performs various physiological functions in the body. The primary functions of haemocytes are: coagulation to prevent loss of blood, phagocytosis, encapsulation of foreign bodies in the insect body cavity, nodule formation, detoxification of metabolites and biological active materials and distribution of nutritive materials to various tissues and stored them also and may be hormones (for more detail, see: Garcia and Rosales [71], Zhou et al. [72], Ling and Yu [73], Ribeiro and Brehelin [74], Siddiqui and Al-Khalifa [75], Chavan et al. [76]).

The insect haemogram serves as a good indicator of the insect physiology during growth and adulthood [104], as well as the environmental adaptability in each developmental stage of insects [105-107]. Also, the insect haemogram is suggested to be a useful tool for investigation of toxic effects of toxic materials on biocontrol agents because alterations in structure, types and number of cells reflect changes in physiological and biochemical processes [84,85,108].

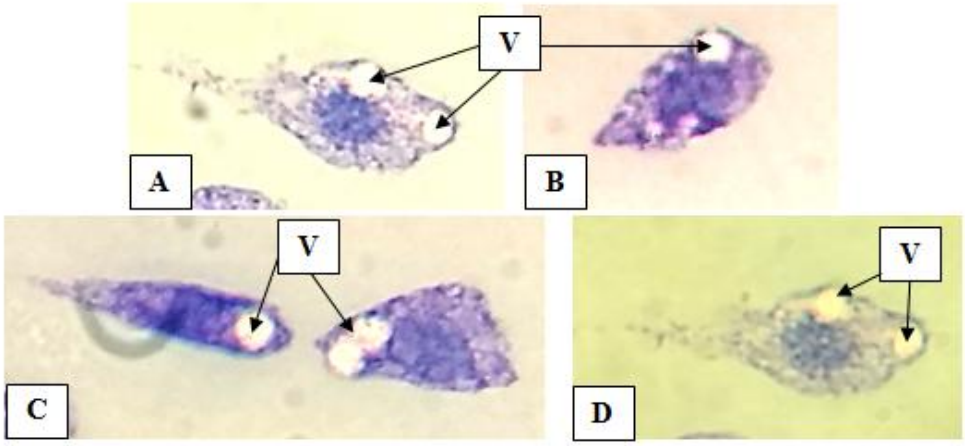


**Fig. 4. Photomicrographs of Plasmatocytes (PLs) in the haemolymph of last (7th) instar larvae of *G. mellonella* (Geimsa stain, 1000x). [C] & [D]: PLs deformations by LC50 of Apitoxin: darkly stained degenerated nuclei and vacuolated cytoplasm. V: vacuole.**

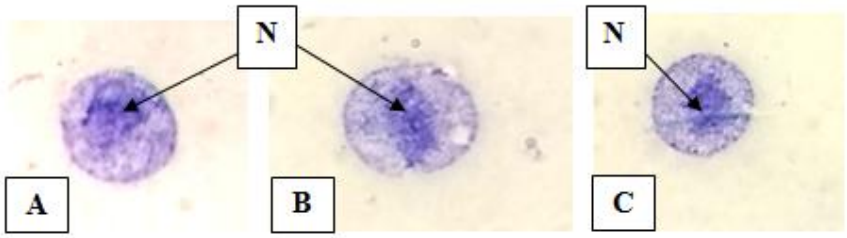


**Fig. 5. Photomicrographs of Granulocytes (GRs) in the haemolymph of last (7th) instar larvae of *G. mellonella* (Geimsa stain, 1000x). [A], [B] & [C]: Typical normal cells. N: nucleus. All tested venoms failed to cause cytopathological effect on GRs**

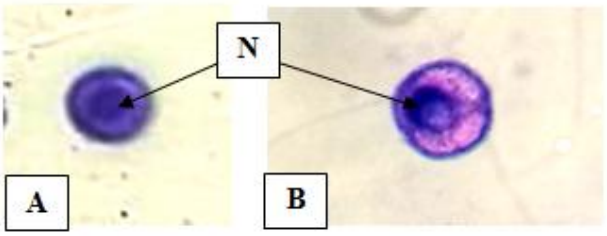


**Fig. 6. Photomicrographs of Spherulocytes (SPs) in the haemolymph of last (7th) instar larvae of *G. mellonella* (Geimsa stain, 1000x). [A] & [B]: Typical normal cells. N: nucleus. All tested venoms failed to cause cytopathological effect on SPs**

### 4.1 Identification of Normal Circulating Haemocytes in Larvae of *G. mellonella*

Since hemocytes are involved in the key insect physiological functions, circulating hemocytes provide an excellent model system to study the cell development, differentiation and their role in the immune system [79,109,110]. In other words, the knowledge of normal haemocytes of an insect is necessary to physiologists, toxicologists and biochemists [84,111].

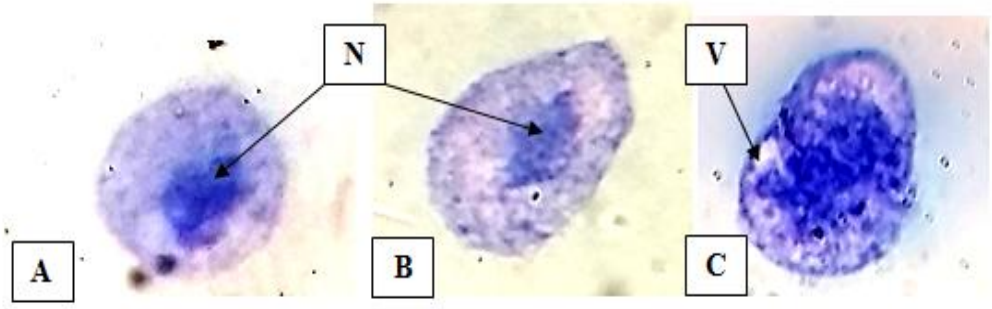


**Fig. 7. Photomicrographs of Oenocytoids (OEs) in the haemolymph of last (7th) instar larvae of *G. mellonella* (Geimsa stain, 1000x). [A] & [B]: Typical normal cells. N: nucleus. All tested venoms failed to cause cytopathological effect on OEs**

In insects, the most common types are prohaemocytes (PRs), plasmatocytes (PLs), granulocytes (GRs), spherulocytes (SPs), adiphohaemocytes (ADs), coagulocytes (CGs) and oenocytoids (OEs). It is important to emphasize that not all these hemocyte types exist in all insect species [112-115]. However, their characteristic features are slightly differing in various insect species [74,116,117]. Also, there is confusion between various haemocyte types, such as PRs and PLs as well as GRs and ADs [118]. For detail, see review of Ghoneim [119].

In the present study, different diagnostic characteristics, such as the cell shape and size, cytoplasmic ratio, cytoplasmic inclusions and shape of nucleus, were used to identify five basic types of the freely circulating haemocytes in last instar (7th) larvae of the greater wax moth *Galleria mellonella*: PRs, PLs, GRs, SPs and OEs. The most important descriptive characters of each main type were given. This result was in agreement with the five hemocyte types distinguished by an earlier study of Ashhurst and Richards [120] in larvae of *G. mellonella*: PRs, PLs, ADs, OEs, and SPs. Also, some researchers [97-99] identified five hemocyte types in the same insect: PRs, PLs, GRs, OEs and SPs. In addition, Sezer and Ozalp [121] identified five hemocyte types in the pupal haemolymph of *G. mellonella*: PRs, PLs, GRs, SPs and OEs. On the other hand, the present result disagreed with many reported results being distinguished other number of circulating haemocytes in *G. mellonella*, such as Shrivastava and Richards [122] who reported at least three types of hemocytes; PRs, GRs and PLs. Identification of each type by light microscope had often been perplexing, especially for GRs which were difficult to be distinguished from PRs [123,124]. Also, three hemocyte types in haemolymph of larvae were observed under fluorescence microscope: PLs, GRs, and PRs [125]. On the other hand, Er et al. [27] distinguished four types of circulating hemocytes in the last instar larvae of the same insect: GRs, PLs, PRs and OEs.

To understand the controversial number and types of the circulating hemocytes in haemolymph of *G. mellonella* larvae, it is important to point out that the used nomenclature or terminology for hemocytes has often complicated comparisons of hemocyte categories in different insect orders [126,127]. For example, the larval hemocytes of Lepidoptera are typically identified by field or phase microscopy whereas this conventional method of hemocyte classification has been the source of frequent controversy in other insect orders [123]; since the hemocyte terminology bases on morphological features which often differ from order to order. There are over 70 different names used for just 6-9 hemocyte types [128]. Thus, there is a need to develop a more uniform terminology for naming hemocytes in different insect species (for review, see Ghoneim [119].

On the other hand, the non-uniformity and considerable differences in haemocyte classification in insects may arise from several causes, such as differences in experimental treatments, observation of living haemocytes as opposed to fixed specimens, morphological changes of haemocytes after withdrawal, and the tendency of some researchers to simplify haemocyte classification [129]. Also, the number, type and morphology of haemocytes vary with the developmental stages of the test insects and their physiological conditions, i.e., there is an

inherent variability of haemocyte types within a species as well as among closely related species [130-132]. Also, the hemocyte classification is often influenced by some factors affecting the haemolymph physical properties or biochemical composition [133]. In addition, the differences in number and types of identified hemocytes in insects may be attributed to several technical difficulties and the characters adopted by other researchers [74,129]. Moreover, many erroneous descriptions of certain hemocytes may be attributed to the rapid transformation of certain hemocytes during or soon after haemolymph collection [102]. Various techniques often yield profound different information about types, number, distribution and functions of haemocytes (for more detail, see Qamar and Jamal [84], Ling et al. [124], Pandey and Tiwari [134], Pandey and Tiwari [110]). In the light of the reported diverse or contradictory results, none of the individual methods for studying the various morphological types of haemocytes was entirely satisfactory for all types of cells within a given insect [135]. Therefore, the hemocyte classification has been recommended to be revised several times in the same insect species [75,119,136-138].

## 4.2 Total Hemocyte Count (THC) in *G. mellonella*

Haemogram is a statement of the haemocyte population picture in an insect at a given time. It is a quantitative (Total haemocyte count, THC) and qualitative (Differential haemocyte count, DHC) expression of the haemolymph and its constituent inclusions [139]. Haemogram parameters include, also, haemolymph (blood) volume, mitotic index and cytological features of hemocytes. The THC, or total hemocyte population, has been found to be quite variable depending upon the insect species, developmental stage, physiological state and the technique followed [140].

### 4.2.1 THC in Normal Larvae of *G. mellonella*

In insects, the THC, or total hemocyte population, has been found to be quite variable depending upon the insect species, developmental stage, physiological state and the used technique [140]. In the present study, THC in normal 5$^{th}$ and 7$^{th}$ instar larvae of *G. mellonella* slightly increased with the larval age (27400±38.6 and 28900±28.7 cells/mm$^3$ in the 5$^{th}$ instar larvae and 7$^{th}$ instar larvae, respectively). This result disagreed with other estimates reported for different insect species, since Hassan [141] determined THC in haemolymph of normal larvae of *Tryporyza* sp. as average 22475cells/mm$^3$. The same author recorded THC in haemolymph of *Meladera* sp. as average 22300cells/mm$^3$ in males and 29100cells/mm$^3$ in females. On the other hand, Mall and Gupta [142] estimated THC of red pumpkin beetle *Aulacophora foveicollis* (Lucas) as average 5500cells/mm$^3$. Sabri and Tariq [143] determined THC of the same beetle as 4372 cells/mm$^3$. Chavan et al. [76] estimated the THC in haemolymph of normal larvae of the beetle *Platynotus belli* Fairmere in an average of 26233.33±251.66 cells/mm$^3$. On the other hand, our result in *G. mellonella* was found in agreement with that increasing THC in the pink bollworm *Pectinophora gossypiella* (Saunders) larvae, since the averages of 7213±716.91 cells/mm$^3$ and 10138±918.67 cells/mm$^3$ had been recorded in 6 hr and 48 hr full grown larvae, respectively [144]. Thus, the total hemocyte population in normal larvae of *G. mellonella*, in the present study, increased toward the prepupae as a physiological event for preparation to moult into the pupal stage.

It is important to shed some light on the varying hemocyte populations in the haemolymph of some insects, as reported in the available literature. The largest hemocyte count in haemolymph of last instar larvae of *Spodoptera mauritia* was estimated for PLs, followed by other hemocyte types [145]. In normal larvae of the beetle *P. belli*, Chavan et al. [76] estimated GRs count as the highest population, followed by PRs, ADs, OEs, PLs, Coagulocyte and SPs, respectively. As recorded by Ghoneim et al. [144] for *P. gossypiella*, the circulating ADs had been observed with the largest count, followed by other hemocyte types, regardless the age of larvae while the least hemocyte population was estimated for OEs, regardless the age. In the present study, the largest hemocyte population in haemolymph of the normal 5$^{th}$ instar larvae of *G. mellonella* was estimated for PRs, followed with OEs, SPs, GRs and PLs, respectively. In addition, the largest hemocyte population in haemolymph of the normal 7$^{th}$ instar larvae was estimated for PRs, followed with SPs, OEs, GRs and PLs, respectively.

### 4.2.2 Inhibited THC in larvae of *G. mellonella* by arthropod venoms

It may be important to mention that the brain endocrine complex is involved in haemocyte accumulation following some initial stimulus [146]. Jones [147] suggested that ecdysteroids

can regulate the number of haemocytes. Hormones, synthetic pesticides, insect growth regulators, and toxins intervene in the intermediary metabolism and immune capability of insects as observed in changes in hemocyte number, differentiation and phagocytosis [84]. Responses of the total hemocyte count to chemicals, phagocytosis, encapsulation and metamorphosis in insects had been reviewed by Siddiqui and Al-Khalifa [148].

For investigating the effects of tested arthropod venoms on THC in haemolymph of 5th and 7th instar larvae of *G. mellonella*, in the present study, the 3rd instar larvae were treated with $LC_{50}$ of death stalker scorpion, *Leiurus quinquestriatus*, oriental (Hornet) wasp, *Vespa orientalis* or Apitoxin of honey bee *Apis mellifera*. All venoms unexceptionally prohibited the larvae to produce normal hemocyte population (count). According to the inhibitory potency, the tested arthropod venoms could be arranged as Apitoxin, *V. orientalis* venom and *L. quinquestriatus* venom, respectively, in both larval instars. These results corroborated with those reported results of decreased THC in larvae of different insects, as response to various insecticides or insect growth regulators (IGRs), such as *Rhynocoris kumarii* Ambrose and Livingstone by endosulfan [129]; *Schistocerca gregaria* Forsskål by spinosad and Proclaim® insecticide [149]; *Papilio demoleus* Linnaeus by Methoprene [150]; *Dysderus koenigii* (Fabricius) by Penfluron [151]; *Agrotis ipsilon* (Hufnagel) by Diflubenzuron [152], *Eurygaster integriceps* Puton by Pyriproxyfen [153]; *Ephestia kuehniella* Zeller by Pyriproxyfen [154], *Spodoptera littoralis* (Boisduval) by Cyromazine [136]; *etc*.

The predominant inhibitory effect of arthropod venoms on THC in *G. mellonella*, in the present investigation, might be correlated with the decrease of some hemocyte types involved in phagocytosis and nodule formation. Reduction of THC might be due to the toxicities of the tested venoms and their inhibitory effects on the insect endocrine organs and secretion, nodule formation, larval hematopoietic function or the cell proliferation [155,143,156,157,153]. In addition, THC declination may be attributed to the death of pathological cells by degeneration [150].

## 4.3 Influenced Differential Hemocyte Counts by Arthropod Venoms

It is important to point out that the increasing DHC of certain haemocyte types and decreasing DHC of others may be due to the transformation of some types into other ones for achieving the phagocytic function or other tasks for defense against the foreign biotic targets, like bacteria, yeast and apoptic bodies as well as the abiotic materials, such as particles of Indian ink or toxic plant products [158,159]. The particular hemocytes reported to be phagocytic varies among insect taxa, and in some cases discrepancies even exist in the literature among studies on the same species [160]. For more detail, see review of Ghoneim [119].

### 4.3.1 Fluctuated PRs population in haemolymph of larvae

In the present study, the differential hemocyte count (DHC) of PRs gradually decreased with the age of normal larvae of *G. mellonella*. After treatment of 3rd instar larvae with $LC_{50}$ values of the tested arthropod venoms, *L. quinquestriatus* venom was only the venom enhancing the 5th instar larvae to produce high PRs population while other venoms suppressed the larvae to produce normal PRs population. With regard to the PRs population in 7th instar larvae, all venoms prohibited them to produce normal PRs population. These results were found in agreement with many reported results of inhibitory actions of different insecticides and IGRs on PRs population in haemolymph of some insects, such as *S. littoralis* by Cyromazine [136], *A. ipsilon* by Diflubenzuron [152], *Philosamia ricini* Watson by Dimethoate [161], *Spodoptera mauritia* (Boisduval) by Flufenoxuron [145], *P. gossypiella* by Novaluron [144], *etc*. However, the general reduction of PRs population in larvae of *G. mellonella*, in the present study, may be attributed either to the cytotoxic effects of the tested arthropod venoms on mitotic division of PRs, conversion of PRs to other hemocyte types or to the inhibitory effects on the activity of haematopoietic organs responsible for PRs production [153].

### 4.3.2 Fluctuated PLs population in haemolymph of larvae

The role of PLs in phagocytosis is disputed because some authors believed that they are phagocytes [73,160] but other authors reported no phagocytic function [118,162]. In the present study, PLs population gradually decreased in haemolymph of *G. mellonella* with the larval instar. In the present study, also, treatment of 3rd instar larvae with $LC_{50}$ of each of the arthropod venoms resulted in the reduction of PLs population in 5th instar larvae. With regard to the

7th instar larvae, only *L. quinquestriatus* venom exhibited reducing effect on PLs count but Apitoxin and *V. orientalis* venom enhanced the PLs population.

The decreasing PLs population in larval haemolymph of *G. mellonella*, as response of certain arthropod venoms in the present study, was in accordance with those reported decreasing PLs count in haemolymph of some insects by various IGRs or insecticides, such as *S. littoralis* by Flufenoxuron [163] or Novaluron [136] as well as *S. gregaria* by Spinosad and proclaim [149] and *S. mauritia* by Flufenoxuron [145]. On the other hand, the enhanced PLs population in larval haemolymph of *G. mellonella* by certain arthropod venoms, in the present study, agreed with some results of increasing PLs in some insects by certain toxins and IGRs, such as *S. littoralis* by Cyromazine [136]; *S. gregaria* nymphs by Deltamethrin [164], *R. kumarii* by endosulfan [129]; *A. ipsilon* by Diflubenzuron [152]; *S. litura* by hexaflumuron [157]; *etc*. The decreasing PLs population in the current work on *G. mellonella* can be explained by their transformation into other types of hemocytes [135], since they are highly polymorphic cells [164]. Also, certain arthropod venoms may impaired the haematopoietic organs which responsible for the production of PLs [165]. However, we cannot provide an appreciable interpretation to the enhanced PLs population, by some of tested venoms, at the present time!!

#### 4.3.3 Fluctuated GRs population in haemolymph of larvae

One of the main functions of GRs is phagocytosis as reported by several authors in different insects, such as Tojo et al. [160] in *G. mellonella*, Pendland and Boucias [166] in *Spodoptera exigua* (Hübner), Butt and Shields [167] in *Lymantria dispar* (Linnaeus), Nardi et al. [168] in *M. sexta*, and Costa et al. [169] in *S. littoralis*. In the present study, a slight decrease of GRs population was recorded from 5th to 7th instar of normal larvae of *G. mellonella*. As shown in the present study, Apitoxin suppressed the 5th instar larvae to produce normal GRs population while *V. orientalis* venom and *L. quinquestriatus* venom enhanced the larvae to produce more GRs population. In respect of 7th instar larvae, Apitoxin and *L. quinquestriatus* venom reduced the GRs population while *V. orientalis* venom enhanced the larvae to gain more GRs population. However, the reduction of GRs population in *G. mellonella* larvae by certain arthropod venoms, in the present study, may be interpreted by the death of a lot of them due to their detoxification activity against the toxic molecules [129,168-170]. Also, it might be due to their differentiation into other types of hemocytes since GRs can differentiate into SPs in another lepidopteran *Bombyx mori* (Linnaeus) [111]. However, we have no exact interpretation to the increasing GRs population in *G. mellonella* larvae after treatment with some of the tested arthropod venoms, right now!!

#### 4.3.4 Fluctuated SPs population in haemolymph of larvae

In Lepidoptera, SPs are quite different from GRs overloaded with phagocytosed material. The functions of SPs are unknown until now [74] but Sass et al. [171] suggested their responsibility for transporting cuticular components. In the present study, SPs population gradually increased in normal larvae of *G. mellonella* with age. After treatment of 3rd instar larvae with $LC_{50}$ values of the tested venoms, diverse effects had been recorded. In the 5th instar larvae, SPs population significantly increased by Apitoxin while *L. quinquestriatus* venom and *V. orientalis* venom suppressed the SPs population. In connection with the 7th instar larvae, only Apitoxin stimulated these larvae to produce increasing population of SPs while other venoms prevented the larvae to attain the normal SPs population. However, the enhanced SPs population in haemolymph of *G. mellonella* larvae after treatment with certain arthropod venoms might be due to their enhancing effects on the differentiation of SPs or transformation of other hemocytes into SPs in the treated larvae of *G. mellonella*. Unfortunately the interpretation of declined SPs population is still obscure!!

#### 4.3.5 Fluctuated OEs population in haemolymph of larvae

In the present study, a slight increase of OEs population was estimated in the normal larvae with the age. The 5th instar larvae had been enhanced to produce increasing OEs in haemolymph after treatment of 3rd instar larvae with Apitoxin and *L. quinquestriatus* venom. On the contrary, *V. orientalis* venom exhibited an inhibitory effect on OEs population in larvae. With regard to 7th instar larvae, *V. orientalis* venom and Apitoxin enhanced them to produce an increasing OEs population while *L. quinquestriatus* venom reduced it. The

decreasing OEs population in the haemolymph of *G. mellonella* larvae after treatment with certain venoms, in the present study, might be due to degeneration of some OEs for releasing precursors of prophenoloxidase that likely play a role in melanization of haemolymph and an important immunity protein in insects [172]. On the other hand, increasing of OEs population in the larval haemolymph of larvae, after treatment with other arthropod venoms, might be due to their role in the detoxification of toxic materials and activating action of some tested products on the hematopoietic organs or cell mitotic division. In conclusion, no certain trend of the disturbance in different hemocyte populations (counts) had been caused by the tested arthropod venoms. The increasing or decreasing population of the circulating hemocytes seemed to depend on the potency of the venoms, hemocyte type and the larval instar. In other words, the tested venoms exerted diverse actions on the differentiated hemocyte counts.

### 4.4 Qualitatively Impaired Hemocyte Profile by Arthropod Venoms

As reported by El-Kattan [173] for *Plodia interpunctella* (Hübner), Qamar and Jamal [84] for *Dysdercus cingulatus* (Fabricius), Teleb [174] for *S. gregaria*, Ghoneim et al. [136] for *S. littoralis* and Manogem et al. [145] for *S. mauritia*, various insecticides or IGRs caused some disruptive alterations in haemocytes basing on the changes in plasma membrane (erosion and extrusion of their cytoplasmic contents), vacuolization and lysis of the cytoplasm and nuclear disorders. To shed some light on the cytopathological impacts of the tested arthropod venoms on the hemocyte profile in haemolymph of *G. mellonella*, the last ($7^{th}$) instar larvae were used. In PRs, some deformations had been observed after treatment with Apitoxin, such as darkly stained cells with degenerated nucleus, destroyed membrane and extruded cytoplasmic contents. The *V. orientalis* venom caused different features of deranged PRs, such as degenerated nuclei, destroyed membranes and extruded cytoplasmic contents. In contrast, *L. quinquestriatus* venom failed to cause cytopathological features in this hemocyte type. With regard to the cytopathological features in PLs after treatment with the tested venoms, Apitoxin caused darkly stained degenerated nuclei and vacuolated cytoplasm. In contrast, both *L. quinquestriatus* venom and *V. orientalis* venom failed to cause cytopathological features in this hemocyte type. No venom exhibited cytopathological effect on GRs, SPs or OEs.

The cytopathological features in *G. mellonella* haemocytes, in the present study, may be attributed to the action of certain arthropod toxins on the 'actin' which localized in the lamellar extensions of the cells [175]. The exact interpretation of the intracellular disturbances in hemocytes has not been available now!! Also, the question whether the hemocytes were affected directly or *via* some physiological or endocrinological pathway is yet to be answered.

## 5. CONCLUSION

As shown in the present study, the arthropod venoms, *viz*., death stalker scorpion *Leiurus quinquestriatus* venom, oriental Hornet (wasp) *Vespa orientalis* venom and Apitoxin of honey bee *Apis mellifera* exhibited quantitative and qualitative impairing effects on the larval haemogram of the greater wax moth, *Galleria mellonella*. Since the primary functions of haemocytes are coagulation, phagocytosis, encapsulation, nodule formation, detoxification of metabolites and biological active materials and distribution of nutritive materials to various tissues, the disturbance or impairment of these hemocytes, by the arthropod venoms, can be considered as an effective approach for controlling *G. mellonella*. At least, these venoms may be used as tools in the Integrated Pest Management for the present pest of the honey bee *Apis mellifera*.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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