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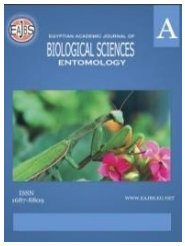

Quantitatively Disturbed Larval Haemogram of *Galleria mellonella* L. (Lepidoptera: Pyralidae) by Certain Plant Growth Regulators

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ABSTRACT

The greater wax moth *Galleria mellonella* L. (Lepidoptera: Pyralidae) is distributed in different parts of the world. Because of the voracious feeding of larvae and their tunnelling behaviour, this pest destructs the honeycomb and other bee-hive products. The objective of the present study was to evaluate the impacts of the plant growth regulators (PGRs), *viz*., indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), 2,4-Dichlorophenoxy acetic acid (2,4-D) and 6-benzyladenine (6-BA), on the important quantitative characters of the larval haemogram, *viz*., total hemocyte count (THC) and differential hemocyte counts (DHCs). For this purpose, the 3rd instar larvae were force-fed on diet mixed with LC_{50} of each PGR (0.24, 0.022, 0.16 & 0.085 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 main types of freely circulating hemocytes in the haemolymph of the 5th instar and 7th (last) instar larvae had been identified as Prohemocytes, Plasmatocytes, Granulocytes, Spherulocytes and Oenocytoids. The THC in haemolymph of control larvae slightly decreased with the instar. The feeding of 3rd instar larvae on diet mixed with IBA, 2,4-D, or 6-BA resulted in increasing THC in haemolymph of 5th instar larvae. Also, IAA, 2,4-D and 6-BA promoted 7th instar larvae to produce considerably increasing THC. In contrast, IAA exhibited a slight reducing effect on 5th instar larvae, since decreasing THC was counted. Also, IBA exerted a suppressing action on larvae to produce normal THC in 7th instar larvae. The tested PGRs exerted diverse actions on DHCs since no certain trend was detected in the fluctuated hemocyte populations. Moreover, some of the PGRs failed to affect the DHCs of certain hemocyte types. However, the auxin PGR, IAA and IBA, may be effective agents in the IPM program against *G. mellonella*.

INTRODUCTION

The greater wax moth or honeycomb moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) is widely distributed throughout the world since it has been recorded in more than 77 countries (Kwadha *et al*., 2017; Roh*et al*., 2020). Larvae of this insectare used as a powerful model organism to test the ecotoxicological, immune and physiological effects of

environmental pollutants (Altuntaş *et al*., 2022) as well as to screen the immunotoxic effects of food preservative agents (Piatek *et al*., 2021;Erbaş *et al*., 2022). Its maintenance and rearing are both easy and inexpensive. Also, its use is considered to be more ethically acceptable than other models, and its immune system has multiple similarities with mammalian immune systems (Admella and Torrents, 2022). On the other hand, *G. mellonella* causes serious problems in temperate, tropical and subtropical beekeeping regions, where the warm temperature favour the rapid development of the moth (Chandel *et al*., 2003;Mohamed *et al*., 2014; Makori *et al*., 2017). It is the major destructive pest of wax comb because of its feeding habits and tunnelling through the combs (Viraktamath, 2010; Ellis *et al*., 2013).

For the control of *G. mellonella*, various physical methods have been adopted (Christen *et al*., 2008; Akyol *et al*., 2009; James, 2011). Also, several biological control agents (Armendariz *et al*., 2002; Hussaini, 2003; Ellis *et al*., 2013; George *et al*., 2019) and the sterile insect technique (or inherited sterility) have been assessed against this pest (Carpenter *et al*., 2005; El-Kholy and Mikhaiel, 2008). In addition, some insect hormone analogues and insect growth regulators had been assessed against *G. mellonella* (Awasthi and Sharma, 2012; Pamita and Priyanka, 2013). However, the control of this pest still relies on insecticides. Although the use of these chemicals is somewhat easy and effective, some precautions for the safety and contamination of bee products are considered. In general, the discriminate uses of many broad-spectrum currently marketed insecticides have led to several drastic problems for the environment (Naqqash *et al*., 2016) and caused serious toxicological hazards to humans (Costa *et al*., 2008; Mosallanejad and Smagghe, 2009; Chaubey, 2015) in addition to the development of insect resistance toward the used insecticides (Pereira *et al*., 2006; Sparks and Nauen, 2015; Maazoun *et al*., 2017). Also, pesticides lead to oxidative stress on plants, inducing toxicity, impeding photosynthesis and negatively affecting the yield of crops (Jan *et al*., 2020).

To avoid the previously mentioned hazards of chemically synthetic insecticides, it is important to search for new effective and safe ways with negligible effects on the ecosystem (Chandler *et al*., 2011; Korrat *et al*., 2012). Plant-derived products, or botanicals, in general, could be efficient alternatives against *G. mellonella* because of their low toxicity to mammals, fast degradability properties and regional availability (Rajendran and Sriranjini, 2008; Abbasipour *et al*., 2009; Mahmoudvand *et al*., 2011). Over the past few decades, many researchers and institutions in the world have focused on plant growth regulators (PGRs) due to their effective control effects on various herbivores (Abdellaoui *et al*., 2015). PGRs are naturally occurring or synthetic compounds that have the potential to control several insect pests through their chemosterilant activity (BecerikliAksan *et al*., 2022). Also, they have received increasing attention recently because they are environmentally friendly compounds of plant origin (Er and Keskin, 2016). However, PGRs have been classified into different categories. Hopkins and Hüner (2004) classified the PGRs into six classes: Gibberellins (GAs), Auxins (Auxs), Ethylene (ET), Cytokinins (CTKs), Abscisic acid (ABA) and Brassinosteroids (BRs). Some years later, Stamm*et al*. (2011) classified the PGRs into main nine classes: Auxs, GAs, CTKs, ET, ABA, Brassinosteroids (BRs), salicylic acid (SA), Jasmonates (JAs) and Strigolactones (SLs). Many authors (Kaur and Rup, 2003; Uçkan*et al*., 2014; Uçkan *et al*., 2015; Çelik *et al*., 2017; Nagaratna *et al*., 2022) reported that the indolic PGRs caused serious effects on survival, development, adult longevity, reproductive potential, hemocytes responses and haemolymph metabolites of various lepidopterous pest species. Against *G. mellonella*, certain PGRs drastically affected the survival, growth (Abo Elsoud *et al*., 2021a) and adult performance (Abo Elsoud *et al*., 2021 b), as well as disturbed the activities of acid and alkaline phosphatases (Ghoneim *et al*., 2022) and qualitative characters of larval haemogram (Hamadah *et al*., 2022).

Haemogram is a statement of the hemocyte population picture in an insect at a given time. It is a quantitative (Total hemocyte count, THC) and qualitative expression (Differential hemocyte count, DHC) of the haemolymph and its constituent inclusions (Arnold, 1972). In addition, the estimation of total haemogram in insects includes the determination of mitotic index, cytological features of hemocytes and blood volume or haemolymph volume (Bardoloi *et al*., 2016; Ghoneim, 2019). The insect haemogram is suggested to be a useful tool for the investigation of the effects of toxic materials on biocontrol agents because alterations in structure, types and the number of cells reflect changes in physiological and biochemical processes (Qamar and Jamal, 2009; Berger and Jurčová, 2012; Ghoneim *et al*., 2021a). The objective of the present study was to evaluate the impacts of the PGRs, *viz*., indole-3-acetic acid, indole-3-butyric acid, 2,4-Dichlorophenoxy acetic acid and 6-benzyladenine, on the most important quantitative characters of the larval haemogram of *G. mellonella*, *viz*., total and differential hemocyte counts.

MATERIALS AND METHODS

1.The Experimental Insect:

A culture of a susceptible strain of the greater wax worm *Galleria mellonella* L. (Lepidoptera: Pyralidae) was established in the Department of Zoology, Faculty of Science, Al-Azhar University, Cairo, Egypt, and maintained for several successive generations under controlled conditions (27±2^{o}C, 65±5% R.H., photoperiod 14 h L and 10 h D). This culture was originally initiated by a sample of larvae kindly obtained from Desert Research Centre, Cairo, Egypt. Larvae were transferred into glass containers, tightly covered with a muslin cloth. Different techniques for preparing the artificial diet had been described by some authors (Metwally *et al*., 2012; Nitin *et al*., 2012). In the present culture of *G. mellonella*, an artificial diet was formulated depending on the method of Bhatnagar and Bareth (2004). The diet contained maize flour (400 g), wheat flour, wheat bran and milk powder, 200 g of each. Also, it was provided with glycerol (400g), bee honey (400g), and yeast (100g). However, some improvements had been manipulated according to Ghoneim *et al*. (2019).

2. Plant Growth Regulators (PGRs):

Four PGRs were selected to be tested against *G. mellonella*, *viz*., (1) Indole-3-Acetic Acid (IAA), a synthetic auxin compound with the chemical name: 2-(1H-indol-3-yl) ethanoic acid. (2) Indole-3-butyric acid (IBA), a synthetic auxin compound with the chemical name: 4-(1H-Indol-3-yl) butanoic acid. (3) 2,4-Dichlorophenoxy acetic acid (2,4-D), a synthetic auxin compound. (4) 6-Benzyladenine (6-BA)(or 6-Benzylaminopurine), a synthetic cytokinin with the chemical name: 4-hydroxyphenethyl alcohol. These PGRs were purchased from Milipore Sigma, Burlington, MA 01803, USA Merk Ltd., Cairo, Egypt.

3. Larval Treatment with PGRs:

Depending on a toxicity bioassay using 100.0, 10.0, 1.0, 0.1, 0.01 and 0.001 ppmof each PGR, LC_{50} values were calculated as 0.24, 0.022, 0.16 and 0.085 ppm for IAA, IBA, 2,4-D and 6-BA, respectively. the evaluation of the disturbing effects of these PGRs on the total and differential hemocytes counts of *G. mellonella*, the 3rd instar larvae were force-fed on an artificial diet supplemented with LC_{50} concentration of each PGR. The control larvae were fed on an untreated artificial diet. Haemolymph of the successfully moulted 5th and 7th (last) instar larvae (treated or control) was subjected to examine the possibly affected hemocyte counts.

4. Collection of Haemolymph:

The haemolymph samples were collected from the treated and control 5th and 7th instar larvae. Each haemolymph sample was obtained by amputation of one or two

prothoracic legs, from the coxa of the larva using fine scissors. Gentle pressure was done on the thorax for obtaining haemolymph drops by a non-heparinized capillary tube. Seven replicates were used and the haemolymph from two individuals was never mixed.

5. HemocyteIdentification and Influenced Hemocyte Counts:

5.1. Hemocyte Identification:

Depending on the cell morphology, cytoplasmic ratio, cytoplasmic inclusions, shape of the nucleus and dye-staining properties, the freely circulating hemocytes in the haemolymph of 5th and 7th (last) instar larvae of *G. mellonella* had been identified and distinguished based on the technique described by some researchers (Altuntaş *et al*., 2012; Blanco, 2016).

5.2. Total Hemocyte 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 the counting slide. After three minutes, the total number of cells recognized in 64 squares of the four corners was counted. If the cells were clumped or unevenly distributed, the preparation was discarded. The number of hemocytes per cubic millimetre was calculated according to the formula of Jones (1962) as follows:

$$\frac{\text{Number of haemocytes counted per champer x dilution x depth factor}}{\text{Number of 1 mm squares counted}}$$

Where the depth factor is usually 10.

5.3. Differential Hemocyte Counts:

Stained haemolymph preparations were carried out, according to Arnold and Hinks (1979). The haemolymph was smeared on clean glass slides, allowed to dry for one minute, and fixed for two 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 a slip cover. The hemocytes were viewed under the light microscope at a magnification of 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 hemocytes using the classification scheme of Brehelin and Zachary (1986).The percentages of hemocyte types were calculated by the formula:

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

6. Statistical Analysis Of Data:

Data obtained were analyzed by the Student's *t*-distribution, and refined by Bessel correction (Moroney, 1956) for the test significance of difference between means using GraphPadInStat© v. 3.01 (1998).

RESULTS

In the present study, the freely circulating hemocytes in haemolymph of the5th and 7th (last) instar larvae of *G. mellonella* had been identified into five main types, *viz*., Prohemocytes (PRs), Plasmatocytes (PLs), Granulocytes (GRs), Spherulocytes (SPs) and Oenocytoids (OEs), depending on the cell shape, cytoplasmic ratio, cytoplasmic inclusions and shape of the nucleus.

1. Effects of Plant Growth Regulators (PGRs) on the Total Hemocyte Count (THC) of *G. mellonella*:

After force-feeding of 3rd instar larvae on diet mixed with LC_{50} concentrations of Indole-3-Acetic Acid (IAA), Indole-3-butyric acid (IBA), 2,4-Dichlorophenoxy acetic acid (2.4-D) and 6-Benzyladenine (6-BA), data of THC were determined in the circulating haemolymph of both 5th instar and 7th instar larvae and assorted in Table (1). Depending on these data, THC in haemolymph of control larvae slightly decreased with the instar (20217±76.37 & 15000±100.0 cell/mm^3, in the 5th instar and 7th instar larvae, respectively). As obviously shown in the same table, feeding of 3rd instar larvae on diet mixed with IBA, 2,4-D, or 6-BA resulted in increasing THC in haemolymph of 5th instar larvae (41.38, 0.57 & 48.05% increments of THC, by IBA, 2,4-D and 6-BA, respectively). According to these data, 6-BA exhibited the most potent enhancing effect on these larvae to produce increasing THC, while 2,4-D exhibited the least enhancing effect. As an exception, IAA exhibited a slight prohibitory effect on 5th instar larvae to produce normal THC (3.54% reduction of THC).
In respect of the THC in haemolymph of 7th instar larvae, data of the same table demonstrated that IBA exerted a suppressing action on larvae to produce normal THC (27.4% reduction of THC) while IAA, 2,4-D and 6-BA promoted the larvae to produce considerably increased THC in their haemolymph (2.0, 19.78 & 17.17% increments of THC, by IAA, 2,4-D and 6-BA, respectively). The strongest promoting effect on THC was exhibited by the compound 2,4-D.

Table 1: Total hemocyte count (THC, cell/mm^3±SD) in 5th instar and 7th instar larvae of *G. mellonella* after force-feeding of newly moulted 3rdinstar larvae on diet mixed with LC_{50} concentrations of plant growth regulator (PGR)*.

PGR		Larval instar	
		5th instar	7th instar
Indole-3-Acetic Acid	Mean±SD	19500±500.00 a	15300±904.1 a
	Change (%)	-3.54	+2.00
Indole-3-butyric acid	Mean±SD	28583±1454.6 d	10867±152.7 d
	Change (%)	+41.38	-27.4
2,4-Dichlorophenoxy acetic acid	Mean±SD	20333±251.66 a	17967±2289.8 a
	Change (%)	+0.57	+19.78
6-Benzyladenine	Mean±SD	29933±378.59 d	17567±1301.3 d
	Change (%)	+48.05	+17.17
Control	Mean±SD	20217±76.37	15000±100.0

*: LC_{50} values were: 0.24, 0.022, 0.16and 0.085ppm for Indole-3-Acetic Acid, Indole-3-butyric acid, 2,4-Dichlorophenoxy acetic acid and Benzyladenine, respectively. Mean±SD followed with (a): insignificantly different (P>0.05). (d): very highly significantly different (P<0.001).

2. Effects of PGRs on the differential hemocyte counts (DHCs) of *G. mellonella*:

2.1. Effects of PGRs on PRs population:

Data of PRs counts in haemolymph of control larvae and treated larvae were assorted in Table (2). Depending on these data, the PRs population slightly increased in control larvae with the instar. All PGRs remarkably promoted the 5th instar larvae to produce a higher PRs population (55.4, 18.1, 7.4 & 59.1% increments of PRs, after treatment with IAA, IBA, 2,4-D and 6-BA, respectively). On the basis of increasing PRs count, 6-BA exhibited the strongest enhancing effect while 2,4-D exhibited the least enhancing effect.

With regard to the 7th instar larvae, data of the same table revealed that IAA, IBA & 6-BA slightly prohibited the treated larvae to produce normal PRs population (28.9, 13.1 & 13.1%

reductions of PRs count, after treatment with IAA, IBA & 6-BA, respectively). In contrast, 2,4-D exceptionally stimulated the 7th instar larvae to produce an unremarkable increase of PRs (5.2% increment of PRs).

Table2: Differential Prohemocyte count (Mean±SD) in 5th instar and 7th instar larvae of *G. mellonella* after force-feeding of newly moulted 3rdinstar larvae on diet mixed with LC_{50} concentrations of PGRs*.

Larval instar		PGR			
		Indole-3-acetic acid	Indole-3-butyric acid	2,4-Dichlorophenoxy acetic acid	6-Benzyladenine
5th	Treated larvae	42.5±2.0 c	33.2±1.0 b	29.2±1.0 a	43.4±3.0 c
	Control	27.4±3.0	27.1±3.0	27.4±3.0	27.5±3.0
	Change (%)	+55.4	+18.1	+7.4	+59.1
7th	Treated larvae	27.3±3.0 b	33.3±3.0 a	40.2±4.0 a	33.3±1.0 a
	Control	38.4±4.0	38.4±4.0	38.1±4.0	38.5±4.0
	Change (%)	-28.9	-13.1	+5.2	-13.1

*, a: see footnote of Table (**1**). (b): significantly different (P<0.05), (c): highly significantly different (P<0.01).

2.2. Effects of PGRs on PLs Population:

Data arranged in Table (3) exiguously displayed a general decrease in PLs population in the control larvae with the instar. After force-feeding of 3rd instar larvae on diet mixed with LC_{50} concentrations of the present PGRs, data of the same table obviously revealed little inhibitory effects of IAA, 2,4-D and 6-BA on PLs population (31.25, 6.25 & 37.5% reductions of PLs, by IAA, 2,4-D and 6-BA, respectively). As an exception, IBA enhanced larvae to produce a higher PLs population (6.25% increased PLs).

In connection with the disturbed PLs population in haemolymph of 7th instar larvae, IAA, IBA and 2,4-D induced larvae produce an increasing population of PLs (7.4, 28.5 & 21.4% increments of PLs, by IAA, IBA and 2,4-D, respectively) while 6-BA suppressed larvae to produce a normal population of PLs (28.5% decrease of PLs).

Table 3: Differential Plasmatocyte count (Mean±SD) in 5th instar and 7th instar larvae of *G. mellonella* after force-feeding of newly moulted 3rdinstar larvae on diet mixed with LC_{50} concentrations of PGRs*.

Larval instar		PGR			
		Indole-3-acetic acid	Indole-3-butyric acid	2,4-Dichlorophenoxy acetic acid	6-Benzyladenine
5th	Treated	11.6±3.0 a	17.3±4.3 a	15.2±2.0 a	10.2±3.0 b
	Control	16.7±2.0	16.4±2.0	16.3±2.0	16.3±2.0
	Change (%)	-31.25	+6.25	-6.25	-37.5
7th	Treated	15.9±4.0 a	18.5±5.2 a	17.7±3.0 a	10.8±3.6 a
	Control	14.8±1.0	14.1±1.0	14.6±1.0	14.7±1.0
	Change (%)	+7.4	+28.5	+21.4	-28.5

*, a: see footnote of Table (**1**), b: see footnote of Table (**2**).

2.3. Effects of PGRs on GRs Population:

Data of differential GRs count were distributed in Table (4). According to these data, GRs population in haemolymph of control larvae slightly decreased with the instar. All tested compounds prohibited 5th instar larvae to produce a normal population of GRs (39.2, 53.5, 10.7 & 17.9% reduction of GRs population, by IAA, IBA, 2,4-D and 6-BA, respectively). The strongest reducing effect was exhibited by IBA. In contrast, all compounds promoted the 7th instar larvae to produce increasing GRs population (9.5, 23.8, 14.2 & 28.5% increments of GRs, by IAA, IBA, 2,4-D and 6-BA, respectively). As clearly seen in the same table, the most potent promoting effect was displayed by 6-BA.

Table 4: Differential Granulocyte count (Mean±SD) in 5th instar and 7th instar larvae of *G. mellonella* after force-feeding of newly moulted 3rdinstar larvae on diet mixed with LC_{50} concentrations of PGRs*.

Larval instar		PGR			
		Indole-3-acetic acid	Indole-3-butyric acid	2,4-Dichlorophenoxy acetic acid	6-Benzyladenine
5th	Treated	17.2±3.0 b	13.7±4.35 b	25.7±4.35 a	23.9±2.0 a
	Control	28.0±5.0	28.8±5.0	28.8±5.0	28.0±5.0
	Change (%)	-39.2	-53.5	-10.7	-17.8
7th	Treated	23.1±2.64 a	26.2±6.0 a	24.6±3.0 a	27.9±2.0 b
	Control	21.5±1.5	21.3±1.5	21.7±1.5	21.3±1.5
	Change (%)	+9.5	+23.8	+14.2	+28.5

*, a: see footnote of Table (**1**), b: see footnote of Table (**2**).

2.4. Effects of PGRs on SPs Population:

Data of SPs population in haemolymph of 5th instar and 7th instar larvae were summarized in Table (5). As obviously seen in this table, the SPs population in larval haemolymph remarkably decreased with the instar. With regard to the disturbing effects of PGRs on the population of these hemocytes, data of the same table exiguously revealed diverse effects, since IBA and 2,4-D induced larvae to produce more SPs (50.0 & 21.4% increment of SPs, by IBA and 2,4-D, respectively) while IAA and 6-BA failed to affect the SPs population.

Also, diverse effects were detected on SPs count in haemolymph of 7th instar larvae, since IAA and IBA suppressed the larvae to produce a normal population of SPs (14.2 & 28.5% reduction of SPs, by IAA and IBA, respectively) while 6-BA elicited larvae to produce a slight increase of SPs (28.5% increment of SPs). On the other hand, 2,4-D failed to affect the SPs population in haemolymph of 7th instar larvae.

Table 5: Differential Spherulocyte count (Mean±SD) in 5th instar and 7th instar larvae of *G. mellonella* after force-feeding of newly moulted 3rdinstar larvae on diet mixed with LC_{50} concentrations of PGRs*.

Larval instar		PGR			
		Indole-3-acetic acid	Indole-3-butyric acid	2,4-Dichlorophenoxy acetic acid	6-Benzyladenine
5th	Treated	14.5±2.0 a	21.1±4.0 b	17.2±3.0 a	14.8±3.0 a
	Control	14.5±1.0	14.8±1.0	14.1±1.0	14.8±1.0
	Change (%)	0.0	+50.0	+21.4	0.0
7th	Treated	6.7±2.0 a	5.7±1.0 a	7.3±2.0 a	5.2±2.0 a
	Control	7.3±1.0	7.8±1.0	7.3±1.0	7.4±1.0
	Change (%)	-14.2	-28.5	0.0	+28.5

*, a: see footnote of Table (**1**), b: see footnote of Table (**2**).

2.5. Effects of PGRs on OEs Population:

Data of OEs population in haemolymph of both 5th instar and 7th instar larvae were assorted in Table (6). Depending on these data, the OEs population in haemolymph of control larvae considerably increased with the instar. Contradictory effects of PGRs were exhibited on the OEs population in haemolymph of 5th instar larvae since IAA and IBA induced 5th instar larvae to produce a higher OEs population (6.88% increment of OEs, by both IAA and IBA) while 2,4-D and 6-BA suppressed larvae to have a normal count of OEs (6.66 & 33.33% reductions of OEs, by 2,4-D and 6-BA, respectively).

In respect of the disturbing effects of PGRs on OEs population in haemolymph of 7th instar larvae, data from the aforementioned table revealed stimulatory effects of IBA, 2,4-D and 6-BA on larvae to gain more OEs in haemolymph (15.0, 20.0 & 25.0% increments of OEs, by IBA, 2,4-D and 6-BA, respectively). IAA was the exceptional compound to prohibit the 7th instar larvae to produce a normal population of OEs (40% reduction of OEs).

Table 6: Differential Oenocyte count (Mean±SD) in 5th instar and 7th instar larvae of *G. mellonella* after force-feeding of newly moulted 3rdinstar larvae on diet mixed with LC_{50} concentrations of PGRs*.

Larval instar		PGR			
		Indole-3-acetic acid	Indole-3-butyric acid	2,4-Dichlorophenoxy acetic acid	6-Benzyladenine
5th	Treated	16.5±4.0 a	16..2±3.0 a	14.2±3.0 a	10.1±1.0 c
	Control	15.4±1.0	15.1±1.0	15.4±1.0	15.7±1.0
	Change (%)	+6.66	+6.66	-6.66	-33.33
7th	Treated	23.2±4.3 a	24.9±3.6 a	12.2±3.0 b	25.2±4.0 a
	Control	20.3±1.0	20.8±1.0	20.5±1.0	20.4±1.0
	Change (%)	-40.0	+15.0	+20.0	+25.0

*, a: see footnote of Table (**1**), b: see footnote of Table (**2**).

Data distributed in Tables 2 – 6 revealed no certain trend of the disturbance in populations of the differential hemocyte types because increasing or decreasing population of each type depended on the potency of the tested compounds and the hemocyte type itself. In other words, the tested PGRs exerted diverse actions on the differential hemocyte counts.

DISCUSSION

Basically, haemogram characters include quantitative (Total hemocyte count, THC) and qualitative description (Differential hemocyte count, DHC) (Arnold, 1972), haemolymph (blood) volume, mitotic index and cytological features of hemocytes. The THC and DHC have been found to be quite variable depending upon the insect species, its developmental stage, physiological state and the applied technique (for recent reviews, see Ghoneim, 2019; Ghoneim *et al*., 2021a).

1. THC as A Quantitative Character of the Larval Haemogram of *G. mellonella*:

1.1. THC in Normal Larvae of *G. mellonella*:

On the basis of the available literature, Mall and Gupta (1979) estimated the THC of the normal red pumpkin beetle *Aulacophora foveicollis* as an average 5500cells/mm^3. Hassan (1985) determined THC in haemolymph of normal larvae of the rice stem borer *Tryporyza* sp. as an average 22475cells/mm^3. The same author recorded THC in haemolymph of the beetle *Meladera* sp. as an average 22300cells/mm^3 in males and 29100cells/mm^3 in females. Sabri and Tariq (2004) determined THC of *A. foveicollis* as 4372 cells/mm^3. Some years later, Chavan *et al*. (2017) estimated the THC in haemolymph of normal larvae of the beetle *Platynotus belli* in an average of 26233.33±251.66 cells/mm^3. In the light of these reported results, THC in haemolymph of normal larvae of *G. mellonella* slightly decreased with the instar number (20217±76.37 and 15000±100.0 cells/mm^3, of 5th and 7th larval instars, respectively)in the present study. Our result disagreed with the reported result of slightly increasing THC in *G. mellonella* (Ghoneim *et al*., 2021b) who estimated the averages of 27400±38.6 and 28900±28.7 cells/mm^3 in 5th instar and 7th instar larvae, respectively. Also, our result disagreed with some reported results of increasing THC in some of the other insects with the age, such as the desert locust *Schistocerca gregaria* (2550.0±180.3, 6266.7±125.8 and 6366.7±125.8 cells/mm^3, of early-, mid- and late-aged nymphs, respectively)(Ghoneim *et al*. 2015 a); the pink bollworm *Pectinophora gossypiella* (7213±716.91 cells/mm^3 and 10138±918.67 cells/mm^3, in 6 hr and 48 hr full-grown larvae, respectively (Ghoneim *et al.*, 2017) and Egyptian cotton leafworm *Spodoptera littoralis* (9276.64±54.76 and 9441.33±28.45 cells/mm^3, at 24 and 72 h of last instar larvae, respectively)(Waheeb, 2020).

It may be important to shed some light on the varying populations of the hemocyte types in haemolymph of some insects, as reported in the current literature. The largest hemocyte population in haemolymph of the last instar larvae of the lawn armyworm *Spodoptera mauritia* was estimated for Plasmatocytes (PLs), followed by other hemocyte types (Manogem*et al*., 2016). In normal larvae of *P. belli*, Chavan, *et al*. (2017) estimated the largest population of Granulocytes (GRs), followed by Prohemocytes (PRs), Adipohemocytes (ADs), Oenocytoids (OEs), PLs, Coagulocyte (CGs) and Spherulocytes (SPs), respectively. As recorded by Ghoneim *et al*. (2015 a) for *S. gregaria*, the circulating CGs had been observed as the largest population, followedby other hemocyte types, regardless of the age of nymphs. As found by Ghoneim *et al*. (2017) for *P. gossypiella*, the circulating ADs had been observed as the largest population, followed by other hemocyte types, regardless of the age of larvae. As recorded by Waheeb (2020) for *S. littoralis*, the circulating PLs had been observed as the largest population, followed by other hemocyte types, regardless of the age of larvae. In the current study, the largest hemocyte population

in haemolymph of the normal 5th instar larvae of *G. mellonella* was estimated for GRs, followed by PRs, PLs, OEs and SPs, respectively. In addition, the largest hemocyte population in haemolymph of the normal 7th instar larvae was estimated for PRs, followed by GRs, OEs, PLs and SPs, respectively.

1.2. Disturbed THC in Larvae of *G. mellonella* by Plant Growth Regulators (PGRs):

Within the same insect species, the population of each hemocyte type varies over development and adaptability in response to environmental stress (Bergin *et al*., 2005; Brayner*et al*., 2007). Therefore, any stress condition resulting in changes in THC, hemocyte morphology and functions would ultimately have an adverse influence on the overall physiology and survival of the insect (Sharma *et al*., 2008; Feitosa *et al*., 2015; Haszcz, 2016). It is important to point out that the changes in THC indicated the stresses of synthetic pesticides, insect growth regulators, plant-derived products and toxins intervening in the intermediary metabolism and immune capability of insects (Qamar and Jamal, 2009). However, responses of THC to chemicals, phagocytosis, encapsulation and metamorphosis in insects were reviewed by Siddiqui and Al-Khalifa (2014) and Ghoneim *et al*. (2021a).

In the present study, the effects of PGRs [indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), 2,4-Dichlorophenoxy acetic acid (2,4-D) and 6-benzyladenine (6-BA)] on THC in haemolymph of 5th and 7th instar larvae of *G. mellonella* were investigated. The 3rd instar larvae were force-fed on diet supplemented with doseLC_{50} of each PGR until the 5th instar. The force-feeding of 3rd instar larvae on diet mixed with IBA, 2,4-D, or 6-BA resulted in an increasing THC in haemolymph of 5th instar larvae. Also, IAA, 2,4-D and 6-BA promoted 7th instar larvae to produce considerably increasing THC. These results were in agreement with some reported results of increasing THC in various insects after treatment with certain PGRs. For example, THC increased in the *G. mellonella* larvae after treatment with different doses of Gibberellic acid (GA_3) (Altuntaş *et al*., 2012). THC increased in the larvae of the lesser wax moth *Achoria grisella* after treatment with IAA (Çelik *et al*., 2017). Apart from PGRs, THC increased in haemolymph of different insects after treatment with some plant-derived products, such as *S. littoralis* (Rizk *et al*., 2001), the flesh fly *Parasarcophaga surcoufi* (Ayaad *et al*., 2001), the tobacco cutworm *Spodoptera litura* (Sharma *et al*., 2003), the seven-spot ladybird *Coccinella septempunctata* (Suhail *et al*., 2007) after treatment with Azadirachtin (Azt) and the red palm weevil *Rhynchophorus ferrugineus* after treatment with Neemazal (Azt formulation) (Hamadah and Tanani, 2017). Also, THC increased in the black cutworm *Agrotis ipsilon* larvae after treatment with acetone extract of *Melia azedarach* (Shaurub and Sabbour, 2017).

For understanding the increasing THC in haemolymph of *G. mellonella* 5th instar larvae, after treatment with IBA, 2,4-D or 6-BA or in haemolymph of 7th instar larvae after treatment with IAA, 2,4-D and 6-BA, in the current investigation, some scenarios could be provided. This THC increase might be due to a defensive action against the tested PGRs (George and Ambrose, 2004). Also, increasing THC has been proposed owing to the promotion of haematopoiesis (Kurt and Kayis, 2015) or the release of hemocytes that adhered on surfaces (sessile haemocytes) within the haemocoel (Ghasemi *et al*., 2013a). In addition, the increase in THC might be due to the activation of the mitotic division of hemocytes which has been activated in response to the tested PGRs (Ratcliffe and George, 1976).Moreover, increasing THC can be regarded as an immune response of an insect against a pathogen or other foreign materials (Anderson *et al*., 1995; Ordas *et al*., 2000), since THC increase indicated that the hemocytes exhibit positive stress immunity in response to the tested PGRs or toxic effect on the immunocytes (certain types of hemocytes) (Zibaee and Bandani, 2010a; Ghasemi et al., 2013b; Shaurub *et al*., 2014). It may be important to mention that the endocrine complex is involved in hemocyte accumulation following some initial stimulus (Nappi, 1974). Also, Jones (1967) suggested the role of ecdysteroids in the

regulation of hemocyte number. Therefore, the present tested PGRs might act as a responsible factor for the modification of haemolymph ecdysteroidtiters (Barnby and Klocke, 1990).

On the contrary, feeding of *G. mellonella* 3rd instar larvae on diet mixed with IAA, in the present study, resulted in decreasing THC in haemolymph of 5th instar larvae. Also, IBA exerted an inhibitory action on 7th instar larvae to produce lower THC. Thus, both IAA and IBA caused a reduction of THC in larvae. These results were in corroboration with a few results of decreasing THC in the larval haemolymph of the same insect after treatment with Abscisic acid (ABA)(Er and Keskin, 2016) and Ethephon (ETF) (Altuntaş*et al*., 2022). Also, feeding of *A. grisella* larvae on diets mixed with doses of 2 - 1,000 ppm of IAA led to a decrease in THC (Çelik *et al*., 2017). Injection of kinetin into the haemocoel of *A. grisella* larvae led to a noticeable decrease in THC (Çelik and Sak, 2021).

Apart from PGRs, some plant-derived compounds caused a considerable decrease in THC in the larval haemolymph of several insects after treatment with Azt, such as *G. mellonella* (Er *et al*., 2017), American cockroach *Periplaneta americana* (Qadri and Narsaiah, 1978), the kissing bug *Rhodnius prolixus* (Azambuja *et al*., 1991), *S. litura* (Sharma *et al*., 2003), the red cotton bug *Dysdercus koenigii* (Tiwari *et al*., 2006), *S. littoralis* (Shaurub *et al*., 2014), the brown spotted locust *Cyrtacanthacris tatarica* (John and Ananthackrishnan, 1995), red cotton stainer *Dysdercus cingulatus* (Pandey and Tiwari, 2011), African monarch *Danaus chrysippus* (Pandey *et al*., 2008), *R. prolixus* (Azambuja and Garcia, 1992) and the banana rhizome weevil *Cosmopolites sordidus* (Sahayaraj and Kombiah, 2010). Also, detrimentally decreased THC in haemolymph of *S. litura* larvae (Rao *et al*., 1984) and *D. cingulatus* nymphs (Ahmad, 1995) had been recorded after treatment with the hormone β-ecdysone. The force-feeding of *G. mellonella* larvae in diets mixed with the food preservatives, sodium benzoate (SB, E211), sodium nitrate (SNa, E251) and sodium nitrite (SNi, E250) resulted in significantly reduced larval THC (Erbaş *et al*., 2022). On the other hand, treatment of *G. mellonella* larvae with two different methods (automated cell counter and hemocytometer) of four doses of Zinc oxide nano-particles (ZnO NPs) resulted in no significant change of THC (Eskin *et al*., 2019).

To interpret the decreasing THC in haemolymph of 5th and 7th instar larvae of *G. mellonella* after feeding of 3rd instar larvae on diets mixed with IAA or IBA, in the current investigation, some conceivable suggestions could be given. (1) It is important to point out that cell proliferation and hemocyte populations are influenced by the mitotic division of the circulating hemocytes (Gardiner and Strand, 2000; Er *et al*., 2010). Thus, THC reduction in *G. mellonella* larvae might be due to the antimitotic effects of IAA and IBA or arrest the of cell proliferation (Pandey *et al*., 2007; Zhu *et al*., 2012; Huang *et al*., 2011; Er *et al*., 2017). (2) The THC reduction might be due to the cytotoxicity of the tested IAA and IBA and the death of pathologically degenerated cells (Pandey *et al*., 2007; Sendi and Salehi, 2010; Zibaee *et al*., 2012) or due to the induction of autophagic or apoptotic pathways resulting in cell death (Huang *et al*., 2011; Shu *et al*., 2015). (3) The reduction in THC after treatment with IAA or IBA may be attributed to the nodulation, encapsulation and phagocytosis (Pandey *et al*., 2007) and/or their toxic effects on the immune cells (Sadeghi *et al*., 2017). (4) It is possible to explain the reduction of THC by the interference of IAA and IBA with endocrine physiology (Azambuja *et al*., 1991; Sharma *et al*., 2003) or the interference with the relationship between the endocrine and immune systems (Figueiredo *et al*., 2006).

2. DHC in the Larval Haemogram of *G. mellonella* as Disturbed by PGRs:

It is important to point out that the increasing DHC of certain hemocyte 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 defence against the foreign biotic targets, like bacteria, yeast and apoptotic bodies as well as the abiotic materials, such as

particles of Indian ink or toxic plant products (Hernandez *et al*., 1999; de Silva *et al*., 2000). The particular phagocytic hemocytes were reported to vary among the insect taxa, and in some cases, discrepancies even exist in the literature among studies on the same species (for detail, see recent reviews of Ghoneim, 2019; Ghoneim *et al*., 2021 a). Moreover, DHCs fluctuate not only as a consequence of different instars of the insect but also within a given instar. These changes may be a result of developmental processes (Gelbic *et al*., 2006).

As shown in the current literature, there are many reported results of variousDHC fluctuations in different insects after treatment with PGRs or plant-derived compounds. For example, PLs population decreased but GR population increased and no fluctuation was recorded for SP, PR and OE populations of *A. Grisella* larvae after treatment with IAA (Çelik *et al*., 2017). In a recent study, Altuntaş *et al*. (2021) investigated the effects of force-fed ETF on the cellular-mediated immune system of *G. mellonella* larvae using lethal doses. According to their results, the populations of PRs, SPs and OEs increased while the GRs population decreased in circulation but PLs population did not alter. A significant reduction of GRs, but an increase of PLs, had been detected in haemolymph of *G. mellonella* larvae after treatment with 100 ppm Azt, while no difference was observed for PRs or OEs (Er *et al*., 2017).

2.1. PRs Population in the Larval Haemolymph As Disturbed by PGRs:

In the present study on *G. mellonella*, PRs population slightly increased in the control larvae with the instar number. This result disagreed with some reported results of decreasing PRs population in larvae with the instar or age, such as a gradual decrease of PRs with the instar of the same insect (Ghoneim *et al*., 2021b); decreasing PRs in larvae of *P. gossypiella* with the age (Ghoneim *et al*., 2017) and decreasing PRs in larvae of *S. littoralis* with the age (Waheeb, 2020).

Depending on the available literature, the reported results of disturbed PRs population in haemolymph, after treatment with PGRs or plant-derived products, are scarce. In the present study, force-feeding of 3rd instar larvae of *G. mellonella* on diets mixed with IAA, IBA, 2,4-D, or 6-BA led to a remarkably increasing PR population in the 5th instar larvae. This result was in agreement with the prevalent inducing effects of ETF on the PR production in larval haemolymph of *G. mellonella* (Altuntaş *et al*., 2022) and the sesquiterpene compounds, Farnesol, Nerolidol and Bisabolol, on the PR production in larval haemolymph of *S. littoralis* (Waheeb, 2020).

In contrast, IAA, IBA and 6-BA slightly reduced the PR population in the haemolymph of *G. mellonella* 7th instar larvae, with an exception of 2,4-D, in the present study. This result was in corroboration with some reported results of decreasing PR population in different insects by some insecticides or insect growth regulators, such as *S. littoralis* by Cyromazine (Ghoneim *et al*., 2015 b), *A. ipsilon* by Diflubenzuron (Abdel-Aziz and Awad, 2010), Eri silkworm *Philosamia ricini* by Dimethoate (Bhagawathi and Mahanthy, 2012), *S. mauritia* by Flufenoxuron (Manogem *et al*., 2016), *P. gossypiella* by Novaluron (Ghoneim *et al*., 2017), *etc*. Also, our result agreed with the reported result of decreasing PRs population in *G. mellonella* larvae after treatment with certain arthropod venoms (Ghoneim *et al*.,2021b) or the food preservatives, SB, E211, SNa, E251 and SNi, E250 (Erbaş *et al*., 2022). On the other hand, our result disagreed with the reported result of Er *et al*. (2017) who found no difference in the PRs population between treated larvae and control larvae of *G. mellonella*, after treatment with NeemAzal (Azt formulation).

For understanding the contradictory results of PR increasing in 5th instar larvae and decreasing in 7th instar larvae of *G. mellonella*, after treatment with the tested PGRs, in the current study, it is interesting to mention that the fluctuation of PR population may be attributed to some factors including inhibition of their mitotic division, their conversion to other cell types or to the inhibition of the activity of hematopoeitic organs responsible for

their production (Pandey *et al*., 2012; Zibaee *et al*., 2012).Although PRs are progenitor stem cells that can differentiate into other types of hemocytes (Yamashita and Iwabuchi, 2001; Lavine and Strand, 2002), their exact function is still unknown (Ribeiro and Brehelin, 2006). Unfortunately, we could not provide an exact interpretation of this fluctuation of PR population in *G. mellonella*, by the present tested PGRs, at the present time!!

2.2. PLs Population in The Larval Haemolymph as Disturbed by PGRs:

In the current study on *G. mellonella*, PLs population in haemolymph of normal larvae generally decreased with the instar number. This result was in accordance with a similar result reported by Ghoneim *et al*. (2021b) for the same insect. It was, also, agreed with some reported results of decreasing PLs with the larval age of *S. gregaria* (Ghoneim*et al*., 2015 a), *P. gossypiella* (Ghoneim *et al*., 2017) and *S. littoralis* (Waheeb, 2020).

In the present investigation, also, force-feeding of 3rd instar larvae of *G. mellonella* on diets mixed with IAA, IBA, 2,4-D and 6-BA led to a slight decrease of PL population in 5th instar larvae by IAA, 2,4-D and 6-BA with an exception of IBA. This result was in accordance with the reported result of decreasing PL population in *A. grisella* larvae after feeding on a diet mixed with IAA (Çelik *et al*., 2017). Also, PL population in haemolymph of larvae of the number of insects decreased after treatment with certain plant-derived compounds, such as the sunn pest *Eurygaster integriceps* and *R. prolixus* (Azambuja*et al*., 1991; Zibaee and Bandani, 2010 a, b); *S. littoralis* (Rizk *et al*., 2001), *C. tatarica* (John and Ananthackrishnan, 1995) and *P. surcoufi* (Ayaad *et al*., 2001) after treatment with Azt. Also, the decreasing PL population in larval haemolymph of *G. mellonella*, in the present study, was in accordance with those reported decreasing PL count in haemolymph of some insects by different insect growth regulators or insecticides, such as *S. littoralis* by Flufenoxuron (Bakr *et al*., 2007) or Novaluron (Ghoneim *et al*., 2015 b) as well as *S. gregaria* by Spinosad and proclaim (Halawa *et al*., 2007) and *S. mauritia* by Flufenoxuron (Manogem *et al*., 2016). In addition, PL population in the 5th instar larvae of *G. mellonella* decreased after treatment with some arthropod toxins (Ghoneim *et al*., 2021b).

The decreasing PL population in the 5th instar larvae of *G. mellonella*, in the current study, could be explained by the transformation of these hemocytes into other types (George, 1996), since they are highly polymorphic cells (Gupta and Sutherland, 1966). Also, the tested PGRs might impair the haematopoietic organs which are responsible for the production of PLs (Tiwari *et al*., 2002). However, we could not provide the exact interpretation of the decreasing PL population in 5th instar larvae, after treatment with the tested IAA, 2,4-D and 6-BA, right now!!

On the other hand, force-feeding of 3rd instar larvae of *G. mellonella* on diets mixed with IAA, IBA, 2,4-D and 6-BA, in the current study, led to an increasing population of PLs in the haemolymph of 7thinstar larvae by IAA, IBA and 2,4-D with an exception of 6-BA. This result was, to some extent, in agreement with few reported results of significantly increasing PLs in *G. mellonella* after injection of ABA into haemocoel of larvae (Er and Keskin, 2016), after the force-feeding of larvae with the food preservatives, SB, E211, SNa, E251 and SNi, E250 (Erbaş *et al*., 2022) and after topical application of 100 ppm of NeemAzal onto last instar larvae (Er *et al*., 2017). Also, our result agreed with the result of Waheeb (2020) in *S. littoralis* larvae after treatment with Farnesol, Bisabolol and Nerolidol. On the other hand, ETF did not affect PLs count in haemolymph of *G. mellonella* larvae (Altuntaş *et al*., 2022).

In addition, the present result of increasing PL population in the haemolymph of 7th instar larvae was, to a great extent, in corroboration with some reported results of the induced PL population in larval haemolymph of some insects by certain toxins and insect growth regulators, such as *S. littoralis* by Cyromazine (Ghoneim *et al*., 2015 b); *S. gregaria* by Deltamethrin (Al-Hariri and Suhail, 2001), *R. kumarii* by endosulfan (George and Ambrose,

2004); *A. ipsilon* by Diflubenzuron (Abdel-Aziz and Awad, 2010); *S. litura* by hexaflumuron (Zhu *et al*., 2012); *etc*.

To understand the induction of PL production in larval haemolymph of *G. mellonella*, in the present study, it is worth mentioning that the role of PLs in phagocytosis is disputed, since some authors (Tojo *et al*., 2000; Ling and Yu, 2006) suggested their act as phagocytes, and thus they should be highly produced while other authors (Nruwirth, 1973; Beaulaton, 1979) reported no phagocytic function of PLs. The increasing PL population could be attributed to the differentiation of hemocytes by mitosis (Kurihara *et al*., 1992). However, a conceivable interpretation of the induced PLs population in *G. mellonella* larval haemolymph, as a response to certain PGRs, could not be provided at the present time!!

2.3. GRs Population in The Larval Haemolymph As Disturbed by PGRs:

In the present work, a slight decrease in GRs population was recorded in haemolymph from normal 5th to 7th instar larvae of *G. mellonella*. This finding coincided with a similar result of Ghoneim *et al*., 2021b) for the same insect. Also, Ghoneim *et al*. (2017) recorded decreasing GRs in larvae of *P. gossypiella* with the age. On the contrary, our result disagreed with some reported results of increasing GR population with the larval age, such as *S. gregaria* (Ghoneim *et al*., 2015 a) and *S. littoralis* (Waheeb, 2020).

In the present investigation, also, force-feeding of 3rd instar larvae of *G. mellonella* on diets mixed with the PGRs, *viz*., IAA, IBA, 2,4-D and 6-BA led to a considerable decrease of GR population in 5th instar larvae. This result was, to a great extent, in accordance with some reported results of decreasing GR count in *G. mellonella* larvae after treatment with ETF (Altuntaş *et al*., 2022) or force-feeding of larvae with the food preservatives, SB, E211, SNa, E251 and SNi, E250 (Erbaş *et al*., 2022), as well as other insects, after treatment with certain plant-derived compounds, such as *P. surcoufi* (Ayaad *et al*., 2001), both bugs *E. integriceps* and *R. prolixus* (Azambuja *et al*., 1991; Zibaee and Bandani, 2010 a, b) after treatment with Azt and *S. littoralis* after treatment with some compounds derived from urea waste and rice straw (Hassan *et al*., 2013).

However, the decreasing GRs population in *G. mellonella* 5th instar larvae by the tested PGRs, in the present study, might be interpreted as the death of a lot of them due to their detoxification activity against these toxic molecules (Barakat *et al*., 2002; George and Ambrose, 2004; Costa *et al*., 2005), since this type of hemocytes performs different functions, like phagocytosis, as reported by several authors in different insects, such as Tojo*et al*. (2000) in *G. mellonella*, Pendland and Boucias 1996) in the beet armyworm *Spodoptera exigua*, Butt and Shields (1996) in the gypsy moth *Lymantria dispar*, Nardi *et al*. (2001) and Costa *et al*. (2005) in *S. littoralis*. Also, it might be due to their differentiation into other types of hemocytes, since GRs can differentiate into SPs in the mulberry silk moth *Bombyx mori* (Liu *et al*., 2013).

On the other hand, force-feeding of 3rd instar larvae of *G. mellonella* on diets mixed with IAA, IBA, 2,4-D and 6-BA, in the present study, led to a remarkable increase of GR population in 7th instar larvae, regardless of the PGR. This result agreed with few reported results of increasing GRs in some insects, as a response to certain PGRs or plant-derived products, such as *A. grisella* as a response to feeding of larvae on a diet supplemented with IAA (Çelik *et al*., 2017); *S. littoralis* as a response to Margosan-0 (Azt formulation) (Rizk *et al*., 2001) and *S. littoralis* as a response to certain Sesquiterpene compounds (Waheeb, 2020).

The increasing count of GRs in larval haemolymph of 7th instar larvae of *G. mellonella*, in the present study, might be explained by the transformation of some hemocytes (may be PLs and PRs) into GRs, since some authors (Kurihara *et al*., 1992; George and Ambrose, 2004) reported the role of GRs in the detoxification of toxic compounds.

2.4. SPs Population in The Larval Haemolymph As Disturbed by PGRs:

In the current study, SP population in the haemolymph of *G. mellonella* normal larvae remarkably decreased with the instar number. This result was in agreement with Ghoneim *et al*. (2017) who recorded decreasing SPs in larvae of *P. gossypiella* with the age. In contrast, this result disagreed with the result of Ghoneim *et al*. (2021b) who recorded a gradually increasing SP population in normal larvae of *G. mellonella* with the instar and Waheeb (2020) who recorded an increasing SPs population in normal larvae of *S. littoralis*. In the present study, also, IBA and 2,4-D-induced 5th instar larvae produced a larger SP population and 6-BA-induced 7th instar larvae to produce increasing SP population. To the best of our knowledge, scarce results of the effects of PGRs on the SP population exist in the current literature. Altuntaş *et al*. (2022) determined a significant increase in SP count in haemolymph of *G. mellonella* after treatment with ETF.

Outside PGRs, some arthropod toxins enhanced the *G. mellonella* larvae to produce more SPs but other toxins prohibited them to produce the normal SPs population (Ghoneim *et al*., 2021a). The force-feeding of *G. mellonella* larvae with the food preservatives, SB, E211, SNa, E251 and SNi, E250 resulted in significantly increased larval SPs (Erbaş *et al*., 2022).

In the present study, the increasing SP population in haemolymph of *G. mellonella* larvae, after treatment with IBA, 2,4-D and 6-BA, 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*. In Lepidoptera, the functions of SPs are unknown until now (Ribeiro and Brehelin, 2006) but Sass *et al*. (1994) suggested their responsibility for transporting cuticular components. Unfortunately, the exact interpretation of the reduced SPs population in the present study is still obscure!!

On the other hand, results of the current investigation revealed that IAA and IBA suppressed the 7th instar larvae to produce a normal SP population. Moreover, IAA and 6-BA failed to affect the SP population in the 5th instar larvae and 2,4-D failed to affect the count of this type of hemocytes in the7th instar larvae. No results of the effects of PGRs on SPs population exist in the available literature. However, Waheeb (2020) recorded suppressive effects of Farnesol, Bisabolol and Nerolidol on SPs population in haemolymph of *S. littoralis* larvae. Unfortunately, the interpretation of the reduced SP population in the present study is still obscure!!

2.5. OEs Population in The Larval Haemolymph as Disturbed by PGRs:

In the present study, OEs population in haemolymph of normal *G. mellonella* larvae considerably increased with the instar number. This result agreed, to some extent, with the result of Ghoneim *et al*. (2021b) who recorded a slight increase of OEs population in the normal larvae with the instar number of the same insect. Also, Ghoneim *et al*. (2017) recorded increasing OEs in larvae of *P. gossypiella* with the age. On the other hand, OE population in haemolymph of *S. littoralis* did not alter with age (Waheeb, 2020).

In the present study, IAA and IBA induced 5th instar larvae of *G. mellonella* to produce larger OE population. Also, IBA, 2,4-D and 6-BA induced 7thinstar larvae to produce a larger OEs population. This result was in agreement with the reported results of significantly increased OEs count in *G. mellonella* larvae after treatment with ETF (Altuntaş *et al*. 2022) or the food preservatives, SB, E211, SNa, E251 and SNi, E250 (Erbaş *et al*., 2022).

To understand the increasing OE population in larval haemolymph of *G. mellonella* after treatment with certain PGRs, it is believed that OEs play a crucial role in phenoloxidase (PO) cascade when an immune challenge occurs (Beckage, 2008; Strand, 2008). Therefore, the increase in their population could be led to the stimulation of the immune system of the treated larvae to the secretion of PO to achieve the defensive function (Kurihara *et al*., 1992;

Ghasemi *et al*., 2013b). Also, the increasing OE population in the larval haemolymph of larvae might be due to their role in the detoxification of toxic compounds and activating action of some tested PGRS on the hematopoietic organs or cell mitotic division.

On the other hand, the present results revealed that 2,4-D and 6-BA suppressed 5th instar larvae of *G. mellonella* to have a normal OEs population and IAA prohibited 7th instar larvae to produce a normal OEs population. This decreasing OE population in the larval haemolymph might be due to the degeneration of some OEs for releasing precursors of prophenoloxidase that likely play a role in the melanization of haemolymph and an important immunity protein in insects (Ribeiro and Brehelin, 2006**)**.

Conclusion:

As clearly shown in the present study on *G. mellonella*, feeding of 3rd instar larvae on diet mixed with 2,4-D or 6-BA resulted in increasing THC in haemolymph of 5th instar larvae and feeding on diets mixed with 2,4-D or 6-BA resulted in increasing THC in 7th instar larvae. In contrast, feeding on diets mixed with IAA resulted in decreasing THC in 5th instar larvae and feeding on diets mixed with IBA resulted in decreasing THC in 7th instar larvae. Because hemocytes in insects perform various functions, such as phagocytosis, encapsulation of foreign bodies and detoxification of metabolites and biologically active materials, decreasing THC in haemolymph of larvae denotes the disrupted immunological and physiological functions of hemocytes. Therefore, the auxin PGR compounds, IAA and IBA, may be effective agents in the IPM program against *G. mellonella*.

REFERENCES

Abbasipour, H.; Mahmoudvand, M.; Deylami, A. and Hosseinpour, M.H. (2009): Fumigant toxicity of essential oils of *Rosemarinus officinalis* L. and *Eucalyptus camodulensis* Deh. against some stored products pests. Proceeding of the 6th Asia-Pacific Congress of Entomology, *"Entomology in Health, Agriculture and Environment"*, October 18-22, 2009, Beijing, China.

Abd El-Aziz, N.M. and Awad, H. (2010): Changes in the haemocytes of *Agrotis ipsilon* larvae (Lepidoptera: Noctuidae) in relation to Dimilin and *Bacillus thuringiensis* infection. *Micron*, 41: 203-209.https://doi.org/10.1016/j. micron.2009.11.001

Abdellaoui, K.; Ben Halima-Kamel, M.; Acheuk, F.; Soltani, N.; Aribi, N. and Ben Hamouda, M.H. (2015): Effects of gibberellic acid on ovarian biochemical composition and ecdysteroid amounts in the migratory locust *Locusta migratoria* (Orthoptera, Acrididae). *International Journal of Pest Management*, 61: 68-72. https://doi.org/ 10.1080/09670874.2014.995746

Abo Elsoud, A.A.; Ghoneim, K.; Hamadah, Kh. and El-Hela, A. (2021a): Toxicity and bio-efficacy of indole-3-acetic acid, a plant growth regulator, against the greater wax moth, *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae). *Egyptian Academic Journal of Biological Sciences (A. Entomology),* 14(2):173-190.Doi: 10.21608/EAJBSA.2021.185451

Abo Elsoud, A.A.; Ghoneim, K.; Hamadah, Kh. and El-Hela, A. (2021b): Suppressive impact of 6-Benzyladenine, a plant growth regulator, on the adult performance and reproductive potential of *Galleria mellonella* L. (Lepidoptera: Pyralidae). *Egyptian Academic Journal of Biological Sciences (F. Toxicology & Pest Control),* 13(2): 49-70. Doi: 10.21608/EAJBSF.2021.186039

Admella, J. and Torrents, E.A. (2022): Straightforward method for the isolation and cultivation of *Galleria mellonella* hemocytes. *International Journal of Molecular Sciences*, 23, 13483. https:// doi.org/10.3390/ijms232113483

Ahmad, A. (1995): Changes in hemocyte counts following topical application of β-ecdysone

and makisterone A on 5th instar nymphs of *Dysdercuscingulatus*Fabr. (Hemiptera: Pyrrhocoridae). *Entomologia Croatica,* 1: 41-48.

Akyol, E.; Yeninar, H.; Şahinler, N. and Ceylan, D.A. (2009): The using of carbon dioxide (CO_2) on controlling of the greater wax moth's *Galleria mellonella* L. (Lepidoptera: Pyralidae) damages. *Uludag Bee Journal,* 9(1): 26-31. (In Turkish with English abstract). http://hdl.handle.net/11452/15881

Al-Hariri, M.K. and Suhail, A. (2001): Effect of lambdacyhalothrin and deltamethrin on the haemocytes of desert locust, *Schistocerca gregaria* Forsk. *International Journal of Agriculture and Biology,* 3(1): 81-84.

Altuntaş, H.; Kiliç, A.Y.; Uçkan, F. and Ergin, E. (2012): Effects of gibberellic acid on hemocytes of *Galleria mellonella* L. (Lepidoptera: Pyralidae). *Environmental Entomology,* 41(3): 688-696. https://doi. org/10.1603/EN11307

Altuntaş, H.; Gwokyalya, R. and Bayram, N. (2022): Immunotoxic effects of force-fed ethephon on model organism *Galleria mellonella* (Lepidoptera: Pyralidae). *Drug and Chemical Toxicology,* 45(4):1761-1768. doi: 10.1080/01480545.2021. 1873358.

Anderson, R.S.; Burreson, E.M. and Paynter, K.T. (1995): Defense responses of haemocytes withdrawn from *Crassostrea virginica* infected with *Perkinsus marinus*. *Journal of Invertebrate Pathology,* 66, 82-89. https://doi.org/10.1006/jipa.1995.1065

Armendariz, I.; Downes, M.J. and Griffin, C.T. (2002): Effect of timber condition on parasitization of pine weevil (*Hylobius abietis* L.) larvae by entomopathogenic nematodes under laboratory conditions. *Biocontrol Science and Technology,* 12: 225-233. https://doi.org/10. 1080/09583150120124487

Arnold, J.W. (1972): A comparative study of the haemocytes (blood cells) of cockroaches (Insecta, Dictyoptera, Blattari), with a view of their significance in taxonomy. *Canadian Entomologist,* 104: 309-348. Doi: 10.4039/Ent104309-3

Arnold, J.W. and Hinks, C.F. (1979): Insect haemocytes under light microscopy: technique. In: "Insect Haemocytes" (Gupta, A.P., ed.). Cambridge Univ. Press, Cambridge. Pp: 531-538.

Awasthi, P. and Sharma, P. (2012): Docking Study of synthesized juvenile hormone analogues as an Insect Growth Regulators. 2012 UK Sim 14th International Conference on Computer Modelling and Simulation. Cambridge, Cambridgeshire United Kingdom, March 28-March 30

Ayaad, T.H.; Dorrah, M.A.; Shaurub, E.H. and El-Sadawy, H.A. (2001): Effects of the entomopathogenic nematode, *Heterohabditis bacteriophora* HP88 and azadirachtin on the immune defense response and prophenoloxidase of *Parasarcophaga surcoufi* larvae (Diptera: Sarcophagidae). *Journal of Egyptian Society of Parasitolgy,* 31(1): 295-325.

Azambuja, P.D. and Garcia, E.S. (1992): Effects of azadirachtin on *Rhodniusprolixus*: immunity and *Trypanosoma* infection. *Memórias do Instituto Oswaldo Cruz Rio de Janeiro,* 87 (Suppl. 5): 69–72. Doi: 10.1590/s0074-02761992000900009

Azambuja, P.D.; Garcia, E.S.; Ratcliffe, N.A. and Warthen, J.D. (1991): Immune-depression in *Rhodniusprolixus* induced by the growth inhibitor azadirachtin. *Journal of Insect Physiology,* 37: 771–777. https://doi. org/ 10.1016/0022-1910(91)90112-D

Bakr, R.F.A.; Soliman, F.E.l.; El-Sayed, M.F.; Hassan, H.A. and Zohry, N.M.H. (2007): Effects of subleathal dosages of flufenoxuron and chlorfluazuron on haemolymph changes of *Spodoptera littoralis*. *Proceedings of 2nd International Conference, Entomological Society of Egypt,* 2: 211-238.

Barakat, E.M.S.; Meshrif, W.S. and Shehata, M.G. (2002): Changes in the haemolymph of the desert locust, *Schistocerca gregaria* after injection with *Bacillus thuringiensis*.

Journal of Egyptian Academic Society of Environment and Development, 2(1): 95-115.

Bardoloi, S.; Desdimona, K. and Mazid, S. (2016). Comparative study of the changes in haemogram of *Antheraea assama* Ww reared on two host plants, Som (*Machilus bombycina* King) and Soalu (*Litsea polyantha* Juss*). International Journal of Pure and Applied Bioscience,* 4(5): 144-152. Doi: 10.18782/2320-7051.2368

Barnby M.A. and Klocke, J.A. (1990): Effects of azadirachtin on levels of ecdysteroids and prothoracicotropic hormone-like activity in *Heliothis virescens* (Fabr) larvae. *Journal of Insect Physiology,* 36: 125-131. Doi:10.1016/0022-1910(90)90183-G

Beaulaton, J. (1979): Haemocytes and haemocytopoiesis in silkworms. Biochimie, 61: 157-164. Doi: 10.1016/S0300-9084(79)80064-4

BecerikliAksan, G.; Uckan, F. and Er, A. (2022): Influence of dietary indole-3-acetic acid on phenoloxidase and hemolytic activities in *Pimpla turionellae* L., 1758 (Hymenoptera: Ichneumonidae) and *Galleria mellonella* L., 1758 (Lepidoptera: Pyralidae) in a host-parasitoid system. *Turkish Journal of Entology,* 46 (2): 149-158. Doi: http://dx.doi.org/10.16970/entoted.1039327

Beckage, N.E. (2008): Insect Immunology. Academic press, 348 pp.

Berger, J. and Jurčová, M. (2012): Phagocytosis of insect haemocytes as a new alternative model. *Journal of Applied Biomedicine,* 10: 35-40. https://doi. org/ 10.2478/v10136-012-0003-1

Bergin, D., Reeves, E.P., Renwick, J., Wientjes, F.B., Kavanagh, K. (2005). Superoxide production in *Galleria mellonella* hemocytes: identification of proteins homologous to the NADPH oxidase complex of human neutrophils. *Infection and Immunity,* 73: 4161-4170. doi: 10.1128/IAI.73.7.4161-4170.2005.

Bhagawati, N. and Mahanta, R. (2012): Changes in haemocyte count in haemolymph of different larval stages of Eri silkworm on application of dimethoate, organophosphorus pesticide. *International Journal of Recent Scientific Research,* 5(3): 396-399.

Bhatnagar, A. and Bareth, S.S. (2004): Development of low-cost, high-quality diet for greater wax moth, *Galleria mellonella* (Linnaeus). *Indian Journal of Entomology,* 66(3): 251-255.

Blanco, L.A.A. (2016): Differential cellular immune response of hemocyte of *Galleria mellonella* larvae against *Actinobacillus pleuropneumoniae* Strains. M.Sc. Thesis, Universidade Federal de Viçosa, Brazil.

Brayner, F.A.; Araújo, H.R.; Santos, S.S.; Cavalcanti, M.G.; Alves, L.C.; Souza, J.R. and Peixoto, C.A. (2007): Hemocyte population and ultrastructural changes during the immune response of *Culex quinquefasciatus* (Diptera: Culicidae) to microfilariae of *Wuchereria bancrofti*. *Medical and Veterinary Entomology,* 21: 112-120. DOI: 10.1111/j.1365-2915. 2007.00673.x

Brehélin, M. and Zachary, D. (1986): Insect haemocytes: a new classification to rule out the controversy. In: "Immunity invertebrates, cells, molecules and defense reactions"(Brehélin M., ed.). Heidelberg: Spring Verlag. Pp: 37-48.

Butt, T.M. and Shields, K.S. (1996): The structure and behaviour of Gypsy moth (*Lymantria dispar*) hemocytes. *Journal of Invertebrate Pathology,* 68: 1-14. https://doi.org/10.1006/jipa.1996.0052

Carpenter, J.; Bloem, S. and Marec, F. (2005): Inherited sterility in insects. In: "Sterile Insect Technique" (Dyck, V.A.; Hendrichs, J.; and Robinson, A.S., eds.). pp. 115-146.

Çelik, E. and Sak, O. (2021): Effects of kinetin on biological parameters and hemocytes of *Achroiagrisella* (Lepidoptera: Pyralidae). *Archives of Biological Sciences,* 72(2):181-192. https://doi.org/10.2298/ ABS200107012C

Çelik D.; Özbek R. and Uçkan F. (2017): Effects of indole-3-acetic acid on hemocytes of *Achoria grisella* Fabr. (Lepidoptera: Pyralidae). *Journal of Entomological Research Society,* 19(2): 83-93.

Chandel, Y.S.; Sharma, S. and Verma, K.S. (2003): Comparative biology of the greater wax moth, *Galleria mellonella* L. and lesser wax moth, *Achoria grisella*. *Forest Pest Management and Economic Zoology,* 11: 69-74.

Chandler, D.; Bailey, A.S.; Tatchell, G.M.; Davidson, G.; Greaves, J. and Grant, W.P. (2011): The development, regulation and use of biopesticides for integrated pest management. *Philosophical Transactions of the Royal Society B,* 366: https://doi.org/10. 1098/rstb.2010.0390.

Chaubey, M.K. (2015): Biological activities of terpenes against pulse beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae). *Entomology and Applied Science Letters,* 2(1): 50-61.

Chavan, J.A.; Chougale, A.K. and Bhawane, G.P. (2017): Toxicity of Dimethoate and Chlorpyriphos on haemocyte count in male *Platynotus belli* Fairmaire (Coleoptera: Tenebrionidae). *Journal of Entomology and Zoology Studies,* 5(1): 126-133.

Christen, J.M.; Campbell, J.F.; Zurek, L.; Shapiro-Ilan, D.I.; Lewis, E.E.; Ramaswamy, S.B. (2008): Role of symbiotic and non-symbiotic bacteria in carbon dioxide production from hosts infected with *Steinernema riobrave*. *Journal of invertebrate pathology,* 99(1): 35-42.https://doi.org/10.1016/j.jip.2008.05.008

Costa, S.C.P.; Ribeiro, C.; Girard, P.A.; Zumbihl, R. and Brehelin, M. (2005): Modes of phagocytosis of Gram-positive and Gram-negative bacteria by *Spodoptera littoralis* granular haemocytes. *Journal of Insect Physiology,* 51: 39-46. https://doi.org/ 10.1016/j.jinsphys. 2004. 10.014

Costa, L.G.; Giordano, G.; Guizzetti, M. and Vitalone, A. (2008): Neurotoxicity of pesticides: a brief review. Frontiers BioScience, 13: 1240-1249.DOI: 10.2741/2758

de Silva, C.; Dunphy, G.B. and Rau, M.E. (2000):Interaction of hemocytes and prophenoloxidase system of fifth instar nymphs of *Acheta domesticus* with bacteria. *Developmental and Comparative Immunology,* 24: 367-379. https://doi.org/10.1016/S0145-305X(99)00063-4

El-Kholy, E.M.S. and Mikhaiel, A.A. (2008): Scanning electron microscopy on the male antennae of the greater wax moth, *Galleria mellonella* (L.), treated with gamma radiation. *Isotope and Radiation Research,* 40(3): 603-613.

Ellis, J.D.; Graham, J.R. and Mortensen, A. (2013): Standard methods for wax moth research. *Journal of Apiculture Research,* 52: 01-17.https://doi.org/10.3896/ IBRA.1.52.1.10

Er A. and Keskin M. (2016): Influence of abscisic acid on the biology and hemocytes of the model insect *Galleria mellonella* (Lepidoptera: Pyralidae). *Annals of Entomological Society of America,* 109: 244-251. https://doi.org/10.1093/ aesa/sav122

Er, A.; Uçkan, F.; Rivers, D.B.; Ergin, E. and Sak, O. (2010): Effects of parasitization and envenomation by the endoparasitic wasp *Pimpla turionellae* (Hymenoptera: Ichneumonidae) on hemocyte numbers, morphology, and viability of its host *Galleria mellonella* (Lepidoptera: Pyralidae). *Annals of the Entomological Society of America,* 103: 273-82. https://doi.org/10.1603/AN09065

Er, A.; Taşkıran, D. and Sak, O. (2017): Azadirachtin-induced effects on various life history traits and cellular immune reactions of *Galleria mellonella* (Lepidoptera: Pyralidae). *Archives of Biological Sciences,* 69(2): 335-344. https://doi.org/ 10.2298/ABS160421108E.

Erbaş, E.D.; Gwokyalya, R.; Altuntaş, H. and Kutrup, B. (2022): Screening the

immunotoxicity of different food preservative agents on the model organism *Galleria mellonella* L. (Lepidoptera: Pyralidae) larvae. *Drug and Chemical Toxicology*, 27: 1-11. doi: 10.1080/01480545.2022.2091589.

Eskin, A.; Özturk, Ş. and Korukcu, M. (2019): Determination of the acute toxic effects of zinc oxide nanoparticles (ZnO NPs) in total hemocytes counts of *Galleria mellonella* (Lepidoptera: Pyralidae) with two different methods. *Ecotoxicology,* 28: 801- 808. https://doi.org/10.1007/s10646-019-02078-2

Feitosa, A.P.; Alves, L.C.; Chaves, M.M.; Veras, D.L.; Silva, E.M.; Aliança, A.S.; França, I.R.; Gonçalves, G.G.; Lima-Filho, J.L. and Brayner, F.A. (2015): Hemocytes of *Rhipicephalus sanguineus* (Acari: Ixodidae): characterization, population abundance, and ultrastructural changes following challenge with *Leishmania infantum*. *Journal of Medical Entomology,* 52: 1193-202. https://doi.org/ 10.1093/ jme/ tjv125

Figueiredo, M.B.; Castro, D.P.; Nogueira, N.F.S.; Garcia, E.S. and Azambuja, P. (2006): Cellular immune response in *Rhodnius prolixus*: role of ecdysone in haemocyte phagocytosis. *Journal of Insect Physiology,* 52: 711-716. https://doi.org/10.1016/ j.jinsphys. 2006.03.011

Gardiner, E.M.M. and Strand, M.R. (2000): Hematopoiesis in larval *Pseudoplusia includes* and *Spodoptera frugiperda*. *Archives of Insect Biochemistry and Physiology*, 43:147–164.https://doi.org/10. 1002/(SICI)1520 -6327(200004)43: 4<147::AID-ARCH1>3.0.CO;2-J

Gelbič, I.; Strbáčkova, J. and Berger, J. (2006): Influence of metyrapone on the morphology of hemocytes of the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd). *Zoological Studies,* 45(3): 371-377.

George, P.J.E. (1996).Impact of chosen insecticides on three non-target reduviid biocontrol agents (Insecta: Heteroptera: Reduviilidae). Ph.D. Thesis, Triunelveli: Manonmaniam Sundaranar University,Tamil Nadu, India. 117pp.

George, P.J.E. and Ambrose, D.P. (2004): Impact of insecticides on the haemogram of *Rhynocoris kumarii* Ambrose & Livingstone (Hem. Reduviidae). *Journal of Applied Entomology,* 128(9-10): 600-604. https://doi.org/10.1111/j.1439-0418. 2004.00896.x

George, J.; G. Devi, and B. Bhattacharyya, (2019): Survival and infectivity of entomopathogenic nematode *Oscheius rugaoensis* in different formulations against wax moth, *Galleria mellonella*. *Journal of Entomology and Zoology Studies,* 7 (3): 241-244.

Ghasemi, V.; Yazdib, A.K.; Tavallaie, F.Z. and Sendi, J.J. (2013a): Effect of essential oils from *Callistemon viminalis* and *Ferula gummosa* on toxicity and on the hemocyte profile of *Ephestia kuehniella* (Lep.: Pyralidae). *Archives of Phytopathology and Plant Protection*, 47(3): 268-278. doi:10.1080/ 03235408.2013.808856

Ghasemi, V.; Moharramipour, S. and Sendi, J.J. (2013b): Circulating hemocytes of Mediterranean flour moth, *Ephestia kuehniella* Zell. (Lep: Pyralidae) and their response to thermal stress. *Invertebrate Survival Journal,* 10: 128-140.

Ghoneim, K. (2019): Characterization of qualitative and quantitative haemogram parameters in insects: a review of current concepts and future prospects. *Egyptian Academic Journal of Biological Sciences (A. Entomology),*12(1): 9-63. Doi: 10. 21608/ EAJBSA.2019.25088

Ghoneim, K.; Hamadah, Kh.; Amer, M.; El-Hela, A. and Mohammad, A. (2015a): Qualitative and quantitative changes in the haemogram of desert locust *Schistocerca gregaria* (Orthoptera: Acrididae) by extracts of *Nigella sativa* (Ranunculaceae). *Journal of Advances in Biology,* 7(2): 1275-1292. https://

doi.org/10.24297/jab. v7i2.4566

Ghoneim, K., Tanani, M., Hamadah, Kh., Basiouny, A. and Waheeb, H. (2015b): Effects of Novaluron and Cyromazine, chitin synthesis inhibitors, on the larval haemogram of *Spodoptera littoralis* (Boisd.)(Lepidoptera: Noctuidae). *International Journal of Advanced Research,* 3(1), 554 -576.

Ghoneim, K.; Hassan, H.A.; Tanani, M.A. and Bakr, N.A. (2017): Deteriorated larval haemogram in the pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) by the chitin synthesis inhibitors, Novaluron and Diofenolan. *International Journal of Modern Research and Reviews*,5(2): 1487-1504.

Ghoneim, K.; Hamadah, Kh.; Tanani, M.; Abdel-Khaliq, A. and Emam, D. (2019): Toxicity and disruptive impacts of the honeybee Apitoxin on growth and development of the greater wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae). *Egyptian Academic Journal of Biological Sciences (F. Toxicology & Pest Control),* 11(2): 97-106. Doi: 10.21608/EAJBSF.2019.45537

Ghoneim, K.; Bakr, R.F.A. and Hamadah, Kh. (2021 a): Disturbing effects of botanicals on the haemogram and immune parameters of insects: recent progress of the search for effective biopesticides. *Egyptian Academic Journal of Biological Sciences (A. Entomology),* 14(1): 147-193. Doi: 10.21608/EAJBSA.2021.157363

Ghoneim, K.; Hamadah, Kh.; Tanani, M. and Emam, D. (2021b): Quantitative and qualitative impacts of selected arthropod venoms on the larval haemogram of the greater wax moth, *Galleria mellonella* (Linnaeus) (Lepidoptera: Pyralidae). *Journal of Applied Life Sciences International,* 24(2): 1-26. DOI: 10. 9734/ JALSI/2021/ v24i230219

Ghoneim, K.; Hamadah, Kh.; El-Hela, A. and Abo Elsoud, A. (2022): Disturbed activities of the detoxifying enzymes, acid and alkaline phosphatases, of *Galleria mellonella* L. (Lepidoptera: Pyralidae) by four plant growth regulators. *International Journal of Biosciences,* 21(4): 44-60.

GraphPad InStat© v. 3.01 (1998): GraphPad Software, Inc. 7825 Fay Avenue, Suite 230 La Jolla, CA 92037 USA. Available online at: http://www.graphpad.com/scientific-software/instat/

Gupta, A.P. and Sutherland, D.J. (1966): *In vitro* transformations of the insect plasmatocyte in some insects. *Journal of Insect Physiology,* 12: 1369-1375.https://doi.org/ 10.1016/0022-1910(66)90151-X

Halawa, S.; Gaaboub, I.; Gad, A.A. and El-Aswad, A.F. (2007): Effect of some insecticides on the haemolymph of desert locust *Schistocerca gregaria* Forskal. *Journal of Egyptian Society of Toxicology,* 36: 61-66.

Hamadah, Kh. Sh. and Tanani, M.A. (2017): Disruptive impacts of selected insecticides on larval haemogram parameters of the red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae). *Egyptian Academic Journal of Biological Sciences (A. Entomology),* 10(8): 85– 97. Doi: 10.21608/EAJBSA.2017.24847

Hamadah, Kh.; Ghoneim, K.; El-Hela, A. and Abo Elsoud, A.A. (2022): Cytopathological impacts of certain plant growth regulators on the circulating hemocytes of *Galleria mellonella* L. (Lepidoptera: Pyralidae). *Egyptian Academic Journal of Biological Sciences (A. Entomology),* 15(3):85-101. DOI: 10.21608/EAJBSA. 2022.259860

Hassan, M.U. (1985): Studies on the effect of some pyrethroids on the haemocyte of *Tryporyza* sp. M.Sc. Thesis, Deptt. Agri. Ento., Univ. Agric., Faisalabad, Pakistan.

Hassan, H.A.; Bakr, R.F.A.; Abd El-Bar, M.M.; Nawar, G.A. and Elbanna, H.M. (2013): Changes of cotton leaf worm haemocytes and esterases after exposure to compounds derived from urea and rice straw. *Egyptian Academic Journal of*

Biological Sciences (A. Entomology),5(2): 35-48. Doi: 10.21608/EAJBSC. 2013.16094

Haszcz, K. (2016): Impairing the insect immune system with plant-derived substances. M.Sc. Thesis, the Graduate College of Missouri State University, 43pp. http://bearworks. missouristate. edu/theses/ 2383

Hernandez, S.; Lanz, H.; Rodriguez, M.H.; Torres, J.A.; Martinez, P.A. and Tsutsumi, V. (1999): Morphological and cytochemical characterization of female *Anopheles albimanus* (Diptera: Culicidae) hemocytes. *Journal of Medical Entomology,* 36: 426-434. https://doi. org/10.1093/jmedent/36.4.426

Hopkins, W.G. and Hüner, N.P. (2004): Introduction to Plant Physiology. 3rd Edition. John Wiley and Sons, Inc. 560pp.

Huang J.F.; Shui K.J.; Li H.Y.; Hu M.Y. and Zhong G.H. (2011): Antiproliferative effect of azadirachtin A on *Spodoptera litura* Sl-1 cell line through cell cycle arrest and apoptosis induced by up-regulation of p53. *Pesticide Biochemistry and Physiology*, 99: 16-24. https://doi.org/10.1016/j.pestbp.2010.08.002

Hussaini, S.S. (2003): Progress of research work on entomopathogenic nematodes in India. In: "Current status of research entomopathogenic Nematodes in India" (Hussaini, S.S.; Rabindra, R.J. and Nagesh, M., eds.). PdbC, publication, Bangalore, India, pp. 27-69.

James, R.R. (2011): Potential of ozone as a fumigant to control pests in honey bee (Hymenoptera: Apidae) hives. *Journal of Economic Entomology*, 104: 353-359. doi: 10.1603/ec10385.

Jan, S.; Singh, R.; Bhardwaj, R.; Ahmad, P. and Kapoor, D. (**2020**): Plant growth regulators: a sustainable approach to combat pesticide toxicity. *Biotechnology,* 10(11). Doi: 10.1007/s13205-020-02454-4

John, P.A. and Ananthakrishnan, T.N. (1995): Impact of azadirachtin on the haemodynamics of *Cyrtacanthacris tatarica* L. (Acrididae: Orthoptera). *Journal of Entomological Research,* 19(4): 285-290. http://oar.icrisat.org/id/eprint/6879

Jones, J.C. (1962): Current concepts concerning insect haemocytes. *Integrative and Comparative Biology,* 2(2): 209-246. https://doi. org/ 10. 1093/icb/2.2.209

Jones, J.C. (1967): Normal differential count of haemocytes in relation to ecdysis and feeding in *Rhodnius prolixus*. *Journal of Insect Physiology*, 13: 1133-1143. https://doi.org/10.1016/0022-1910(67)90087- X

Kaur, R. and Rup, P.J. (2003): Influence of four plant growth regulators on development of the melon fruit fly, *Bactrocera cucurbitae* (Coquillett). *Insect Science and its Application,* 23(2):121-125.DOI: https://doi.org/10.1017/S1742758400020336

Korrat, E.E.E.; Abdelmonem, A.E.; Helalia, A.A.R. and Khalifa, H.M.S. (2012): Toxicological study of some conventional and non-conventional insecticides and their mixtures against cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). *Annals of Agricultural Science,* 57: 145-152.

Kurihara, Y.; Shimazu, T. and Wago, H. (1992): Classification of hemocytes in the common cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae): I. Phase microscopic study. *Applied Entomology and Zoology,* 27(2): 225-235. https://doi.org/10.1303/aez. 27.225

Kurt, D. and Kayis, T. (2015): Effects of the pyrethroid insecticide deltamethrin on the hemocytes of *Galleria mellonella*. *Turkish Journal of Zoology,* 39: 452-457. DOI: 10.3906/zoo-1405-66

Kwadha, C.A.; Ongamo, G.O.; Ndegwa, P.N.; Raina, S.K. and Fombong, A.T. (2017): The biology and control of the greater wax moth, *Galleria mellonella*. *Insects,* 9, 8(2): 61. doi: 10.3390/ insects8020061

Lavine, M.D. and Strand, M.R. (2002):Insect haemocytes and their role in immunity. *Insect Biochemistry and Molecular Biology,* 32: 1295-1309. https://doi.org/10. 1016/S0965-1748(02)00092-9

Ling, E. and Yu, X.Q. (2006):Hemocytes from the tobacco hornworm *Manduca sexta*have distinct functions in phagocytosis of foreign particles and self-dead cells. *Developmental and Comparative Immunology,* 30: 301- 309. doi: 10.1016/j.dci. 2005.05.006.

Liu, F.; Xu, Q.; Zhang, Q.; Lu, A.; Beerntsen, B.T. and Ling E. (2013): Hemocytes and hematopoiesis in the silkworm, *Bombyx mori*. *Invertebrate Survival Journal,* 10: 102-109.

Maazoun, A.M.; Ben Hlel, T.; Hamdi, S.H.; Belhadj, F.; Ben Jemâa, J.M. and Marzouki, M.N. (2017): Screening for insecticidal potential and acetylcholinesterase activity inhibition of *Urginea maritima* bulbs extract for the control of *Sitophilus oryzae* (L.). *Journal of Asia-Pacific Entomology,* 20: 752-760.https://doi.org/10.1016/ j.aspen. 2017.04.004

Mahmoudvand, M.; Abbasipour, H.; Hosseinpour, M.H.; Rastegar, F. and Basij, M. (2011): Using some plant essential oils as natural fumigants against adults of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *Munis Entomology & Zoology,* 6(1): 150-154.

Makori, D.M.; Fombong, A.T.; Abdel-Rahman, E.M.; Nkoba, K.; Ongus, J.; Irungu, J.; Mosomtai, G.; Makau, S.; Mutanga, O. and Odindi, J. (2017): Predicting spatial distribution of key honeybee pests in Kenya using remotely sensed and bioclimatic variables: Key honeybee pests distribution models. *ISPRS International Journal of Geo-Information,* 6: 66. https://doi.org/10.3390/ ijgi6030066

Mall, S.B. and Gupta, S.P. (1979): Free haemocytes in the adult red pumpkin beetle *Aulacophora foveicollis* Lucas. *Indian Journal of Entomology*, 41: 223-230.

Manogem E.M.; Cheruparambath, P.; Shibi, P.; Arathi, S. and Banu, A. (2016): Effect of chitin synthesis inhibitor, Flufenoxuron, on haemocytes of *Spodoptera mauritia* (Boisd.)(Lepidoptera: Noctuidae). International Journal of Plant, *Animal and Environmental Sciences,* 6(1): 68-75.

Metwally, H.M.S.; Hafez, G.A.; Hussein, M.A.; Salem, H.A.; Saleh, M.M.E. (2012): Low cost artificial diet for rearing the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) as a host for entomopathogenic nematodes. *Egyptian Journal of Biological Pest Control,* 22(1): 15. http://www.esbcp.org/index.asp

Mohamed, H.F.; El-Naggar, S.E.M.; Elbarky, N.M.; Ibrahim, A.A.; Salama, M.S. (2014): The impact of each of the essential oils of marjoram and lemon grass in conjunction with gamma irradiation against the greater wax moth, *Galleria mellonella*. *IOSR Journal of Pharmacy and Biological Sciences,* 9(5): 92-106. DOI: 10.9790/3008-095492106

Moroney, M.J. (1956): Facts from figures (3rd ed.). Penguin Books Ltd., Harmondsworth. Middle Sex.

Mosallanejad, H. and Smagghe, G. (2009): Biochemical mechanisms of methoxyfenozide resistance in the cotton leafworm *Spodoptera littoralis*. *Pest Management Science,* 65: 732-736. DOI:10.1002/ ps.1753

Nagaratna, W.; Kalleshwaraswamy, C.M.; Dhananjaya, B.C.; Sharanabasappa and Prakash, N.B. (2022): Effect of Silicon and Plant Growth Regulators on the Biology and Fitness of Fall Armyworm, *Spodoptera frugiperda*, a Recently Invaded Pest of Maize in India. *Silicon,* 14:783–793. https://doi.org/10.1007/ s12633-020-00901-8

Nappi, J.A. (1974): Insect haemocytes and the problem of host recognition of foreigners. In: "Contemporary Topics in Immuonology-Invertebrate immunity"(Cooper, E.L.,

ed.), IV, pp: 207-224. Plenum Press, New York and London.

Naqqash, M.N.; Gökçe, A.; Bakhsh, A. and Salim, M. (2016): Insecticide resistance and its molecular basis in urban insect pests. *Parasitology Research,* 115: 1363–1373.doi: 10.1007/s00436-015-4898-9.

Nardi, J.B.; Gao, C. and Kanost, M.R. (2001): The extracellular matrix protein lacunin is expressed by a subset of hemocytes involved in basal lamina morphogenesis. *Journal of Insect Physiology,* 47: 997-1006. https://doi.org/10.1016/S0022-1910(01)00074-9

Nitin, K.; Kumar, K.D.; Kumar, M.V. and Sanjay, P. (2012): Effect of economical modification in artificial diet of greater wax moth *Galleria mellonella* (Lepidoptera: Pyralidae). *Indian Journal of Entomology,* 74(4): 369-374.

Nruwirth, M. (1973): The structure of the haemocytes of *Galleria mellonella* (Lepidoptera). *Journal of Morphology,* 139: 105-124.doi: 10.1002/jmor. 1051390107.

Ordas, M.C.; Ordas, A.; Belosa, C. and Figueras, A. (2000): Immune parameters in carpet shell clams naturally infected with *Perkinsus atlanticus*. *Fish Shellfish Immunology,* 10(7): 597-609. https://doi. org/10.1006/fsim.2000.0274

Pamita, A. and Priyanka, S. (2013): Designing and binding mode prediction of juvenile hormone analogues as potential inhibitor for *Galleria mellonella*. *Journal of Computer Science & Systems Biology, 6*: 106-111. DOI: 10.4172/jcsb.1000107

Pandey, J.P. and Tiwari, R.K. (2011): Neem based insecticides interaction with development and fecundity of red cotton bug, *Dysdercus cingulatus* Fab. *International Journal of Agricultural Research,* 6(4): 335-346. DOI:10.3923/ ijar.2011.335.346

Pandey, J.P.; Upadhyay, A.K. and Tiwari, R.K. (2007): Effect of some plant extracts on haemocyte count and moulting of *Danaus chrysippus* larvae. *Journal of Advances in Zoology,* 28: 14-20. DOI: 10.3923/je.2012.23.31

Pandey, J.P.; Tiwari, R.K. and Kumar, D. (2008): Reduction in hemocyte mediated immune response in *Danaus chrysippus* following treatment with neem based insecticides. *Journal of Entomology,* 5: 200-206. DOI: 10.3923/ je.2008.200.206

Pandey, S.; Pandey, J.P. and Tiwari, R.K. (2012): Effect of botanicals on hemocytes and molting of *Papilio demoleus* larvae. *Journal of Entomology,* 9(1): 23-31. DOI:10.3923/ je.2012.23.31

Pendland, J.C. and Boucias, D.G. (1996): Phagocytosis of lectin-opsonized fungal cells and endocytosis of the ligand by insect *Spodoptera exigua* granular hemocytes: An ultrastructural and immunocytochemical study. *Cell Tissue Research,* 285: 57-67.

Pereira, S.G.; Sanaveerappanavar, V.T. and Murthy, M.S. (2006): Geographical variation in the susceptibility of the diamond back moth *Ptlutella xylostella* L. to *Bacillus thuringiensis* products and acylurea compounds. *Pest Management,* 15: 26–26.

Piatek, M.; Sheehan, G. and Kavanagh, K. (2021): *Galleria mellonella*: The versatile host for drug discovery, *In Vivo* toxicity testing and characterising host-pathogen interactions. *Antibiotics (Basel),* 10(12):1545. doi: 10.3390/antibiotics10121545.

Qadri, S.S.H. and Narsaiah, J. (1978): Effect of azadirachtin on the moulting processes of last instar nymphs of *Periplaneta americana* (L.). *Indian Journal of Experimental Biology,* 16: 1141-1143.

Qamar, A. and Jamal, K. (2009): Differential haemocyte counts of 5th instar nymphs and adults of *Dysdercus cingulatus* Fabr (Hemiptera: Pyrrhocoridae) treated with acephate, an organophosphorus insecticide. *Biology and Medicine,* 1(2):116-121.

Rajendran, S. and Sriranjini, V. (2008): Plant products as fumigants for control of stored-product insects. *Journal of Stored Products Research,* 44: 126-35. https://doi.org/10.1016/j.jspr.2007.08.003

Rao, P.C.G.; Ray, A. and Ramamurty, P.S. (1984): Effect of ligation and ecdysone on total

haemocyte count in the tobacco caterpillar, *Spodoptera litura* (Noctuidae: Lepidoptera). *Canadian Journal of Zoology*, 62, 1461-1463. https://doi.org/10. 1139/z84-211

Ratcliffe, N.A. and George, S.J. (1976): Cellular defense reactions of insect haemocytes *in vivo*: nodule formation and development in *Galleria mellonella* and *Pieris brassicae* larvae. *Journal of Invertebrate Pathology,* 28: 373- 382. https://doi.org/10.1016/0022-2011(76)90013-6

Ribeiro, C. and Brehelin, M. (2006):Insect haemocytes: what type of cell is that?.*Journal of Insect Physiology*, 52: 417- 429. https://doi.org/ 10.1016/ j.jinsphys. 2006.01.005

Rizk, S.A.; El–Halfawy, N.A. and Salem, H.M. (2001): Toxicity and effect of Margosan–O and azadirachtin on haemocytes of *Spodoptera littoralis* (Boisd.) larvae. *Bulletin of Entomological Society of Egypt (Economic Series)*, 28: 39-48.

Roch, M.; Varela, M.C.; Taglialegna, A. and Rosato, A.E. (2020): Tedizolid is a promising antimicrobial option for the treatment of *Staphylococcus aureus* infections in cystic fibrosis patients. *Journal of Antimicrobial Chemistry,* 75:126-134. DOI: 10.1093/jac/dkz418

Sabri, M.A. and Tariq, B. (2004): Toxicity of some insecticides on the haemocytes of red pumpkin beetle, *Aulacophora foveicollis* Lucas. *Journal of Pakistan Entomology*, 26: 109-114.

Sadeghi, R.; Raeisi, N.H. and Jamshidnia, A. (2017): Immunological responses of *Sesamia cretica* to *Ferula ovina* essential oil. *Journal of Insect Science,* 17(1): 1–5.doi:10.1093/jisesa/iew124

Sahayaraj, K. and Kombiah, P. (2010): Insecticidal activities of neem gold on banana rhizome weevil (BRW), *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae). Journal of Biopesticides, 3(1 Special Issue): 304 -308.

Sass, M.; Kiss, A. and Locke, M. (1994): The localization of surface integument peptides in tracheae and tracheoles. *Journal of Insect Physiology,* 40: 561-575. https://doi.org/10.1016/0022-1910(94)90143- 0

Sendi, J.J. and Salehi, R. (2010): The effect of methoprene on total hemocyte counts and histopathology of hemocytes in *Papilio demoleus* L. (Lepidoptera). *Munis Entomology and Zoology*, 5(1): 240-246.

Sharma, P.R., Sharma, O.P. and Saxena, B.P. (2003): Effect of neem glod on hemocytes of the tobacco armyworm, *Spodoptera littura* (Fabricius) (Lepidoptera: Noctuidae). *Current Science,* 84(5): 690-695. https://www.jstor. org/stable/24108506

Sharma, P.R.; Sharma, O.P. and Saxena, B.P. (2008): Effect of sweet flag rhizome oil (*Acorus calamus*) on hemogram and ultrastructure of hemocytes of the tobacco armyworm, *Spodoptera litura* (Lepidoptera: Noctuidae). *Micron,* 39: 544-551. https://doi.org/ 10.1016/ j.micron. 2007. 07.005

Shaurub, E.H. and Sabbour, M.M. (2017): Impacts of pyriproxyfen, flufenoxuron and acetone extract of *Melia azedarach* fruits on the hemolymph picture of the black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae). *Advances in Agricultural Science,* 5(2), 1-9. https://aaasjournal.org/submission/index.php/aaas/article/view/11

Shaurub, E.H.; Abd El-Meguid, A. and Abd El-Aziz, N.M. (2014): Quantitative and ultrastructural changes in the haemocytes of *Spodoptera littoralis* (Boisd.) treated individually or in combination with *Spodoptera littoralis* multicapsidnucleopolyhedrovirus (SpliMNPV) and azadirachtin. *Micron*, 65: 62–68. DOI:10.1016/j.micron.2014.04.010

Shu, B.; Wang, W.; Hu, Q.; Huang, J.; Hu, M. and Zhong, G. (2015): A comprehensive study on apoptosis induction by azadirachtin in *Spodoptera frugiperda* cultured cell line

Sf9. *Archives of Insect Biochemistry,* 89: 153-168.DOI: 10.1002/ arch.21233

Siddiqui, M.I. and Al-Khalifa, M.S. (2014): Review of haemocyte count, response to chemicals, phagocytosis, encapsulation and metamorphosis in insects. *Italian Journal of Zoology,* 81(1): 2-15. doi: 10.1080/11250003.2013.858780

Sparks, T.C. and Nauen, R. (2015): IRAC: mode of action, classification and insecticide resistance management. *Pesticide Biochemistry and Physiology,* 121: 122–128.https://doi.org/10.1016/j.pestbp.2014. 11.014

Stamm, P.; Ramamoorthy, R. and Kumar, P.P. (2011): Feeding the extra billions: strategies to improve crops and enhance future food security. *Plant Biotechnology Reports,* 5: 107-120.DOI: 10.1007/s11816-011-0169-0

Strand, M.R. (2008): The insect cellular immune response. Insect Sci., 15: 1-14. https://doi.org/10.1111/j.1744-7917.2008.00183.x

Suhail, A.; Gogi, M.D.; Arif, M.J.; Rana, M.A. and Sarfraz, M. (2007): Effect of various treatment of azadirachtin, spinosad and abamectin on the haemogram of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). *Pakistan Entomologist,* 29(2): 151-164.

Tiwari, R.K.; Pandey, J.P. and Salehi, R. (2002): Haemopoietic organs and effect of their ablation on total haemocyte count in lemon butterfly, *Papilio demoleus* L. *Indian journal of Experimental Biology,* 40(10):1202-1205.

Tiwari, R.K.; Pandey, J.P. and Kumar, D. (2006): Effects of neem-based insecticides on metamorphosis, haemocytes count and reproductive behavior in red cotton bug, *Dysdercus koenigii* Fabr (Heteroptera: Pyrrhocoridae). *Journal of Entomology and Zoology Studies,* 31: 267-275.

Tojo, S.; Naganuma, F.; Arakawa, K. and Yokoo, S. (2000): Involvement of both granular cells and plasmatocytes in phagocytic reactions in the greater wax moth, *Galleria mellonella*. *Journal of Insect Physiology,* 46: 1129-1135. https://doi.org/10.1016/S0022-1910(99)00223-1

Uçkan, F.; Soydabaş, H.K. and Özbek, R. (2014): Effect of Indol-3-acetic acid on the biochemical parameters of *Achoria grisella* hemolymph and *Apanteles galleriae* larva. *Pakistan Journal of Biotechnology,* 11(2): 163-171.

Uçkan, F.; Özbek, R. and Ergin, E. (2015): Effects of indole-3-acetic acid on the biology of *Galleria mellonella* (Lepidoptera: Pyralidae) and its endoparasitoid *Pimpla turionellae* (Hymenoptera: Ichneumonidae). *Belgian Journal of Zoology,* 145(1): 49-58.

Viraktamath, S. (2010): Incidence, damage potentiality and management of *Galleria mellonella*: seasonal incidence, damage potentiality and management of the greater wax moth, *Galleria mellonella* (L.). VDM Verlag Dr. Müller. 284 pp.

Waheeb, H. (2020): Physiological and haematological responses of the Egyptian cotton leafworm *Spodoptera littoralis* Boisd. (Lepidoptera: Noctuidae) to some Sesquiterpenes. Ph.D. Thesis, Faculty of Science, Al-Azhar University, Cairo, Egypt, 380pp.

Yamashita M. and Iwabuchi K. (2001): *Bombyx mori* prohemocyte division and differentiation in individual microcultures. *Journal of Insect Physiology,* 47: 325-331. https://doi.org/10.1016/S0022-1910(00)00144-X

Zhu, Q.; He, Y.; Yao, J.; Liu, Y.; Tao, L. and Huang, Q. (2012): Effects of sublethal concentrations of the chitin synthesis inhibitor, hexaflumuron, on the development and hemolymph physiology of the cutworm, *Spodoptera litura*. *Journal of Insect Science,* 12(27): 1-13.doi: 10.1673/031.012.2701.

Zibaee, A. and Bandani, A.R. (2010a): Effects of *Artemisia annua* L. (Asteracea) on the digestive enzymatic profiles and the cellular immune reactions of the Sunn pest,

Eurygaster integriceps (Heteroptera: Scutellaridae), against *Beauveria bassiana*. *Bulletin of Entomological Research,* 100(2):185-96. doi: 10.1017/ S0007485309990149

Zibaee, A. and Bandani, A.R. (2010b): A study on the toxicity of the medicinal plant, *Artemisia annua* L. (Astracea) extracts the Sunn pest, *Eurygaster integriceps* Puton (Heteroptera: Scutelleridae). *Journal of Plant Protection Research,* 50: 48-54. DOI: 10.2478/v10045-010-0014-4

Zibaee, A.; Bandani, A.R. and Malagoli, D. (2012): Methoxyfenozide and pyriproxyfen alter the cellular immune reactions of *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae) against *Beauveria bassiana*. *Pesticide Biochemistry and Physiology,* 102: 30-37. DOI: 10.1016/j.pestbp.2011.10.006