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ORIGINAL ARTICLE

DETERIORATED LARVAL HAEMOGRAM IN THE PINK BOLLWORM *Pectinophora gossypiella* (SAUNDERS) (LEPIDOPTERA: GELECHIIDAE) BY THE CHITIN SYNTHESIS INHIBITORS, NOVALURON AND DIOFENOLAN.

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ABSTRACT

The pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) is one of the most destructive insects attacking cotton fields world-wide. It acquired resistance against most of the conventional pesticides. Therefore, the present study was conducted to evaluate the disruptive effects of Novaluron and Diofenolan on different parameters of the larval haemogram after treatment of full grown larvae with LC_{50} values of these chitin synthesis inhibitors (0.765 and 0.036 ppm, respectively). Six main hemocyte types had been identified in the larval haemolymph, *viz*., Prohemocytes (PRs), Plasmatocytes (PLs), Spherulocytes (SPs), Oenocytoids (OEs), Granulocytes (GRs) and Adipohemocytes (ADs). The most important diagnostic characteristics of each main type had been described. Both compounds exerted strong promoting actions on larvae (6 and 48 hr post-treatment) to produce increasing total hemocyte population. The differential hemocyte count of each hemocyte type was determined in haemolymph, as response to the enhancing or inhibitory effect of the tested compounds. No effect was exhibited by Novaluron or Diofenolan on the cytological architecture of ADs while other types appeared with different symptoms of malformation. The present study was the first in the world investigating the effects of chitin synthesis inhibitors on the larval haemogram of *P. gossypiella*.

Keywords: Adipohemocyte, Granulocyte, Oenocytoids, Plasmatocytes, Prohemocytes, Spherulocytes.

1.INTRODUCTION

The insect pests may be controlled by disturbing their physiological activities, *viz*. feeding, moulting, reproduction and immune system (Pandey *et al*., 2012**)**. There is an open circulatory system in insects. It contains various types of haemocytes. Insects lack an acquired immune system like of the higher animals but have a well-developed innate response. Due to economical and ethical problems with the use of vertebrates in biomedical studies, insects have been suggested as alternative biomodels for toxicological preclinical studies (Berger *et al*., 2003; Gelbic *et al*., 2006). In addition, insects have been widely used in other fields of biomedical research, such as neuroscience (Bier and Bodmer, 2004; Crowther *et al*., 2002). The cellular defense of insects refers to haemocyte-mediated immune responses (Schmidt *et al*., 2001; Lavine and Strand, 2002). Haematological studies are very important in insect physiology because the haemocyte performs various physiological functions in the body. The primary functions of haemocytes are: coagulation to prevent loss of blood, phagocytosis, encapsulation of foreign bodies in the insect body cavity, nodule formation, detoxification of metabolites and biological active materials and distribution of nutritive materials to various tissues and stored them also and may be hormones (for more detail, see: Garcia and Rosales, 2002; Zhou *et al*., 2004; Ling and Yu, 2006; Ribeiro and Brehelin, 2006; Siddiqui and Al-Khalifa, 2012a; Chavan *et al*., 2017). Therefore, knowledge of normal haemocytes of an insect is necessary to physiologists, toxicologists and biochemists, as alterations in structure, types and number of cells reflects changes in physiological and biochemical processes (Qamar and Jamal, 2009; Berger and Jurčová, 2012).

Worldwide, the pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) is one of the most destructive insect pests that cause terrible damage to the cotton (Patil, 2003). In **Egypt**, this insect pest causes serious damage to cotton bolls resulting in enormous reduction in quantity and quality arising to one million kentar annually (Khidr *et al*., 1996; El-Aswad and Aly, 2007; Kandil *et al*., 2012). In **Egypt**, also, *P. gossypiella* has recently developed

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resistance to several classes of insecticides currently used in cotton fields because it has been known for its ability to detoxify these chemicals (Khurana and Verma, 1990; Abd-Elhady and Abd El-Aal, 2011). In addition, the intensive and discriminate uses of many conventionally synthetic pesticides led to several drastic problems, such as the environmental pollution, hazards to human and animals like birds, fishes and mammals, destruction of the pollinators and all other non-target insects as well as the natural enemies, like parasites and predators (Miles and Lysandrou, 2002; Abo-El Ghar *et al*., 2005; Aydin and Gurkan, 2006; Davies *et al*., 2007; Costa *et al*., 2008; Relyea, 2009; Mosallanejad and Smagghe, 2009; Sabry and Abdel-Aziz, 2013). Therefore, alternative materials have been initiated recently to minimize the serious toxicological problems to humans and the environment hazards (Derbalah *et al.*, 2014) and to delay the resistance development in *P. gossypiella* (Dahi *et al*., 2009; Hussain, 2012; Salama *et al*., 2013).

At present, using insect growth regulators (IGRs) is considered as the possible alternative way of synthetic insecticides for controlling this pest. IGRs are bio-rational compounds that are species-specific and highly selective in action and act by disrupting the normal development of several insect species (Henrick *et al*., 1973; Staal, 1975). The effects of IGRs, more precisely, the chitin synthesis inhibitors (CSIs) which interfere with chitin biosynthesis have been worked out on a number of insect species. CSIs interfere with chitin biosynthesis in insects preventing the moulting process or producing an imperfect cuticle (Hammock and Quistad, 1981). Thus they are effective suppressors of development for the entire life cycle of insects (Verloop and Ferrell, 1977). These compounds, also, affect the hormonal balance resulting in physiological disturbances (DeLoach *et al*., 1981).
Novaluron is a relatively new benzoylphenyl urea CSI with good activity against several insects, such as the Colorado potato beetle (Cutler *et al*., 2005a,b, 2007; Alyokhin *et al*., 2009), *Spodoptera littoralis* (Ghoneim *et al*., 2015 a; Hamadah *et al*., 2015, 2016; Tanani *et al*., 2016; Basiouny *et al*., 2016) and *Pectinophora gossypiella* (Ghoneim *et al*., 2017), as well as it has only low mammalian toxicity (Barazani, 2001; Ishaaya *et al*., 2002, 2003). Its residues tend to dissipate with half-life of 2.08 days and the safe use of it on possibly various crops in Egypt was established (Malhat *et al*., 2014). However, Cutler and Scott-Dupree (2007) reviewed some prospects and limitations Novaluron in insect pest management. Diofenolan is a CSI used for the control of several pests, such as lepidopterous species and scale insects (Paloukis and Navrozidis, 1995; Dhadialla *et al*., 1998), *Papilio demoleus* (Singh and Kumar, 2011), *Musca domestica* (Ghoneim *et al*., 2001, 2003), *Rhynchophorus ferrugineus* (Ghoneim *et al*., 2004) and *Schistocerca gregaria* (Ghoneim *et al.*, 2012; Hamadah *et al*., 2012; Tanani *et al*., 2012). It did not affect the survival of beneficial parasitoids and predators of some pests, such as *Chrysoperla carnea* (Sechser *et al*., 1994).

Among the environmental factors affecting insect hemocytes, morphologically and functionally, are insecticides and other compounds (Zibaee, 2011). Depending on the available literature, no research work had been conducted for investigating the effects of IGRs (including CSIs) on the larval haemogram of *P. gossypiella*. Therefore, the present study was carried out aiming to distinguish the circulating hemocytes in full grown larvae of this insect pest. It was the first research work in the world investigating the effects of CSIs Novaluron and Diofenolan on different parameters of the larval haemogram of *P. gossypiella*.

2. MATERIALS AND METHODS

Experimental insect

A culture of the pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) was originated by a sample of newly hatched larvae from the susceptible culture maintained for several generations along some years in Plant Protection Research Institute, Doqqi, Giza, Egypt. It was reared under constant conditions (27±2°C and 75±5% R.H.) at Department of Zoology and Entomology, Faculty of science, Al-Azhar University, Cairo. Larvae were provided with an artificial diet as described by Abd El-Hafez *et al*. (1982). For rearing details and manipulation of all developmental stages under laboratory controlled conditions (27±2°C and 75±5% R.H.), see Ghoneim *et al*. (2017).

CSIs and larval treatment

The tested compounds in the present study were the chitin synthesis inhibitors, Novaluron and Diofenolan. Novaluron (Rimon) [1-[chloro-4-(1,1,2-trifluoromethoxyethoxy) phenyl] -3- (2,6-difluorobenzoyl) urea] has the molecular formula $C_{17}H_9ClF_8N_2O_4$. Diofenolan (Aware®) (2S,4R)-2-Ethyl-4-[(4-phenoxyphenoxy) methyl]-1,3-dioxolane has the molecular formula $C_{18}H_{20}O_4$. Both compounds were supplied by Sigma-Aldrich Chemicals. In a preliminary experiment on full grown larvae of *P. gossypiella*, LC_{50} values were estimated in 0.765 and 0.036 ppm of Novaluron and Diofenolan, respectively. Four replicates of full grown larvae (10/replicate) were transferred into Petri dishes (one replicate/dish). Each replicate was sprayed with LC_{50} of each CSI using an atomizer. Control replicates were treated with distilled water only using the same technique.

Haematological criteria of study

Collection of haemolymph

After 6 and 48 hrs of treatment, haemolymph pools (3 larvae/ pool) were collected. Similar haemolymph pools were collected from the control congeners. The haemolymph was obtained by amputation of one or two prothoracic legs, before coxa of the larva using fine scissors. Gentle pressure was done on the thorax for obtaining haemolymph drops by non-heparinized capillary tube. Three individual replicates were used and the haemolymph from two individuals was never mixed.

Total haemocyte count

The haemolymph was collected into thoma-white blood cell diluting pipette to the mark (0.5). Diluting solution (Na Cl 4.65 gm, K Cl 0.15 gm, $CaCl_2$ 0.11 gm, Crystal violet 0.05 gm and acetic acid 1.25 ml / liter distilled water) was taken up to the mark (11) on the pipette (dilution is 20 times). The

first three drops were discharged to avoid errors. The mixture was dispended to the chamber of counting slide. After three minutes, the total numbers of cells recognized in 64 squares of the four corners were observed and counted using the light microscope. If the cells clumped or uneven distributed, the preparation was discarded. The number of haemocytes per cubic millimeter was calculated according to the formula of Jones (1962) as follows:

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

Where the depth factor is usually 10.

Differential haemocyte 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 1 minute, and fixed for 2 minutes with drops of absolute methyl alcohol. Fixed cells were stained with Giemsa's solution (diluted 1 : 20 in distilled water) for 20 minutes, washed several times with tap water, and dipped in distilled water. The stained smears were air-dried and mounted in DPX with slip cover. The haemocytes were viewed under oily lens of light microscope at a magnification 10 X 100 = 1000 and 100 cells per slide were examined. The cell shape, cytoplasmic ratio, cytoplasmic inclusions and shape of nucleus were used for classification of haemocytes using the classification scheme of Raina (1976). The percentages of haemocyte types were calculated by the formula:

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

Haemocyte deformations

For recording of the haemocyte deformities caused by CSIs, photomicrographs were obtained by using a light microscope provided with a camera at a magnification 10 X 100 = 1000.

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.

3. RESULTS

Identification and description of normal circulating haemocytes in the full grown larvae of *P. gossypiella*

Depending on the cell shape, cytoplasmic ratio, cytoplasmic inclusions and shape of nucleus, the free haemocytes in the haemolymph of full grown larvae of *P. gossypiella*, in the present study, had been identified and distinguished into six basic types, *viz*., Prohemocytes (PRs), Plasmatocytes (PLs), Spherulocytes (SPs), Oenocytoids (OEs), Granulocytes (GRs) and Adipohemocytes (ADs). It is interesting to give the most important diagnostic characteristics of each main type as follows.

PRs can be described as variable in size (3-7 µm wide and 6-8 µm long). They were observed as ovoid cells but nearly round or spherical in shape. It had a large centrally located nucleus and a prominent nucleolus. This nucleus occupied most of the cell volume. Abundant cytoplasm was deeply stained containing few organelles, such as sparse rough endoplasmic reticulum. Some vesiculation of the plasma membrane was evidently observed in few cases (see Plate 1A). This type of hemocytes was seen single or occasionally in small clusters of 4-8 cells. However, these deviations of the typical form could not be used as additional diagnostic characters to distinguish this type into subtypes (see Plate 1B & C).

PLs were observed as spindle-shaped cells and measured about 16x 4 µm. A large nucleus (occupying 40-50% of the cell volume) was observed as elongate, round or spherical and centric or eccentric in position with a distinct nucleolus. Cytoplasm was basophilic (faintly stained) and rich in organelles, such as a moderate amount of rough endoplasmic reticulum, many pinocytotic vesicles, scattered chromatin masses and several tapering projections (see Plate 2A).

SPs were distinguished as basophilic or acidophilic cells of variable size (8-20 µm wide and 7-24 µm long). They were observed in a round or ovoid shape and characterized by several cytoplasmic inclusions as well as intracytoplasmic spherules occupying almost all the cytoplasm. These spherules contained either granular, fine-textured filaments or flocculent material. Some cells liberated the entire content of their spherules, leaving on the enclosing membranes. Nucleus appeared small, centric or eccentric in position, mostly deformed by the spherules (see Plate 2B).

OEs were the largest hemocytes observed in the haemolymph of full grown larvae of *P. gossypiella*. They were observed as spherical (22-35.5 µm in diameter) or ovoid (18.7-25 µm long and 26.5-35.6 µm wide) cells. When stained with Geimsa stain, cytoplasm was seen homogenous basophilic showing clusters of fibrous structures interspersed with scarce groups of some organelles, including round adipophilic granules. Nucleus was small, slightly eccentric and darkly stained (see Plate 2C).

GRs appeared as spherical to ovoid cells of 10-12 µm in diameter. Nucleus was centrally located and might be centric or eccentric occupying 45-55% of the cell volume. Nucleus had a number of scattered chromatin masses and nucleolus. Cytoplasm was basophilic (deeply stained) and contained few types of granules, endoplasmic reticulum and an occasional lipid droplet. A progressive accumulation of lipid droplets in this type of hemocytes might be give indication to misidentify it as ADs. Some GRs appeared with extrusion of granules (see Plate 3).

ADs were observed in an irregular shape and of a variety of sizes (18-46 µm in diameter with an average of 25.7 µm). They contained variable lipid droplets and several other non-lipid inclusions (see Plate 4).

Effects of CSIs on the total hemocyte count (THC)

In a preliminary experiment, LC_{50} values of Novaluron and Diofenolan were calculated, after treatment of full grown larvae of *P. gossypiella*, in 0.765 and 0.036 ppm, respectively. After treatment of the full grown larvae with LC_{50} values of Novaluron and Diofenolan, the treated full grown larvae (6 and 48 hr post-treatment) were used to investigate the effects on some of most important hematological parameters.

Data of THC in the haemolymph of full grown larvae were assorted in Table (1). As clearly shown, THC in normal (untreated) larvae was estimated in an average of 7213±716.91 cells/mm^3 (6 hr full grown larvae) and 10138±918.67 cells/mm^3 (48 hr full grown larvae).

Depending on data of the same table, both Novaluron and Diofenolan exerted powerful promoting actions on the larvae to produce remarkably increasing hemocyte population. The enhancing effect of each CSI was stronger on larvae 6 hr post-treatment (162.72 & 136.91% increments, after treatment with Novaluron and Diofenolan, respectively) than 48 hr post-treatment (143.03 & 100.72% increments, after treatment with Novaluron and Diofenolan, respectively). As obviously shown in this table, THC in control larvae increased with the age of larvae (7213±716.91 & 10138±918.67 THC, at 6 hr post-treatment and 48 hr post-treatment, respectively). A similar trend was also observed in the treated larvae, regardless the tested compound. In addition, Novaluron exhibited stronger promoting action than Diofenolan on larvae for producing more hemocyte population.

Effects of CSIs on the differential hemocyte counts (DHCs)

As clearly shown in Table (2), the circulating ADs had the highest count in haemolymph of normal full grown larvae, followed by other hemocyte types, regardless the age. Also, this type considerably increased with the larval age (33.33±2.89 & 42.33±2.08, at 6 hr and 48 hr, respectively). In contrast, the least hemocyte population was estimated for **OEs**, regardless the age. In a similar trend, **OEs** slightly increased with the age of larvae (2.33±0.58 & 3.33±0.58, at 6 hr and 48 hr, respectively). Other hemocyte types unexceptionally decreased with the age of larvae.

Data of DHCs of the identified circulating hemocytes in the haemolymph of full grown larvae 6 hr and 48 hr post-treatment with LC_{50} values of Novaluron and Diofenolan were distributed in Table (**3**). According to these data, the affected DHCs of all hemocyte types, *viz*., ADs, PRs, GRs, PLs, SPs and OEs, can be summarized as follows.

With regard to ADs, the DHC significantly increased in haemolymph of larvae 6 hr post-treatment (41.01 & 33.00% increments, by Novaluron and Diofenolan, respectively) but slightly decreased 48 hr post-treatment (5.50 & 7.09% reductions, by Novaluron and Diofenolan, respectively). As obviously shown, Novaluron was more potent than Diofenolan for promoting larvae (6 hr post-treatment) to produce ADs while Diofenolan exhibited stronger action than Novaluron for stimulating the larvae (48 hr post-treatment) to produce large population of ADs.

In respect of PRs, data of the same table clearly revealed that a weak or strong suppressing action of CSIs had been exerted on larvae to produce this type of hemocytes (47.51 & 22.35% reductions in larvae at 6 hr and 48 hr post-treatment with Novaluron, respectively, as well as 1.27% reduction in larvae at 6 hr post-treatment with Diofenolan). An exceptional case of a slight increase of PRs was determined in larvae 48 hr post-treatment with Diofenolan (18.44% increment). As exiguously seen, Novaluron exhibited stronger inhibitory effect on PRs production than Diofenolan.

In connection with GRs, each of the tested CSIs exerted a prevalent stimulatory action on larvae to produce more hemocyte population than that produced by control larvae, regardless the time of counting. On the other hand, such stimulatory action declined with the age of larvae, since GRs population decreased with the age (29.99 & 16.22% increments, in larvae 6 hr & 48 hr post-treatment with Novaluron, respectively, as well as 29.80 & 13.54% increments, in larvae 6 hr & 48 hr post-treatment with Diofenolan, respectively). Novaluron exhibited approximately stronger action than Diofenolan for enhancing the production of GRs in larvae.

In contrast, production of PLs was slightly or considerably prohibited by the tested compounds, regardless the time of counting. The most potent inhibitory effect of Novaluron was exhibited on larvae 6 hr post-treatment (57.16% reduction in PLs) while the most potent inhibitory effect of Diofenolan was exhibited on larvae 48 hr post-treatment (66.70% reduction in PLs). In general, Diofenolan exerted stronger reducing action than Novaluron.

Concerning SPs, data arranged in Table (**3**) clearly revealed, also, that the production of this type of hemocytes was slightly or pronouncedly enhanced, irrespective of the tested compound and time of counting. In other words, increasing SPs population was determined after treatment with Novaluron (14.36 & 134.93% increments, 6 hr & 48 hr post-treatment, respectively) and Diofenolan (14.51 & 29.99% increments, 6 hr & 48 hr post-treatment, respectively).

Considering OEs, data of the same table obviously displayed a difference in effect between the tested compounds. Although Novaluron exhibited a slight prohibitory effect on larvae to produce this type of hemocytes (28.33 & 30.03% reductions, in haemolymph of larvae 6 hr & 48 hr post-treatment, respectively), Diofenolan enhanced the larvae to produce slightly increasing OEs (42.92 & 40.24% increments, in haemolymph of larvae 6 hr & 48 hr post-treatment, respectively).

Qualitative effects of CSIs on the hemocyte profile

To shed some light on the cytopathological effects of CSIs on GRs in haemolymph of full grown larvae of *P. gossypiella*, photomicrographs in Plate (9) clearly demonstrated some morphological deformities, such as cell degranulation and appearance of pycnotic nuclei, as a response to the disruptive action of Novaluron (see Plate 5B). As a response to the disruptive action of Diofenolan, faintly stained cells were observed containing cytoplasmic vacuolation and nucleus lysis (see Plate 5C).

With regard to the cytopathological effects of CSIs on PRs in the same larvae, photomicrographs in Plate (6) obviously displayed darkly stained cells with destroyed membranes, as a response to the disruptive action of Novaluron (see Plate 6B) and lysed cells with pycnotic nuclei, as a response to the disruptive action of Diofenolan (see Plate 6C).

As clearly shown in Plate (7), slight disruptive effects were exhibited by the tested CSIs on PLs in the haemolymph. After treatment of full grown larvae with LC_{50} of Novaluron, some cells had been disintegrated with deeply stained cytoplasm containing pycnotic nuclei (see Plate 7B). After treatment of full grown larvae with LC_{50} of Diofenolan, some cells had been disintegrated and cytoplasm was faintly stained with cytoplasmic vacuolation (see Plate 7C).

In respect of SPs, both Novaluron and Diofenolan exhibited disruptive effects on the cell morphology, since some cells appeared in compressed size with cytoplasm lysis (see Plate 8 B & C).

As clearly demonstrated in Plate (9), Novaluron failed to exhibit a cytopathological effect on OEs but Diofenolan treatment resulted in faintly stained cells with cytoplasm lysis (see Plate 9B). However, neither Novaluron nor Diofenolan exhibited cytopathological effect on ADs.

Table-1. Total hemocyte count (cell/mm^3) in full grown larvae of *P. gossypiella* as affected by treatment with LC_{50} values of CSIs.

CSI		Count Time	
		6 hrs post-treatment	48 hrs post-treatment
Novaluron	(mean±SD)	18950 ± 460.07 d	24638 ± 4228.5 d
	Change(%)	+162.72	+143.03
Diofenolan	(mean±SD)	17088 ± 971.58 d	20350 ± 1003.3 d
	Change(%)	+136.91	+100.73
Control	(mean±SD)	7213 ± 716.91	10138 ± 918.67

Mean±SD followed by letter (a): not significantly different (P>0.05), (b): significantly different (P<0.05), (c): highly significantly different (P<0.01), (d): very highly significantly different (P<0.001).

Table-2. Differential hemocyte counts (mean±SD) in haemolymph of normal full grown larvae of P. *gossypiella*:

Hemocyte type	Larval age (Count Time)	
	6 hrs post-treatment	48 hrs post- treatment
ADs	33.33±2.89	42.33±2.08
PRs	26.67±2.89	25.33±4.51
GRs	16.67±1.53	12.33±0.58
PLs	11.67±3.06	10.00±1.00
SPs	9.33±0.58	6.67±1.53
OEs	2.33±0.58	3.33±0.58

Table-3. Differential hemocyte counts (mean±SD) **in full grown larvae of *P. gossypiella* as affected by treatment with LC_{50} values of CSIs.**
a, b, c, d : see footnote of Table (**1**). ADs: Adipohemocytes, PRs: Prohemocytes, PLs: Plasmatocytes, GRs: Granulocytes, SPs: Spherulocytes, OEs: Oenocytoides.

Hemocyte type		Count Time			
		Novaluron		Diofenolan	
		6 hrs post-treatment	48 hrs post-treatment	6 hrs post-treatment	48 hrs post- treatment
ADs	Treated larvae	47±3.46 c	40±2.00 a	44.33±4.04 b	39.33±1.16 a
	Change (%)	+41.01	-5.50	33.00	-7.09
	Control larvae	33.33±2.89	42.33±2.08	33.33±2.89	42.33±2.08
PRs	Treated larvae	14±3.61 c	19.67±2.52 a	26.33±3.22 a	30±2.00 a
	Change (%)	-47.51	-22.35	-1.27	+18.44
	Control larvae	26.67±2.89	25.33±4.51	26.67±2.89	25.33±4.51
GRs	Treated larvae	21.67±2.89 a	14.33±2.08 a	21.33±1.16 c	14±2.65 a
	Change (%)	+29.99	+16.22	+29.80	+13.54
	Control larvae	16.67±1.53	12.33±0.58	16.67±1.53	12.33±0.58
PLs	Treated larvae	5±1.00 b	8±1.00 a	8±1.00 a	3.33±0.58 d
	Change (%)	-57.16	-20.00	-31.45	-66.70
	Control larvae	11.67±3.06	10±1.00	11.67±3.06	10±1.00
SPs	Treated larvae	10.67±3.51 a	15.67±2.08 c	10.67±1.16 b	8.67±2.08 a
	Change (%)	+14.36	+134.93	+14.51	+29.99
	Control larvae	9.33±0.58	6.67±1.53	9.33±0.58	6.67±1.53
OEs	Treated larvae	1.67±0.58 a	2.33±0.58 a	3.33±0.58 a	4.67±1.53 a
	Change (%)	-28.33	-30.03	+42.92	+40.24
	Control larvae	2.33±0.58	3.33±0.58	2.33±0.58	3.33±0.58

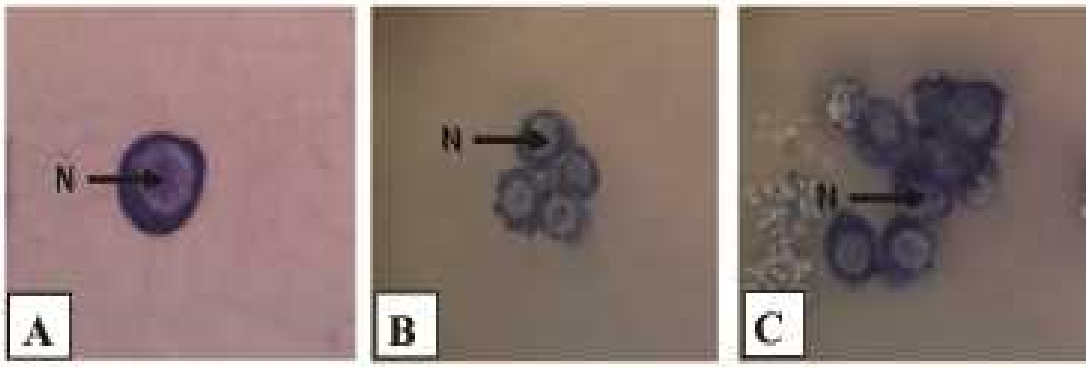

Plate-1. Photomicrographs of Prohemocytes **(PRs)** in the haemolymph of full grown larvae of *P. gossypiella* (Geimsa stain, 1000x). [A]: **Typical Normal** cell, [B]: Normal cell in a cluster of four cells, [C]: Normal cell in a cluster of many cells. N: nucleus.

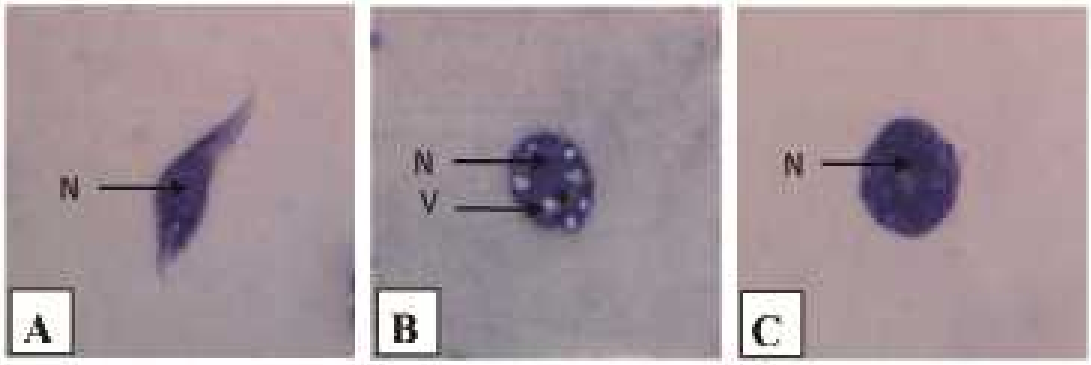

Plate-2. Photomicrographs of some types of normal hemocytes in the haemolymph of full grown larvae of *P. gossypiella* (Geimsa stain, 1000x). [A]: Plasmatocyte **(PL)**, [B]: Spherulocyte **(SP)**, [C]: Oenocytoid **(OE)**. N: nucleus, V: vacuoles.

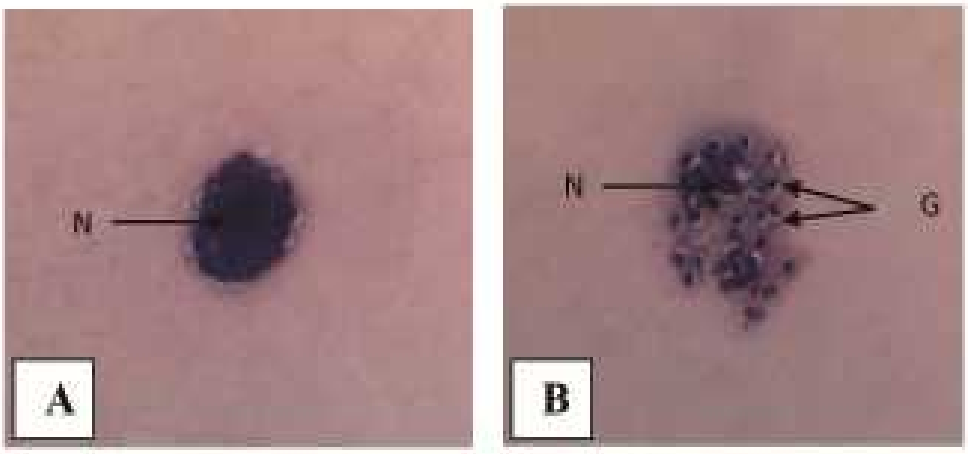

Plate-3. Photomicrographs of Granulocytes **(GRs)** in the haemolymph of full grown larvae of *P. gossypiella* (Geimsa stain, 1000x). [A]: **Typical normal** cell. [B]: **Normal** cell with extrusion of granules. N: nucleus, G: granules.

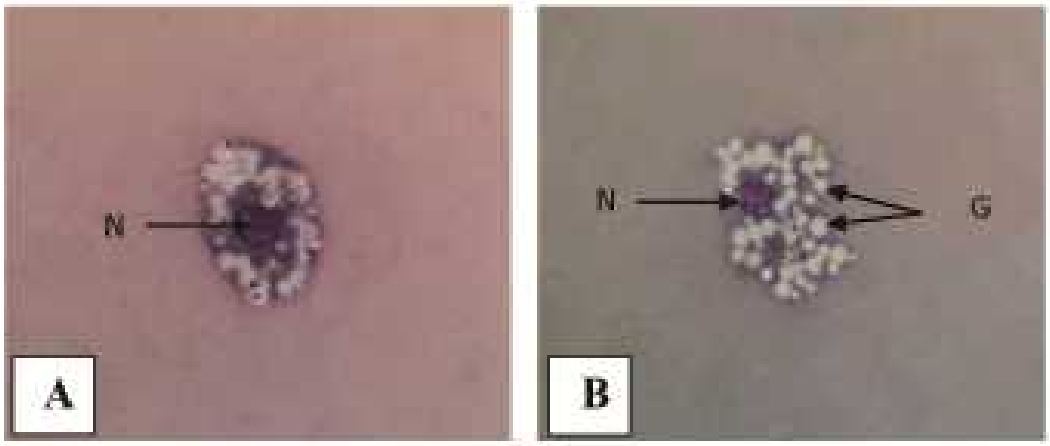

Plate-4. Photomicrographs of Adipohemocytes **(ADs)** in the haemolymph of full grown larvae of *P. gossypiella* (Geimsa stain, 1000x). [A]: **Typical normal** cell. [B]: Normal cell with irregular shape. N: nucleus, G: granules.

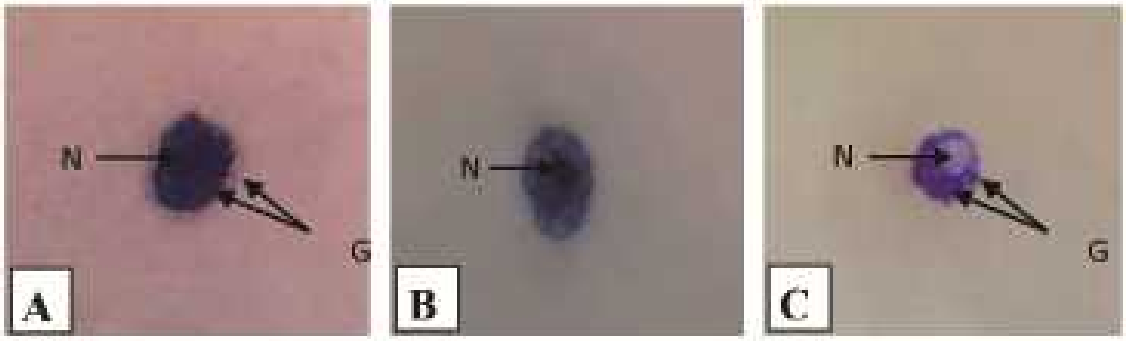

Plate-5. Photomicrographs of the deformed **GRs** in the haemolymph of full grown larvae of *P. gossypiella* (Geimsa stain, 1000x) by LC_{50} values of CSIs. [A]: Normal cell, [B]: Hemocyte deformation by **Novaluron**: a degranulated cell with pycnotic nucleus. [C]: Hemocyte deformation by **Diofenolan**: faintly stained cell and cytoplasmic vacuolation with nucleus lysis. N: nucleus, G: granules.

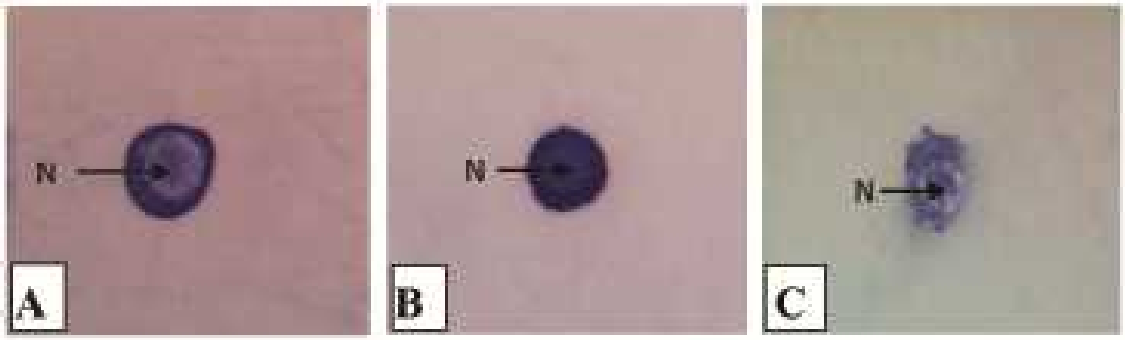

Plate-6. Photomicrographs of the deformed **PRs** in the haemolymph of full grown larvae of *P. gossypiella* (Geimsa stain, 1000x) by LC_{50} values of CSIs. [A]: Normal cell, [B]: Hemocyte deformation by **Novaluron**: darkly stained cell with destroyed cell membrane. [C]: Hemocyte deformation by **Diofenolan**: cell lysis and pycnotic nucleus. N: nucleus.

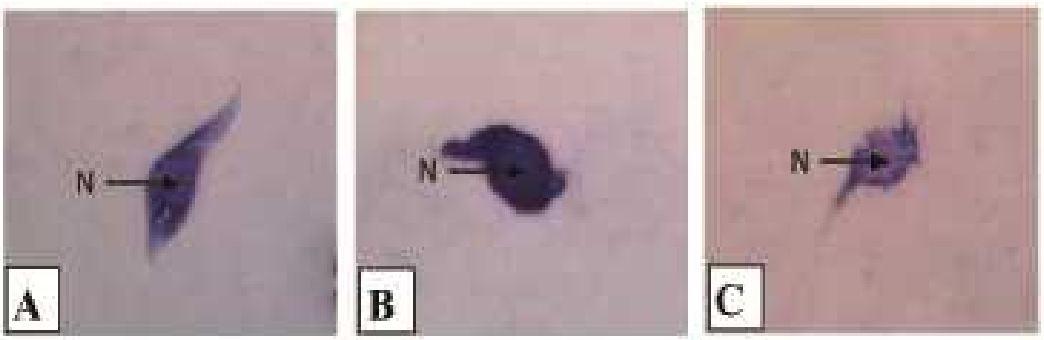

Plate-7. Photomicrographs of the deformed **PLs** in the haemolymph of full grown larvae of *P. gossypiella* (Geimsa stain, 1000x) by LC_{50} values of CSIs. [A]: Normal cell, [B]: Hemocyte deformation by **Novaluron**: cell lysis and deeply stained cytoplasm with pycnotic nucleus. [C]: Hemocyte deformation by **Diofenolan**: cell lysis, faintly stained and cytoplasmic vacuolation. N: nucleus.

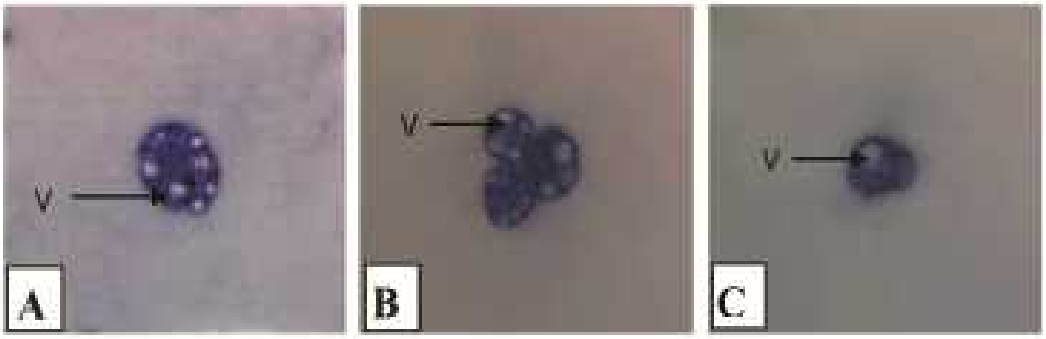

Plate-8. Photomicrographs of the deformed **SPs** in the haemolymph of full grown larvae of *P. gossypiella* (Geimsa stain, 1000x) by LC_{50} values of CSIs. [A]: Normal cell, [B]: Hemocyte deformation by **Novaluron**: compressed size and cytoplasm lysis. [C]: Hemocyte deformation by **Diofenolan**: compressed size and cytoplasm lysis. V: vacuoles.

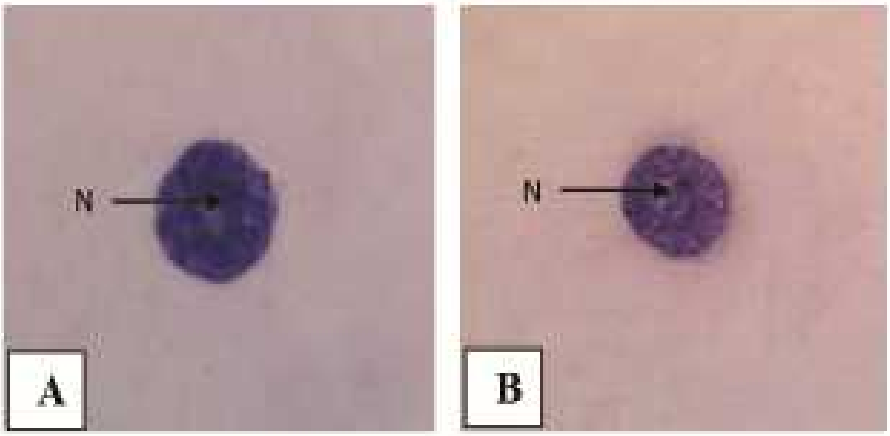

Plate-9. Photomicrographs of the deformed **OEs** in the haemolymph of full grown larvae of *P. gossypiella* (Geimsa stain, 1000x) by LC_{50} of Diofenolan. [A]: Normal cell, [B]: Hemocyte deformation: faintly stained cell with cytoplasm lysis. N: nucleus.

4. DISCUSSION

Circulating haemocytes perform various important physiological functions in the insect body, such as immune system, metabolism, and detoxification of metabolites that eventually play a crucial role in the defense of xenobiotics or microbial infection (for details, see Patton, 1983; Chavan *et al*., 2017). Haemocytes have been studied mostly in Lepidoptera, Hymenoptera, Coleoptera and Diptera (Gupta, 1985; Gurwattan *et al*., 1991; Miller and Stanley, 2000; Ayaad *et al*., 2001; Rizk *et al*., 2001; Lavine and Strand, 2002; El-Sheikh, 2002; Gelbic *et al*., 2006; Zohry, 2006; Ribeiro and Brehelin, 2006; Annuradha and Anuadurai, 2008; Ghoneim *et al*., 2015 b) as well as Dictyoptera (Chiang *et al.*, 1988), Heteroptera (Sanjayan *et al*., 1996), Hemiptera (George and Ambrose, 2004) and Orthoptera (Barakat *et al*., 2002; Tanani, 2010). Sharma *et al*. (2003) reported that the number and proportion of different haemocytes were beneficial for insects to develop environmental fitness. Earlier many studies evaluated the effects of various toxins, such as insecticide stress factors on the haemocyte of various insects. But only, limited work has been carried out on the role of insect growth regulators (IGR), particularly Chitin Synthesis Inhibitor (CSI) on the hematology of insects.

Circulating hemocyte types in *P. gossypiella* larvae

Classified categories of haemocyte types range from four to seven (Gupta, 1979) or between three and nine (Arnold, 1974; Al-Khalifa and Siddiqui, 1985). Otherwise, the available literature shows that the most common types are Prohemocytes (PRs), Granulocytes (GRs) and Oenocytoids (OEs) as described from different species in various orders (Ahmad, 1992; Fenoglio and Gervaso, 1993; Joshi and Lambdin, 1996; Hernandez *et al*., 1999; De Silva *et al*., 2000; Siddiqui and Al-Khalifa, 2012b). There is confusion between various haemocyte types such as PRs and Plasmatocytes (PLs) as well as GRs and Adipohemocytes (ADs) (Nruwirth, 1973).

As reported in the currently available literature, Manogem *et al*. **(**2016) recognized eight distinct classes of haemocytes were in the last instar larvae of *Spodoptera mauritia*: PLs, GRs, PRs, Spherulocytes (SPs), ADs, OEs, Vermicytes and Podocytes. Seven types of hemocytes have been described in various insects (Gupta, 1985; Brehelin and Zachary, 1986). Chavan *et al*. (2017) identified seven types of haemocytes in haemolymph of the beetle *Platynotus belli*, *viz*., PRs, PLs, GRs, OEs, ADs, SPs and Coagulocytes. Six types of hemocytes were identified in *Diatraea saccharalis* (Falleiros *et al*., 2003) and *Papilio demoleus* (Jalali and Salehi, 2008). Five distinct classes of haemocytes were identified in different insect species, such as *Spodoptera littoralis* (Zohry, 2006; Hassan *et al*., 2013; Ghoneim *et al*., 2015 b), *Manduca sexta* (Miller and Stanely, 2000), *Poekilocerus bufonius* (Al-Robai *et al*., 2002), *Spodoptera litura* (Sharma *et al*., 2003), *Ostrinia furnacolis* (Jian *et al*., 2003) and *Bombyx mori* (Han *et al*., 1998; Ling *et al*., 2003a; Tan *et al*., 2013; Liu *et al*., 2013). Four types of haemocytes were identified in some other insect species (Osman *et al*., 1984; Mahmoud and Yousuf, 1985; Masconi *et al*., 1989; Peter and Ananthakrishnan, 1995; Gelbic *et al*., 2006). Three types were characterized in some insects such as *Schistocerca gregaria* (Tanani, 2010). Only two types could be identified in *Drosophila* spp. (Lavine and Strand, 2002) and *Melanoplus sanguinipes* (Gurwattan *et al*., 1991; Meranpuri *et al*., 1991). However, Sendi and Salehi (2010) identified only two major hemocyte types in *P. demoleus* basing on their role in immunity, i.e., PLs and GRs.

With regard to the pink bollworm *P. gossypiella*, Clark and Chadbourne (1960) identified four categories of haemocytes in the last **(**4^{th}) instar larvae: Proleucocytoids, PLs, SPs, and Cystocytes. Raina and Bell (1974) observed seven types in haemocytes of the last larval instar of the same insect: PRs, PLs, GRs, SPs, ADs, OEs and Podocytes. After two years, Raina (1976) used some ultrastructural characteristics and described only five types of hemocytes in the haemolymph of mature last instar larvae of the same insect: PRs, PLs, GRs, SPs and OEs because he could not distinguish ADs or Podocytes.

In the present study, six main hemocyte types had been identified and distinguished in the haemolymph of full grown larvae of *P. gossypiella*, *viz*., PRs, PLs, SPs, OEs, GRs and ADs. The most important diagnostic characteristics of each type had been described. Thus, the present result disagrees with those records previously reported for the same insect. However, the diverse results might be attributed to the differences in insect species or even its developmental stage, several technical difficulties for identification and the characters adopted by other workers (George and Ambrose, 2004; Ribeiro and Brehelin, 2006).

In addition, hemocyte classification, types and morphology are often influenced by some factors affecting the haemolymph physical properties or biochemical composition (Carrel *et al*., 1990), physiological condition of the insect (Chapman, 1998) and the insect developmental stages. Therefore, the hemocyte classification has been recommended to be revised several times in the same species (Dean *et al*., 2004; Ribeiro and Brehelin, 2006; Wood and Jacinto, 2007; Qamar and Jamal, 2009; Siddiqui and Al-Khalifa, 2012a, b; Ghoneim *et al*., 2015 b).

Total hemocyte population in normal larvae of *P. gossypiella*

The total hemocyte count (THC) has been found to be quite variable depending upon the insect species, developmental stage, physiological state and the technique followed (Romosen and Stofolano, 1998). It may be important to mention that the brain endocrine complex is involved in haemocyte accumulation following some initial stimulus (Nappi, 1974).

As far as our literature survey could ascertain, no research work had been conducted for investigating the effects of IGRs on the THC in larvae of *P. gossypiella*. THC in the haemolymph of normal (untreated) full grown larvae of this insect was estimated, in the current study, in an average of 7213±716.91 cells/mm^3 (6 hr full grown larvae and 10138±918.67 cells/mm^3 (48 hr full grown larvae). As clearly seen, the hemocyte population increased toward the prepupae as a physiological event for preparation to moult into the pupal stage.

Our results disagree with some other estimates as reported in the currently available literature, since Hassan (1985) recorded THC of normal larvae of *Tryporyza* sp. in average 22475 cells/mm^3 and in *Meladera* sp. of the average 22300 cells/mm^3 in males and 29100 cells/mm^3 in females. On the other hand, Mall and Gupta (1979) estimated THC of red pumpkin beetle *Aulacophora foveicollis* in an average of 5500 cells/mm^3. Sabri and Tariq (2004) determined THC of the same beetle in 4372 cells/mm^3. Recently, Chavan *et al*. **(**2017) estimated the THC in haemolymph of normal larvae of the beetle *P. belli* in an average of 26233.33±251.66 cells/mm^3.

It is important to shed some light on the varying hemocyte population in the haemolymph of normal full grown larvae of *P. gossypiella*. The circulating ADs had been observed with the largest count, followed by other hemocyte types, regardless the age. Also, this type considerably increased with the larval age. On the other hand, the least hemocyte population was estimated for OEs, regardless the age. OEs slightly increased with the age of larvae. In contrast, other hemocyte types decreased with the age of larvae. As reported in the available literature, the largest hemocyte count in haemolymph of last instar larvae of *Spodoptera mauritia* was estimated for PLs, followed by other types (Manogem *et al*., 2016). In normal (untreated) larvae of the beetle *P. belli* Chavan *et al*. **(**2017) estimated GRs count as the highest population, followed by PRs, ADs, OEs, PLs, Coagulocyte and SPs, respectively.

THC in *P. gossypiella* larvae as affected by Novaluron and Diofenolan

Hormones, synthetic pesticides and insect growth regulators (IGRs) intervene in the intermediary metabolism and immune capability of insects as observed in changes in hemocyte number, differentiation and phagocytosis (Qamar and Jamal, 2009). Responses of haemocyte count to chemicals, phagocytosis, encapsulation and metamorphosis in insects was reviewed by Siddiqui and Al-Khalifa (2014). After treatment of full grown larvae of *P. gossypiella* with LC_{50} values of Novaluron and Diofenolan (0.765 and 0.036 ppm, respectively), in the present study, THC was estimated in the treated larvae (6 and 48 hr post-treatment). Both compounds exerted strong promoting actions on larvae to produce increasing hemocyte population. Novaluron exhibited stronger promoting action than that of Diofenolan. The present result is in agreement with those reported results of pronouncedly increasing THC in larvae of several insect species after treatment with various IGRs, such as *S. littoralis* by Flufenoxuron and Chlorfluazuron (Bakr *et al*., 2007), Teflubenzuron (Abdel-Al *et al*., 2011), Hexaflumuron (Zhu *et al*., 2012), some compounds derived from urea waste (Hassan *et al*., 2013), Novaluron (Ghoneim *et al*., 2015); *S. litura* by ecdysone (Rao *et al*., 1984); *Coccinella septempunctata* by Spinosad (bacteria-based product)(Suhail *et al*., 2007); *Eurygaster integriceps* by Methoxyfenozide (Zibaee *et al*., 2012). After injection of the *Bombyx mori* larvae with 20-ecdysone, hemocyte density significantly increased at approximately 12-18 hr post-injection (Ling *et al*., 2003b). Also, some insecticides promoted the production of THC in larvae of different insect species, such as *Gryllus bimaculatus* (Mahmoud and Yousuf, 1985), *Acanthaspis pedestris* (Ambrose and George, 1996), *S. gregaria* (Al-Hariri and Suhail, 2001), *Rhynocoris kumarii* (George and Ambrose, 2004), *Dysdercus cingulatus* (Haq *et al*., 2005), and *Leptinotarsa decemlineata* (Dubovskiy *et al*., 2014), *etc*. Very recently, insecticides Dimethoate and Chlorpyriphos enhanced the larvae of *P. belli* to produce increasing THC which was inversely proportional to the concentration (Chavan *et al*., 2017).

On the contrary, the increasing hemocyte population in full grown larvae of *P. gossypiella*, after treatment with Novaluron or Diofenolan, in the current work, contradictory to those reported results of declined THC in larvae of different insects, as response to various IGRs or insecticides, such as *R. kumarii* by endosulfan (George and Ambrose, 2004); *S. gregaria* by spinosad and Proclaim® insecticide (Halawa *et al*., 2007); *C. septempunctata* by abamectin (bacteria-based product)(Suhail *et al.*, 2007); *P. demoleus* by Methoprene (Sendi and Salehi, 2010); *Mythima separata* by Hydroprene (Wang *et al*., 1993), *D. cingulatus* by β–ecdysone and maskisterone (phytoecdysone)(Ahmad, 1995); *Dysderus koenigii* by Penfluron (Prakash *et al*., 2007); *Agrotis ipsilon* by Diflubenzuron (Abdel-Aziz and Awad, 2010), *S. gregaria* by Teflubenzuron (Teleb, 2011); *E. integriceps* by Pyriproxyfen (Zibaee *et al*., 2012); *Ephestia kuehniella* by Pyriproxyfen and Hexaflumuron (Rahimi *et al*., 2013), *Glyphodes pyloalis* by a juvenile hormone (Khosravi *et al*., 2014); *S. littoralis* by certain concentration levels of some compounds derived from urea waste (Hassan *et al*., 2013) and Cyromazine (Ghoneim *et al*., 2015 b); *Aulacophora foveicollis* by Spinosad 240 G/L and insecticide Deltaphos-R (Sabri and Tariq, 2004); *Earias insulana* by insecticide Tracer 480 SC (Fareed, 2001) and *Spodoptera mauritia* by certain concentrations of Flufenoxuron (Manogem *et al*., 2016); *etc.* However, the application of Methoprene onto the 20-ecdysone-injected larvae of *B. mori* kept the hemocyte population stable without an obvious change (Ling *et al*., 2003b).

The prevalent promoting effects of Novaluron and Diofenolan on the full grown larvae of *P. gossypiella* to produce high THC, in the present study, can be attributed to the release of adhered hemocytes in general circulation and is response to the tested CSIs, as these cells are directly involved in defense mechanisms (Trehan and Pajni, 1961; Gupta, 1979). Because many insect species possess population of sessile haemocytes, the increasing THC, as recorded in the present study, may be due to the release of these cells and the activation of mitotic division of them (Ratcliffe and George, 1976) which might be activated in response to some insecticides or IGRs. This suggestion may be substantiated by the immune response of insects against pathogen or any foreign body, such as the introduced CSIs, in the present study (Chu *et al*., 1993; Anderson *et al*., 1995; Ordas *et al*., 2000). In this context, increasing THC, in the current investigation, can be attributed, also, to the enhanced encapsulation of Novaluron and Diofenolan molecules through process of melanization because melanin deposition during encapsulation is commonly initiated by haemocytes and/or phenoloxidase enzyme circulation in the plasma (Rolff and Siva-jothy, 2002; Nappi and Christensen, 2005).

Differential hemocyte counts (DHCs) in haemolymph of *P. gossypiella* larvae as disturbed by Novaluron and Diofenolan

It is important to point out that the increasing counts of some haemocyte types and decreasing counts of others may be due to the transformation of some types into other ones for achieving the phagocytic function or other tasks in the battle against the biotic targets like bacteria, yeast and apoptic bodies, as well s against and abiotic materials such as particles of Indian ink (Hernandez *et al*., 1999; De Silva *et al*., 2000). The particular haemocytes reported to be phagocytic varies among insect taxa, and in some cases discrepancies even exist in the literature among studies on the same species (Tojo *et al*., 2000). 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). DHCs in haemolymph of the full grown larvae of *P. gossypiella* had been changed depending on the hemocyte type, tested CSI and age of larvae.

Fluctuated DHC of PRs in haemolymph of larvae

In the present study, treatment of full grown larvae of *P. gossypiella* with LC_{50} of Novaluron or Diofenolan led to slightly or considerably declined PRs count, regardless the time of count, indicating that these larvae had been subjected to suppressing action of each compound. This result is, to a great extent, in conformity with those decreasing PRs as reported in *S. littoralis* by Flufenoxuron (Zohry, 2006) and Cyromazine (Ghoneim *et al*., 2015 b) as well as *A. ipsilon* by Diflubenzuron (Tiwari *et al*., 2002; Abdel-Aziz and Awad, 2010); *Philosamia ricini* by Dimethoate (Bhagawathi and Mahanthy, 2012); *S. mauritia* by Flufenoxuron (Manogem *et al*., 2016). In contrast, the present result disagrees with those reported results of increasing PRs population in some insects after treatment with different IGRs or insecticides, such as *M. separata* by Hydroprene (Wang *et al*., 1993), *R. kumarii* by Endosulfan (George and Ambrose, 2004), *S. gregaria* by Teflubenzuron (Teleb, 2011), *etc*. Although PRs are progenitor stem cells which 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). As reported by Liu *et al*. (2013), PRs in *B. mori* can differentiate into PLs and GRs. However, the general reduction of PRs population in larvae of *P. gossypiella*, in the present study, may be attributed either to the cytotoxic effects of the tested CSIs on mitotic division of PRs, conversion to other types of cells or to the inhibitory effects on the activity of haematopoietic organs responsible for PRs production (Zhu *et al*., 2012; Zibaee *et al*., 2012). Also, reduction of PRs population in larvae of *P. gossypiella*, in the present study, may be due to the destruction of haemopoetic organs which responsible for the production of this type of hemocytes (Saxena and Srivasthava, 2001).

Fluctuated DHC of PLs in haemolymph of larvae

In the current study on *P. gossypiella*, both Novaluron and Diofenolan suppressed the treated larvae to produce normal count of PLs, since their population was slightly or remarkably decreased at both 6 and 48 hr post-treatment. this result is in accordance with those reported decreasing PLs count in haemolymph of some insects by various IGRs 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). On the other hand, the present result is in contrast with those reported results of increasing PLs count in *S. littoralis* by Cyromazine (Ghoneim *et al*., 2015 b); *S. gregaria* nymphs by Lambdacyhalothrin and Deltamethrin (Al-Hariri and Suhail, 2001) and Teflubenzuron (Teleb, 2011); *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*. The role of PLs in phagocytosis is disputed because some authors believed that they are phagocytes (Tojo *et al*., 2000; Ling and Yu, 2006) but other authors reported no phagocytic function (Nruwirth, 1973; Beaulaton, 1979). The decreasing PLs population in the current work on *P. gossypiella* can be explained by their transformation into other types of hemocytes (Beaulaton and Monpeyssin, 1976; George, 1996) since they are highly polymorphic cells (Gupta and Sutherland, 1966). Also, Novaluron and Diofenolan may impaired the haematopoietic organs which responsible for the production of these hemocytes (Tiwari *et al*., 2002).

Fluctuated DHC of GRs in haemolymph of larvae

One of the main functions of GRs is phagocytosis as reported by Wago **(**1980) in *B. mori*, Raina (1976) in *Pectyinophora gossypiella*, Tojo *et al*. (2000) in *Galleria mellonella*, Nardi *et al*. (2001) in *Manduca sexta*; Essawy *et al*. **(**1985) in *Heliothis armigera*, Pendland and Boucias **(**1996) in *Spodoptera exigua*, Butt and Shields (1996) in *Lymantria dispar* and Costa *et al*. **(**2005**)** in *S. littoralis*. In the current investigation, both Novaluron and Diofenolan exhibited stimulatory effects on full grown larvae of *P. gossypiella* to produce larger GRs population, as counted at 6 and 48 hr post-treatment. This result is, to some extent, concomitant to those reported results of enhanced GRs population in *S. gregaria* after treatment with Lambdacyhalothrin and Deltamethrin (Al-Hariri and Suhail, 2001) or Teflubenzuron (Teleb, 2011) and *A. ipsilon* after treatment with Diflubenzuron (Abdel-Aziz and Awad, 2010). On the other hand, our result disagrees with the decreasing GRs in haemolymph of *S. littoralis* larvae after treatment with the drug metyrapone (Gelbic *et al*., 2006), LC_{50} of Flufenoxuron (Bakr *et al*., 2007), or some compounds derived from urea waste (Hassan *et al*., 2013), as well as decreasing GRs count in other insect species, such as *R. kumarii* by Endosulfan (George and Ambrose, 2004) and *S. litura* by hexaflumuron (Zhu *et al*., 2012). Whereas the interpretation of decreasing count of GRs in haemolymph of some insects as response to some foreign bodies and compounds was available in the current literature (Butt and Shields, 1996; Nardi *et al*., 2001; Barakat *et al*., 2002; George and Ambrose, 2004; Costa *et al*., 2005; Liu *et al*., 2013), we have no conceivable interpretation to the increasing GRs population in *P. gossypiella* larvae after treatment with Novaluron and Diofenolan, rightnow!!

Fluctuated DHC of SPs in haemolymph of larvae

Throughput screening of the available literature indicated very few reports of increasing SPs count in insects, as response to IGRs or insecticides, such as *A. ipsilon* by Diflubenzuron (Abdel-Aziz and Awad, 2010) and *S. littoralis* by Novaluron and Cyromazine (Ghoneim *et al*., 2015 b). The present result evidently corresponds to those reported results, since SPs population slightly or remarkably increased in haemolymph of full grown larvae of *P. gossypiella* at 6 and 48 hr post-treatment with Novaluron and Diofenolan. However, the major promoting action of each of the tested CSIs on SPs population, in the present study, disagrees with some reported results of decreasing count of this hemocyte type in haemolymph of some insects, such as *S. gregaria* by Lambdacyhalothrin and Deltamethrin (Al-Hariri and Suhail, 2001) or Tflubenzuron (Teleb, 2011), *M. separata* by KK-42 (Wang *et al*., 1993), *P. demoleus* by methoprene (Sendi and Salehi, 2010) and *S. mauritia* by Flufenoxuron (Manogem *et al*., 2016). In Lepidoptera, SPs are quite different from GRs overloaded with phagocytosed material. The functions of SPs are unknown until now (Ribeiro and Brehelin, 2006) but Sass *et al*. (1994) suggested their responsibility for transporting cuticular components. However, the general increase of SPs population in full grown larvae of *P. gossypiella* after treatment with Novaluron and Diofenolan may be due to their enhancing effects on the differentiation of SPs or transformation of other hemocytes into SPs in the treated larvae of *P. gossypiella*. Unfortunately the exact mode of action is still obscure!!

Fluctuated DHC of OEs in haemolymph of larvae

In the present study, treatment of full grown larvae of *P. gossypiella* with LC_{50} of Novaluron resulted in an insignificantly decreased population of OEs, regardless the time of count. In contrast, treatment with LC_{50} of Diofenolan led to increasing OEs population. This promoting action of Diofenolan on OEs population is concomitant to those reported results of enhanced OEs population in haemolymph of *S. littoralis* by Hexaflumuron (Abu El-Magd *et al*., 1994: Zhu *et al*., 2012) and Novaluron and Cyromazine (Ghoneim *et al*., 2015 b) as well as in other insects, such as *S. gregaria* as response to laminarin (derived from the brown seaweed *Laminaria digitata*)(Abu El-Magd, 1992) or Teflubenzuron (Teleb, 2011), while disagrees with the reported decreasing OEs count in *S. mauritia* after treatment with Flufenoxuron (Manogem *et al*., 2016). However, OEs count in haemolymph of last instar larvae of *S. littoralis* was unaffected after treatment with LC_{50} of Flufenoxuron (Zohry, 2006) or Novaluron and Cyromazine in the 2-day old larvae (Ghoneim *et al*., 2015 b). Increasing of OEs population in the haemolymph of *P. gossypiella* larvae, in the present study, may be attributed to their role in the detoxification of toxic materials and activating action of the tested CSIs on the hematopoietic organs or cell mitotic division.

Fluctuated DHC of ADs in haemolymph of larvae

In the present study, treatment of full grown larvae of *P. gossypiella* with Novaluron and Diofenolan resulted in significantly increased ADs population in larvae 6 hr post-treatment but slightly decreased ADs population in larvae 48 hr post-treatment. As far as our literature survey could ascertain, no information was available on the effects of IGRs or insecticides on ADs population except Manogem *et al*. (2016) who recorded decreasing DHC of ADs at 24 and 48 hr post-treatment of *S. mauritia* with Flufenoxuron. Unfortunately, there is no appreciable interpretation of the diverse effects of the tested CSIs on ADs count in *P. gossypiella* larvae until now!!

Qualitative haemocyte profile in *P. gossypiella* larvae as impaired by Novaluron and Diofenolan

As reported by Miselyunene **(**1976) for *Pieris rapae*, El-Kattan **(**1995) for *Plodia interpunctella*, Barakat *et al*. (2002) for *S. gregaria*, Bakr *et al.* (2007) and Ghoneim *et al*. (2015 b) for *S. littoralis* and Manogem *et al*. (2016) for *S. mauritia*, some pathogenic microorganisms, insecticides or IGRs caused some disruptive alterations in the haemocytes basing on changes in the plasma membrane (erosion and extrusion of their cytoplasmic contents), vacuolization and lysis of the cytoplasm and nuclear disorders. However, OEs were the least affected hemocytes as reported in *D. cingulatus* after treatment with the insecticide Acephate (Qamar and Jamal, 2009), *S. gregaria* after treatment with Teflubenzuron (Teleb, 2011) and *S. littoralis* after treatment with Novaluron and Cyromazine (Ghoneim *et al*., 2015 b).

In the present study on *P. gossypiella*, treatment of full grown larvae with Novaluron and Diofenolan caused some cytopathological aberrations in the majority of circulating hemocytes. With regard to GRs, PRs, PLs, SPs and OEs, some hemocytes appeared with different symptoms of malformation, such as faintly or deeply stained cells, compressed size, cell lysis and/or degranulation, pycnotic and/or lysed nuclei, cytoplasmic lysis and/or vacuolation, cytoplasm. Some differences in the disruptive potencies of the tested CSIs were exhibited. However, no effect was exhibited by Novaluron or Diofenolan on ADs. The morphological disorders of *P. gossypiella* haemocytes, in the present study, may be attributed to the action of CSIs on the 'actin' which localized in the lamellar extensions of the cells. Any naturally originating pesticidal molecule may exert its activity by targeting actins (Anunradha and Annadurai**,** 2008). No appreciable interpretation of the intracellular disturbances in hemocytes by CSIs has been available now!! The question whether the hemocytes are affected directly or *via* some physiological or endocrinological pathway is yet to be answered in spite of reports that developmental effects caused by IGRs and botanicals were attributed to disruption of endocrine events (Schmutterer, 1990).

5. CONCLUSION

As obviously found in the present study on *P. gossypiella*, Novaluron and Diofenolan caused serious disturbance of the total hemocyte population and differential hemocyte counts of the identified hemocyte types as well as exhibited dangerous cytopathological effects on the majority of hemocytes. Therefore, these CSIs exhibited a decrease in the capacity of larval immune defense, thereby altering the hormonal triggers in the treated larvae. Thus, the tested CSIs may be effective agents being included in the integrated management program against *P. gossypiella*.

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