Toxicity of Certain IGRs and Conventional Insecticides against Cotton Leafworm and Their Effects on the Development and Haemocyte Counts

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BSTRACT

Toxicity of two insect growth regulators (IGRs) (novaluron and chlorfluazuron) and two conventional insecticides (chlorpyrifos and lambda-cyhalothrin) were evaluated against 2nd and 4th instars larvae of cotton leafworm (CLW), *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). Joint toxic action of tested IGRs and insecticides against 4th instar larvae of CLW were also evaluated. The sublethal effects of tested insecticides on the development and haemocyte counts were also carried out. Results showed that, chlorfluazuron was the most toxic against both 2nd and 4th larval instars with LC_{50} values 0.12 and 1.4 mg/L after 96 hrs of treatment followed by chlorpyrifos which is followed by novaluron. Lambda-cyhalothrin recorded the least toxicity against both 2nd and 4th larval instars with LC_{50} values 0.86 and 4.6 mg/L after 96 hrs of treatment. Potentiating effect was obtained when chlorpyrifos was mixed with novaluron or chlorfluazuron each at LC_{25} with co-toxicity factors (CTFs) 42.85 and 46.66, respectively. All other mixtures of chlorpyrifos with novaluron or chlorfluazuron resulted in additive effects. All tested insecticides at concentrations equivalent to LC_{10} and LC_{25} significantly reduced larval body weights, percentage of pupation, pupal mean weight and percentage of adult emergence. Total haemocyte count (THC) was significantly decreased and reached to 34.6, 45.2, 72.3 and 87.6×10^3 cell/mm^3 after 24 hrs from treatment with novaluron, chlorfluazuron, chlorpyrifos and lambda-cyhalothrin at concentration equivalent to LC_{25}, respectively, compared to 106.4×10^3 cell/mm^3 in control. All treatments with tested IGRs and insecticides decreased the percentages of Prohaemocytes (PRs) and Spherulocytes (SPs) after 24, 48, 72 and 96 hrs of treatment compared to control. In contrast, the percentages of Granulocytes (GRs) and Oenocytoids (OEs) were slightly increased. While in novaluron and chlorfluazuron treatments, Plasmatocytes (PLs) were significantly increased and reached to 48.3 and 49.2 %, respectively, it was decreased by chlorpyrifos and lambda-cyhalothrin treatments and reached to 39.6 and 40.5 %, respectively, after 24 hrs compared to 44.8 % in control. Finally, the present data suggests that the use of novaluron and chlorfluazuron in binary mixtures with conventional insecticides particularly chlorpyrifos can reduce the rate of used insecticides and consequently reduce environmental pollution. Also, sublethal concentrations of tested insecticides can negatively affect the population of CLW.

Keywords: Cotton leafworm; Insect growth regulators; Insecticides; Binary mixtures; Biological parameters; Total haemocyte count; Differential haemocyte count.

INTRODUCTION

Cotton is one of the most important crops and plays a vital role in social and economic affairs of the world. Cotton plants are liable to be attacked by many pests from the seedling to harvest stage. Among these pests, CLW is a severe polyphagous insect pest causing various ravages not only for cotton plants but also for several cultivated crops, vegetables, ornamentals and orchard trees (Matthews and Tunstall, 1994).

Chemical control is an important tool for CLW management because non-chemical control measures alone usually do not adequately prevent economic damage. Unfortunately, CLW has developed resistance to organophosphates, carbamates and synthetic pyrethroids due to the unwise use of these insecticides (Abo El-Ghar *et al*., 1986; Abdallah, 1991; El-Zemaity *et al*., 2003; Abo-Elghar *et al*., 2005; Abou-Taleb, 2010). In addition, the environmental hazards of conventional insecticides necessitate introduce of other new insecticides from different chemical groups with different modes of action alone or in mixtures. To overcome these problems and achieving an effective control of CLW with lower doses of used insecticides many researchers resorted to the insecticide mixtures with other control agents such as IGRs (Ghoneim, 2002; Kandil *et al*., 2006; Abdel-Rahman and Abou-Taleb, 2007; Ghoneim *et al*., 2012; Abd El-Razik and Mostafa, 2013).

The advantages of IGRs make them highly desirable in integrated pest management programs. They do not persist long in the environment due to their rapid biodegradation. In addition, they exhibit low toxicity for non-target organisms (Zibaee *et*

al., 2011). The IGRs accomplished their mode of action by interfering with development, disrupting the normal activity of the endocrine system, reproduction or metamorphosis of the target insects (Kai *et al*., 2009). The IGRs include juvenile hormone (JH) mimics, ecdysone agonists and chitin synthesis inhibitors (CSIs) (Tunaz and Uygun, 2004). CSIs benzoylphenylureas insecticides such as novaluron and chlorfluazuron act on incorporation of N-acetyl glucosamine monomer into chitin in the integument. This leads to the formation of abnormal new cuticle and death of the insect (Nakagawa *et al*., 1993, 1996). In addition, previous studies have documented the effect of IGRs on the haemolymph components of insects (Zhu, *et al*., 2012; Zibaee *et al*., 2012; Abou-Taleb *et al*., 2015; Ghoneim *et al*., 2015). Haemocytes play multifunctional roles such as coagulation, phagocytosis, encapsulation, nodule formation, detoxification and secretion of humeral immunity factors may render them more sensitive than other cell towards insecticides and internal/external factors (Gupta *et al*., 2005; Saha, 2011).

Therefore, the present work was performed to investigate the toxicity and joint toxic action of novaluron and chlorfluazuron with certain conventional insecticides (chlorpyrifos and lambda-cyhalothrin) against the 2nd and 4th instars larvae of CLW. In addition, the sublethal effects of tested insecticides on the larval and pupal development durations, larval and pupal weights, percentages of pupation and adult emergence of CLW were evaluated. The impact of tested insecticides at concentration equivalent to LC_{25} on the total and differential haemocyte counts of CLW larvae was also tested.

MATERIALS AND METHODS

Experimental insect: Egg masses of CLW were reared on the Department of Cotton Pesticides Bioassay Research, Plant Protection Research Institute, ARC, Al Sabhia, Alex., Egypt. The newly hatched larvae were fed on fresh castor bean leaves, *Ricinus communis* L., as a natural diet under experimental conditions (27±2 °C, 65±5 % RH) for several generations without exposure to insecticides as mentioned by (El-Defrawi *et al*., 1964).

Tested insecticides: Novaluron (Equo® 10% EC) was produced by Isagro Co., Italy. Chlorfluazuron (Topron® 5% EC) and chlorpyrifos (Pestban® 48% EC) were produced by Agrochem Co., Alexandria. Lambda-Cyhalothrin (Lambada® 5% EC) was produced by Dow AgroSciences Co., England.

Toxicity test: Toxicity of novaluron, chlorfluazuron, chlorpyrifos and lambda-cyhalothrin were determined using the leaf dip bioassay method (Eldefrawi *et al.*, 1964). A series of the insecticides concentrations were processed in distilled water. Homogenous castor bean leaf pieces were immersed in each insecticide concentration solution for 10 seconds, dried at room temperature before being offered to newly ecdysed 2nd (2.3±0.1 mg/larvae) and 4th (46.6±0.4 mg/larvae) instar larvae. Untreated larvae were fed on castor bean leaf pieces immersed in distilled water only. Each treatment was replicated four times with 10 larvae per replicate. After 24 hrs, fresh castor bean leaf pieces were added to each replicate. Mortality percentages were recorded after 96 hrs of exposure, corrected using the Abbott equation (Abbott, 1925) and subjected to probit analysis according to (Finney, 1971). LC_{10}, LC_{25} and LC_{50} values, their confidence limits and slope ± SE were calculated.

Joint toxic action studies: Binary mixtures of novaluron or chlorfluazuron with chlorpyrifos and lambda-cyhalothrin against the 4th instar larvae of CLW were investigated after 96 hrs of exposure. LC_{25} of novaluron or chlorfluazuron were mixed with LC_{25} or LC_{10} of chlorpyrifos and λ-cyhalothrin. Also, LC_{10} of novaluron or chlorfluazuron were mixed with LC_{25} of chlorpyrifos and λ-cyhalothrin. Three control groups were subjected to calculate the expected mortalities. The joint action of the different mixtures was expressed at the co-toxicity factors (CTFs), calculated by the equation given by Mansour *et al.,* (1966), as follows:

$$\text{Co-toxicity factor} = \frac{\text{Observed\% mortality - expected \% mortality}}{\text{expected \% mortality}} \times 100$$

This factor was used to categorize the results into three categories as follow: co-toxicity factors ≥ +20 meant potentiation; co-toxicity factors < -20 meant antagonism; and co-toxicity factors between -20 and +20 meant additive effect.

Effects of sublethal concentrations of tested insecticides on some biological parameters of CLW: The sublethal effects of novaluron, chlorfluazuron, chlorpyrifos and lambda-cyhalothrin at their LC_{10} and LC_{25} equivalent concentrations on some biological parameters of CLW were evaluated. Homogenous castor bean leaves were immersed in each insecticide LC_{10} and LC_{25} equivalent concentrations for 10 seconds, dried at room temperature then introduced to the larvae. One hundred newly ecdysed 2nd instar larvae in each replicate were used and provided with treated leaves. The untreated larvae were fed on leaves immersed in distilled water only. Each treatment and control was replicated four times. Surviving larvae were transferred to jars containing fresh untreated leaves after 48 hrs and observed daily for pupation and adult emergence. Larval and pupal development durations were determined.

Larval and pupal weights, percentages of pupation and adult emergence were also recorded.

Haemolymph studies: Castor bean leaves were soaked in the determined LC_{25} equivalent concentration for each of tested insecticides. One hundred 4th instar larvae were used in each replicate and exposed to the treated leaves. Each treatment was replicated four times. Surviving larvae were subjected directly to total and differential haemocyte counts after 24, 48, 72 and 96 hrs from treatment.

Total haemocyte count (THC): To determine the total haemocyte count, 20 µL of the haemolymph was diluted 1:9 (v/v) in chilled saline (7 gL^{-1} NaCl, 0.2 gL^{-1} KCl, 0.2 gL^{-1} $CaCl_2$, 0.1 gL^{-1} $MgCl_2$, 0.15 gL^{-1} $NaHCO_3$, 0.2 gL^{-1} NaH_2PO_4, Glucose 7.0 gL^{-1}), and aliquots were transferred to a Neubauer haemocytometer. Cells were counted using a light microscope and number of total haemocytes per cubic millimeter (mm^3) was calculated according to the formula of Jones (1962), as follows:

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

Where the depth factor is usually 10.

Differential haemocyte count (DHC): Haemolymph preparations were stained, 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 light microscope at magnification 1000X and 100 cells per slide were examined. The cell shape, cytoplasmic ratio, cytoplasmic inclusions and nucleus shape were used for classification of haemocytes using the classification scheme of Brehélin and Zachary (1986). The percentages of haemocyte types were calculated by the formula:

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

Haemocytes deformations: for recording the haemocytes deformities of the treated larvae, photomicrographs were obtained by using a light microscope with a camera at a magnification 1000 X.

Statistical analysis: The SAS 8.0 software was used for analysis of the data obtained from each experiment and the means were tested for significant differences using analysis of variance (ANOVA) test (LSD at $P < 0.05$) (SAS Statistical software, 1999).

RESULTS AND DISCUSSION

Toxicity of novaluron, chlorfluazuron, chlorpyrifos and lambda-cyhalothrin against 2nd and 4th instars larvae of CLW: Data presented in (Table1) demonstrated the LC_{10}, LC_{25} and LC_{50} values, their confidence limits and slope ± SE for the tested insecticides against the 2nd instar larvae of CLW. Results showed that, toxicity of chlorfluazuron (LC_{50} = 0.12 mg/L) is 7.2 times more toxic than λ-cyhalothrin (LC_{50} = 0.86 mg/L), 2.9 times more toxic than novaluron (LC_{50} = 0.35 mg/L) and 2.3 times more toxic than chlorpyrifos (LC_{50} = 0.28 mg/L) against the 2nd instar larvae after 96 hrs of treatment. Chlorpyrifos is 3.1 more toxic than λ-cyhalothrin. Toxicity of chlorpyrifos and novaluron against the 2nd instar larvae is comparable.

Concerning the toxicity of tested insecticides against the 4th instar larvae, same trend of results was recorded (Table 2). Chlorfluazuron is the most toxic (LC_{50} = 1.4 mg/L), followed by chlorpyrifos (LC_{50} = 2.5 mg/L) and novaluron (LC_{50} = 2.8 mg/L) after 96 hrs of treatment. Lambda-cyhalothrin recorded the least toxicity against the 4th instar larvae with LC_{50} value of 4.6 mg/L after 96 hrs of treatment. From these results, it is clear that 4th instar larvae were less susceptible than the 2nd instar larvae. Similar results were observed by Kandil *et al*., (2006) where they reported that, chlorfluazuron achieved high toxicity to CLW compared to chlorpyrifos and profenofos. In addition, Abdien *et al.*, (2016) recorded a higher toxicity of chlorpyrifos compared to λ-cyhalothrin against the 4th instar larvae of CLW. Furthermore, Abdel-Rahman and Abou-Taleb, (2007) recorded higher toxicity of chlorfluazuron compared to spinosad and spinetoram against the 2nd instar larvae of CLW after 72 hrs of treatment. Comparable toxicity was observed between chlorfluazuron and lufenuron against the 4th instar larvae of CLW (Abou-Taleb *et al*., 2015).

Joint toxic action of novaluron or chlorfluazuron with chlorpyrifos and lambda-cyhalothrin against 4th instar larvae of CLW: To determine the effect of binary mixtures of novaluron or chlorfluazuron (LC_{25} and LC_{10}) with chlorpyrifos or lambda-cyhalothrin (LC_{25} and LC_{10}), the expected mortality can be ranged between 50% or 35%, when the mixture was used. Since the average weights of the 4th instar larvae used in each test varied, thus, the expected mortality for the concentrations applied in every test varied accordingly (Abdel-Rahman and Abou-Taleb, 2007). So, the expected mortality was calculated for each insecticide in the mixture in every test by treating the larvae by each one alone.

Table1: Toxicity of novaluron, chlorfluazuron, chlorpyrifos and lambda-cyhalothrin against the 2nd instar larvae of CLW after 96 hrs of treatment:

Insecticide	LC_{10} (mg L^{-1}) Confidence limits	LC_{25} (mg L^{-1}) Confidence limits	LC_{50} (mg L^{-1}) Confidence limits	Slope ± SE*
Novaluron	0.07 0.05-0.09	0.17 0.14-0.20	0.35 0.24-0.46	1.2±0.14
Chlorfluazuron	0.02 0.01-0.03	0.07 0.05-0.08	0.12 0.08-0.16	1.8±0.16
Chlorpyrifos	0.09 0.06-0.12	0.16 0.14-0.18	0.28 0.24-0.32	1.4±0.17
λ-Cyhalothrin	0.19 0.16-0.21	0.38 0.34-0.42	0.86 0.62-1.12	1.6±0.15

*SE means Standard Error

Table2: Toxicity of novaluron, chlorfluazuron, chlorpyrifos and lambda-cyhalothrin against the 4th instar larvae of CLW after 96 hrs of treatment:

Insecticide	LC_{10} (mg L^{-1}) Confidence limits	LC_{25} (mg L^{-1}) Confidence limits	LC_{50} (mg L^{-1}) Confidence limits	Slope ± SE*
Novaluron	0.7 0.5-0.8	1.6 1.4-1.8	2.8 2.6-3.2	2.4±0.23
Chlorfluazuron	0.3 0.1-0.4	0.7 0.4-0.8	1.4 1.2-1.6	2.3±0.21
Chlorpyrifos	0.5 0.4-0.6	1.2 1.1-1.5	2.5 2.3-2.8	1.8±0.22
λ-Cyhalothrin	0.9 0.8-1.2	2.4 2.1-2.7	4.6 4.2-5.2	2.1±0.23

*SE means Standard Error

Therefore, the expected mortality for the mixture of two insecticides was the sum of the mortalities of each of the concentrations used in the mixture. The joint toxic action of novaluron or chlorfluazuron with chlorpyrifos or λ-cyhalothrin at different concentrations after 96 hrs of exposure is shown in (Table 3). It is clear that, the potentiating effect was obtained when chlorpyrifos was mixed with novaluron or chlorfluazuron each at LC_{25} with CTFs 42.85 and 46.66, respectively. All other mixtures of chlorpyrifos with novaluron or chlorfluazuron resulted in additive effects with CTFs ranged between 11.10 to 18.16. All mixtures of λ-cyhalothrin with novaluron or chlorfluazuron each at LC_{10} or LC_{25} resulted to an additive effect with CTFs ranged between -9.11 to 12.50. These results agree partially with Radwan *et al.,* (2009) which reported that, mixtures between chlorpyrifos with chlorfluazuron at different mixing ratios exhibited potentiating action. In addition, Ghoneim *et al.,* (2012) reported that, while mixtures of chlorpyrifos with the IGRs hexaflumuron or triflumuron resulted in potentiation effects, mixtures of chlorpyrifos with the IGR chlorfluazuron resulted in additive effect against resistant field population of the CLW. All λ-cyhalothrin mixtures with the tested IGR compounds resulted in an additive effect. In this point, results of the present study differ with Sufian *et al*., (2013) where they reported that, mixtures of the pyrethroids insecticides deltamethrin or bifenthrin with chlorfluazuron showed antagonistic effect on *Spodoptera litura* larvae.

Sublethal effects of tested insecticides on some biological parameters of CLW: In the field some insects may be exposed to sublethal concentrations of the applied insecticides which can result in sublethal effects on insect pests. In the present study, sublethal effects of novaluron, chlorfluazuron, chlorpyrifos and lambda-cyhalothrin at concentrations equivalent to LC_{10} and LC_{25} on some biological parameters of CLW larvae were carried out and results are shown in (Tables 4 and 5). During the observation period, all treatments significantly reduced the mean larval weights at a concentration dependent manner compared to control. After 10 days of treatment, the mean larval weight reached to 129.4, 125.8, 140.2 and 145.5 mg/larva when larvae were exposed to novaluron, chlorfluazuron, chlorpyrifos and λ-cyhalothrin at concentrations equivalent to LC_{25}, respectively, compared to 189.2 mg/larva in control. The mean larval weights reached to 437.4, 424.8, 453.6 and 478.5 mg/larva when the larvae were exposed to the same treatments after 15 days, respectively, compared to 610.6 mg/larva in control (Table 4).

Table 3: Joint toxic action of novaluron or chlorfluazuron with chlorpyrifos and lambda-cyhalothrin against the 4th instar larvae of CLW after 96 hrs of treatment:

Mixtures	Concentration levels	Expected (%) mortality	Observed (%) mortality	Cotoxicity factor*
Novaluron + Chlorpyrifos	$LC_{25} + LC_{25}$	46.67	66.67	42.85
	$LC_{25} + LC_{10}$	36.67	43.33	18.16
	$LC_{10} + LC_{25}$	30.00	33.33	11.10
Novaluron + λ-Cyhalothrin	$LC_{25} + LC_{25}$	43.33	46.67	7.71
	$LC_{25} + LC_{10}$	40.00	36.67	-8.33
	$LC_{10} + LC_{25}$	36.67	33.33	-9.11
Chlorfluazuron + Chlorpyrifos	$LC_{25} + LC_{25}$	50.00	73.33	46.66
	$LC_{25} + LC_{10}$	40.00	50.00	15.39
	$LC_{10} + LC_{25}$	36.67	43.33	18.16
Chlorfluazuron + λ-Cyhalothrin	$LC_{25} + LC_{25}$	53.33	60.00	12.50
	$LC_{25} + LC_{10}$	33.33	36.67	10.02
	$LC_{10} + LC_{25}$	32.00	34.00	6.25

*Cotoxicity factor = [(observed (%) mortality- expected (%) mortality)/expected (%) mortality] ×100 (Mansour *et al*., 1966).

When the 2nd instar larvae were exposed to novaluron, chlorfluazuron, chlorpyrifos and λ-cyhalothrin at concentrations equivalent to $LC_{25,}$ the average time to pupation was 22.7, 23.9, 22.3 and 21.2 days, respectively, compared to 17.4 days in control (Table 4). The two IGR compounds, novaluron and chlorfluazuron, at concentrations equivalent to LC_{25} had the highest effect on the percentage of pupation. The percentage of pupation was 36.2 and 32.8 % at LC_{25} of novaluron and chlorfluazuron compared to 92.4 % in control. Chlorpyrifos and λ-cyhalothrin at concentrations equivalent to LC_{10} had the least effect on % pupation, where it was 84.6 and 87.2 %, respectively (Table 4).

As shown in Table (5), all treatments significantly reduced the pupal mean weight compared to control treatment. The weight averages of pupae were 248.7, 243.5, 273.2 and 278.3 mg/pupa when the 2nd instar larvae were exposed to novaluron, chlorfluazuron, chlorpyrifos and λ-cyhalothrin at concentrations equivalent to LC_{10}, respectively, compared to 289.3 mg/pupa in control. While, in the LC_{25} treatments, pupae weight averages were 223.4, 218.6, 260.8 and 264.7 mg/pupa, when the larvae were exposed to the same insecticides, respectively. Reduction in the adult emergence was significantly achieved by all treatments where it was 52.4, 46.3, 73.8 and 79.4 % in the LC_{10} of novaluron, chlorfluazuron, chlorpyrifos and λ-cyhalothrin treatments, respectively, compared to 86.7 % in the control treatments. The LC_{25} of novaluron and chlorfluazuron achieved the highest reduction in the adult emergence, where it was 38.2 and 32.6 %, respectively (Table 5). On the other hand, pupal duration did not differ significantly in all insecticides treatments compared to control treatment (Table 5).

However, results of the present study agreed with Abdel-Rahman *et al*., (2007) where they reported that, when the 3rd instar larvae of CLW were treated with lufenuron larvae ceased feeding within 48 hrs and lead to reduction in larval mean weight. Perveen (2000) reported that, at lethal dosages of chlorfluazuron the development of different CLW instars, moulting to pupae and emergence into adults, larval and pupal weights were adversely affected. Novaluron at sublethal concentrations was found to reduce average larval weight, average time to the pupation, percentage of pupation, pupal mean weight and percentage of adult emergence (Metayi *et al.*, 2015). Also, Nasr *et al*., (2010) found that buprofezin and pyriproxyfen decreased body weight, extended the duration of larval and pupal development, and reduced the pupation of CLW. Adel (2012) recorded an antifeedant effect for lufenuron against CLW larvae which affects all biological parameters of treated larvae. This may be the main reason for larval and pupal weight reduction.

Effect of tested insecticides on THC: As shown in Table (6), THC was significantly decreased and reached to 34.6, 45.2, 72.3 and 87.6×10^3 cell/mm^3 after 24 hrs from treatment with novaluron, chlorfluazuron, chlorpyrifos and lambda-cyhalothrin, respectively, compared to 106.4×10^3 cell/mm^3 in control. Also, THC was decreased and reached to 25.2, 32.4, 48.6 and 56.3×10^3 cell/mm^3 compared to 67.3×10^3 cell/mm^3 in control, 43.7, 58.4, 60.6 and 66.5×10^3 cell/mm^3 compared to 77.2×10^3 cell/mm^3 in control and reached to 62.3, 75.6, 83.2 and 98.4×10^3 cell/mm^3 compared to 105.8×10^3 cell/mm^3 in control when the larvae were exposed to the same treatments after 48, 72 and 96 hrs, respectively.

Table 4: Effect of novaluron, chlorfluazuron, chlorpyrifos and lambda-cyhalothrin when applied to the 2nd instar larvae of CLW on the larval weight gain, larval duration and percentage of pupation:

Insecticide	Conc. (mg L^{-1})	Mean weight (mg/larva) ± SE after different days of treatment			Larval duration (days) ± SE	Pupation (%) ± SE
		5	10	15		
Control	-	$48.7^{a} \pm 1.5$	$189.2^{a} \pm 3.2$	$610.6^{a} \pm 5.9$	$17.4^{c} \pm 1.3$	$92.4^{a} \pm 1.2$
Novaluron	0.07	$41.4^{b} \pm 2.8$	$165.3^{c} \pm 3.0$	$518.2^{d} \pm 4.5$	$18.6^{b} \pm 1.4$	$58.6^{d} \pm 1.0$
	0.17	$35.3^{c} \pm 1.6$	$129.4^{e} \pm 2.7$	$437.4^{f} \pm 5.3$	$22.7^{a} \pm 0.8$	$36.2^{e} \pm 1.5$
Chlorfluazuron	0.02	$40.2^{b} \pm 1.9$	$162.9^{c} \pm 4.2$	$509.3^{d} \pm 3.8$	$18.3^{b} \pm 1.5$	$52.4^{d} \pm 1.6$
	0.07	$33.7^{c} \pm 2.1$	$125.8^{e} \pm 3.6$	$424.8^{f} \pm 4.6$	$23.9^{a} \pm 1.7$	$32.8^{e} \pm 1.3$
Chlorpyrifos	0.09	$42.0^{b} \pm 1.4$	$172.3^{b} \pm 2.4$	$540.6^{c} \pm 5.2$	$17.8^{c} \pm 1.6$	$84.6^{b} \pm 1.4$
	0.16	$40.4^{b} \pm 1.3$	$140.2^{d} \pm 3.8$	$453.6^{e} \pm 5.7$	$22.3^{a} \pm 1.2$	$65.3^{c} \pm 1.6$
λ-Cyhalothrin	0.19	$43.8^{b} \pm 1.6$	$177.6^{b} \pm 2.9$	$564.2^{b} \pm 4.8$	$16.4^{d} \pm 1.4$	$87.2^{b} \pm 1.7$
	0.38	$41.6^{b} \pm 2.3$	$145.5^{d} \pm 4.3$	$478.5^{e} \pm 3.6$	$21.2^{a} \pm 1.8$	$68.7^{c} \pm 1.0$

Note: Means followed by the same letter in the same column are not significantly different ($p < 0.05$, LSD test).
SE: Standard Error.

Table 5: Effect of novaluron, chlorfluazuron, chlorpyrifos and lambda-cyhalothrin when applied to the 2nd instar larvae of CLW on the pupal weight gain, pupal duration and percentage of adult emergence:

Insecticide	Conc. (mg L^{-1})	Pupal mean weight (mg/pupa) ± SE	Pupal duration (days) ± SE	% Adult emergence ± SE
Control	-	$289.3^{a} \pm 6.4$	$9.8^{a} \pm 0.7$	$86.7^{a} \pm 2.4$
Novaluron	0.07	$248.7^{d} \pm 5.6$	$9.3^{a} \pm 0.5$	$52.4^{c} \pm 1.8$
	0.17	$223.4^{e} \pm 6.3$	$9.7^{a} \pm 0.5$	$38.2^{e} \pm 2.6$
Chlorfluazuron	0.02	$243.5^{d} \pm 5.2$	$9.3^{a} \pm 0.4$	$46.3^{d} \pm 1.7$
	0.07	$218.6^{e} \pm 4.8$	$9.6^{a} \pm 0.7$	$32.6^{e} \pm 1.5$
Chlorpyrifos	0.09	$273.2^{b} \pm 5.8$	$9.7^{a} \pm 0.6$	$73.8^{b} \pm 2.2$
	0.16	$260.8^{c} \pm 4.9$	$9.4^{a} \pm 0.5$	$54.3^{c} \pm 1.4$
λ-Cyhalothrin	0.19	$278.3^{b} \pm 6.2$	$9.8^{a} \pm 0.6$	$79.4^{b} \pm 2.3$
	0.38	$264.7^{c} \pm 4.6$	$9.5^{a} \pm 0.4$	$58.2^{c} \pm 1.2$

Note: Means followed by the same letter in the same column are not significantly different ($p < 0.05$, LSD test).
SE: Standard Error.

In the present study, the decrease in the haemocyte numbers of treated larvae may be due to nodulation and encapsulation as well as degranulation of some cell types or the inhibition of the brain hormone secretion (Abd El-Aziz and Awad, 2010). These results agreed with Abou-Taleb *et al*., (2015), where they found that lufenuron and chlorfluazuron caused the highest decrease in total haemocyte count in CLW after 72 hrs from treatment. In addition Ghoneim *et al*., (2015), observed that THC in 6th instar larvae of CLW was drastically descended in 0-, 2- and 4-day old larvae after treatment with LC_{50} of novaluron and cyromazine.

Effect of tested insecticides on DHC: Five types of haemocytes were found in the haemolymph of CLW larvae (Jones, 1962). They were identified as Prohaemocytes (PRs), Plasmatocytes (PLs), Granulocytes (GRs), Spherulocytes (SPs) and Oenocytoids (OEs). PRs are usually round in shape and small in size (Photo A&B). According to data distributed in Table (6), percentages of PRs were decreased and amounted to 7.4, 4.8, 9.3 and 11.4 % compared to 15.6 % in control, 9.8, 6.7, 12.6 and 14.2 % compared to 19.3 % in control, 8.2, 5.3, 10.2 and 12.3 % compared to 14.6 % in control and 6.8, 4.3, 7.6 and 8.2 % compared to 9.3 % in control after 24, 48, 72 and 96 hrs from treatment with novaluron, chlorfluazuron, chlorpyrifos and lambda-cyhalothrin, respectively. PLs are usually spindle-shaped (Photo C&D). After 24 hrs from treatment with novaluron and chlorfluazuron, PLs were significantly increased and reached to 48.3 and 49.2 %, respectively.

On the other hand, reduction of PLs by chlorpyrifos and λ-cyhalothrin treatments was 39.6 and 40.5 %, respectively, compared to 44.8 % in control. While, there was no significance different in percentages of PLs between all treatments after 72 and 96 hrs compared in control. GRs are recognized as spherical or oval cells (Photo E&F). In the current investigation, percentages of GRs were increased and reached to 39.7, 40.5, 44.3 and 42.2 % after 24 hrs from treatment with novaluron, chlorfluazuron, chlorpyrifos and λ-cyhalothrin,

respectively, compared to 32.4 % in control. The function process of GRs and PLs is working as defense management cells against bodies. Therefore, the releases of these cells are usually occurred during each moulting stage and larval metamorphosis. While, percentages of SPs were decreased and reached to 2.4, 2.7, 3.2 and 3.5 % compared to 6.24 % in control and reached to 3.6, 3.2, 2.9 and 2.3 % compared to 5.9 % in control after 24 and 96 hrs from treatment with novaluron, chlorfluazuron, chlorpyrifos and λ-cyhalothrin, respectively. Percentages of OEs were increased and reached to 2.2, 1.8, 2.6 and 2.4 % compared to 0.96 % in control and reached to 2.63, 2.14, 3.58 and 3.42 % compared to 1.72 % in control at the same treatments after 24 and 96 hrs, respectively. The general reduction of PRs population in larvae of CLW, in the present study, may be attributed either to the cytotoxic effects of CSIs on the mitotic division of PRs, conversion to other types of cells or to the inhibitory effects on the activity of hematopoietic organs responsible for PRs production (Zhu *et al*., 2012; Zibaee *et al*., 2012). These results were relatively similar to those obtained in (Abou-Taleb *et al*., 2015), suggested that the percentages of PRs, PLs and GRs in the haemolymph of CLW markedly decreased after treatment with both lufenuron and chlorfluazuron. In contrast, OEs and SPs increased after 72h of treatment. Also, Ghoneim *et al*., (2015), suggested that novaluron and cyromazine slightly decreased the percentages of PRs and PLs in last instar larvae of CLW. While, during the second half of larval instar, OEs were enhanced by both CSIs.

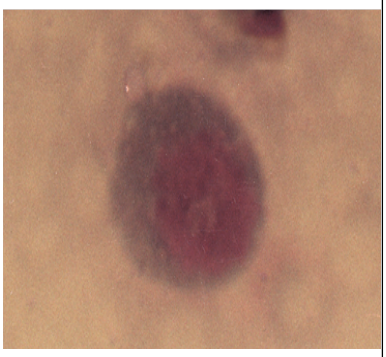
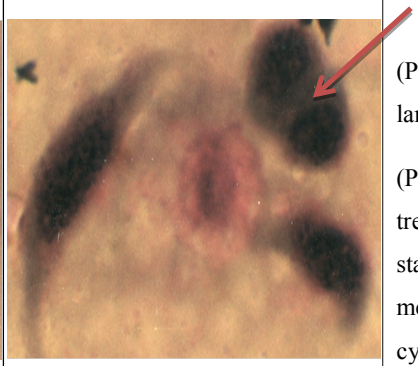
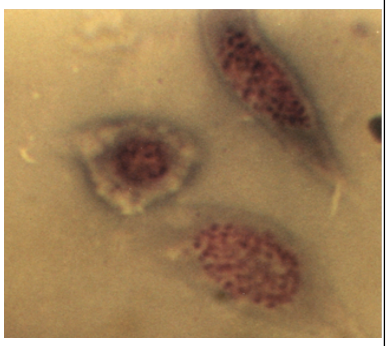
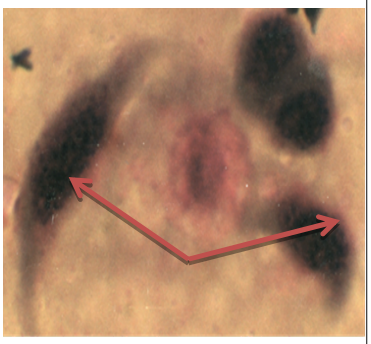
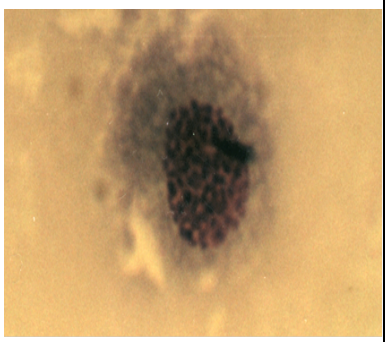
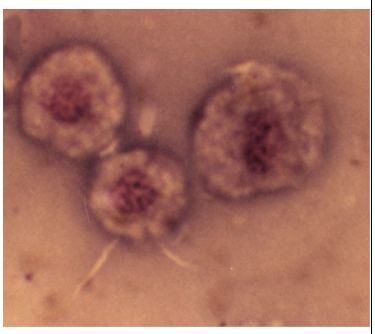
Table 6: Effect of tested insecticides at their LC_{25} on the haemocyte counts of CLW 4th instar larvae after different exposure times:

Time after exposure (hrs)	Treatments	Total haemocyte count ($\times 10^3$ cell/mm^3)	Percentages of different haemocyte types ± SE				
			PRs	PLs	GRs	SPs	OEs
24	Control	106.4 ± 2440^{a}	15.6 ± 1.6^{a}	44.8 ± 3.9^{b}	32.4 ± 3.7^{d}	6.24 ± 3.2^{a}	0.96 ± 0.3^{c}
	Novaluron	34.6 ± 1342^{e}	7.4 ± 1.7^{d}	48.3 ± 3.4^{a}	39.7 ± 2.9^{c}	2.4 ± 2.7^{c}	2.2 ± 0.4^{a}
	Chlorfluazuron	45.2 ± 2069^{d}	4.8 ± 1.3^{e}	49.2 ± 4.6^{a}	40.5 ± 4.8^{bc}	2.7 ± 1.2^{c}	1.8 ± 0.6^{b}
	Chlorpyrifos	72.3 ± 2145^{c}	9.3 ± 1.2^{c}	39.6 ± 4.2^{c}	44.3 ± 2.6^{a}	3.2 ± 2.4^{b}	2.6 ± 0.3^{a}
	λ-Cyhalothrin	87.6 ± 1934^{b}	11.4 ± 1.5^{b}	40.5 ± 3.7^{c}	42.2 ± 3.4^{b}	3.5 ± 1.8^{b}	2.4 ± 0.9^{a}
48	Control	67.3 ± 2449^{a}	19.3 ± 2.2^{a}	46.8 ± 3.9^{b}	27.8 ± 4.2^{d}	5.2 ± 3.4^{a}	0.92 ± 0.5^{a}
	Novaluron	25.2 ± 1341^{e}	9.8 ± 1.3^{d}	54.2 ± 3.2^{a}	32.6 ± 3.4^{c}	2.7 ± 2.6^{b}	0.73 ± 0.8^{c}
	Chlorfluazuron	32.4 ± 1903^{d}	6.7 ± 1.8^{e}	56.5 ± 2.4^{a}	33.8 ± 2.6^{c}	2.3 ± 2.4^{b}	0.68 ± 0.3^{c}
	Chlorpyrifos	48.6 ± 1465^{c}	12.6 ± 1.2^{c}	36.5 ± 2.7^{c}	47.2 ± 3.2^{a}	2.9 ± 3.2^{b}	0.82 ±0.4^{b}
	λ-Cyhalothrin	56.3 ± 1370^{b}	14.2 ± 0.9^{b}	37.8 ± 2.9^{c}	44.3 ± 2.8^{b}	2.4 ± 2.8^{b}	0.84 ±0.6^{b}
72	Control	77.2 ± 1551^{a}	14.6 ± 1.3^{a}	45.3 ± 1.8^{a}	34.7 ± 2.4^{b}	5.6 ± 1.2^{a}	0.86 ± 0.2^{c}
	Novaluron	43.7 ± 2290^{d}	8.2 ± 1.2^{d}	46.7 ± 2.3^{a}	42.3 ± 3.2^{a}	1.7 ± 2.4^{c}	0.93 ± 0.3^{c}
	Chlorfluazuron	58.4 ± 2153^{c}	5.3 ± 1.5^{e}	48.2 ± 2.5^{a}	43.8 ± 3.6^{a}	1.4 ± 1.3^{c}	1.32 ±0.9^{b}
	Chlorpyrifos	60.6 ± 2250^{bc}	10.2 ± 1.3^{c}	42.9 ± 2.1^{a}	41.6 ± 2.8^{a}	3.8 ± 2.7^{b}	2.46 ± 0.7^{a}
	λ-Cyhalothrin	66.5 ± 1864^{b}	12.3 ± 1.6^{b}	44.3 ± 1.6^{a}	36.8 ± 3.4^{b}	4.2 ±1.9^{b}	2.13 ± 0.5^{a}
96	Control	105.8 ± 1209^{a}	9.3 ± 2.4^{a}	42.5 ± 3.3^{a}	41.3 ± 3.7^{a}	5.9 ± 2.3^{a}	1.72 ± 0.4^{c}
	Novaluron	62.3 ± 1795^{e}	6.8 ± 1.8^{d}	44.2 ± 3.6^{a}	42.8 ± 3.3^{a}	3.6 ± 2.8^{b}	2.63 ±0.9^{b}
	Chlorfluazuron	75.6 ± 1522^{d}	4.3 ± 1.7^{e}	46.2 ± 2.5^{a}	44.2 ± 2.5^{a}	3.2 ± 1.4^{b}	2.14 ±0.5^{b}
	Chlorpyrifos	83.2 ± 2118^{c}	7.6 ± 1.2^{bc}	40.6 ± 2.8^{a}	45.3 ± 2.9^{a}	2.9 ± 1.6^{c}	3.58 ± 0.8^{a}
	λ-Cyhalothrin	98.4 ± 1954^{b}	8.2 ± 1.4^{b}	39.4 ± 2.7^{a}	46.7 ± 3.4^{a}	2.3 ± 1.6^{c}	3.42 ± 0.3^{a}

Note: PRs, Prohaemocytes; PLs, Plasmatocytes; GRs, Granulocytes; SPs, Spherulocytes and OEs, Oenocytoids.

*Within the same time after exposure, means followed by the same letter in the same column are not significantly different ($p < 0.05$, LSD test).

Haemocytes deformations:

 (Photo A)	 (Photo B)	(Photo A) PRs of untreated larvae of *S. littoralis* (1000X). (Photo B) PRs deformation of treated larvae, showing darkly stained with destroyed membrane and extruded cytoplasmic contents.
 (Photo C)	 (Photo D)	(Photo C) PLs of untreated larvae of *S. littoralis* (1000X). (Photo D) PLs deformation of treated larvae, Showing destroyed cell membranes and lysed cytoplasm.
 (Photo E)	 (Photo F)	(Photo E) GRs of untreated larvae of *S. littoralis* (1000X). (Photo F) GRs deformation of treated larvae, showing destroyed cell membranes and vacuolated cytoplasm.

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الملخص العربي

سمية بعض منظمات النمو الحشرية والمبيدات التقليدية ضد دودة ورق القطن وتأثيرهم على التطور وأعداد خلايا الدم

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أجريت هذة الدراسة المعملية لتقدير سمية أثنين من منظمات النمو الحشرية (النوفاليرون والكلورفلوازيورون) مع أثنين من المبيدات الحشرية التقليدية (الكلوربيريفوس واللمداسيهالوثرين) ضد الأعمار اليرقية الثانية والرابعة لدودة ورق القطن. كما تم تقييم الفعل السام المشترك للمبيدات المختبرة ضد العمر اليرقى الرابع . وأيضا تم أختبار التركيزات اللازمة لقتل ١٠ و٢٥ % من اليرقات المعاملة على بعض القياسات البيولوجية . وكذلك تأثير المبيدات المختبرة على العدد الكلى ونسبة وجود كل نوع لخلايا الدم. فأوضحت النتائج أن الكلورفلوازيورون كان أكثرهم سمية (التركيز اللازم لقتل ٥٠ % من اليرقات المعاملة ١٢.٠ و ٤.١ملجم/ لتر ضد الأعمار اليرقية الثانية والرابعة، على الترتيب) يليه الكلوربيريفوس ثم النوفاليرون وأقلهم سمية اللمداسيهالوثرين. وعند خلط المبيدات المختبرة وجد ان أكثر الخلطات فاعلية فى زيادة السمية هى الخلط بين الكلوربيريفوس بالتركيزاللازم لقتل ٢٥ % من اليرقات المعامله مع النوفاليرون أوالكلورفلوازيورون بعد ٩٦ ساعة من المعاملة. وأوضحت الدراسة أيضا الخفض لوزن اليرقات مع أطالة فترة الطور اليرقى عند المعاملة بالمبيدات المختبرة. كما لوحظ الخفض المعنوى على كلا من نسبة التعذير ووزن العذارى ونسبة ظهور الحشرات الكاملة عند المعاملة بالتركيزات اللازمة لقتل ١٠ و ٢٥ % من اليرقات وذلك بمقارنتها بالكنترول. وأوضحت الدراسة أيضا الخفض الملحوظ للعدد الكلى لخلايا الدم وكذلك التأثيرعلى نسبة وجود كل نوع من خلايا الدم ليرقات دودة ورق القطن عند معاملتها بالمبيدات المختبرة. ومن هذة الدراسة يتضح فاعلية الخلط بين منظمات النمو الحشرية المختبرة والكلوربيريفوس وإستخدامها فى برامج المكافحة المتكاملة لدودة ورق القطن. وأيضا فاعلية إستخدام التركيزات اللازمة لقتل ١٠ و٢٥ % لليرقات المعاملة مما يؤدى الى تقليل التركيزات المستخدمة من المبيدات المختبرة وبالتالى التقليل من تأثيرها على البيئة.