EVALUATION OF THE EFFICACY OF CERTAIN IGRS ON HAEMOGRAM, HISTOLOGICAL CHANGES, AND PHYSIOLOGICAL ASPECTS IN THE COTTON LEAFWORM, *SPODOPTERA LITTORALIS* (LEPIDOPTERA: NOCTUIDAE)

BEDEWY, A. E.[1] – ESSAWY, M. M.[1] – IBRAHIM, A.[1] – ALRASHIDI, B. M.[2] – ALI, M.[3] – IBRAHIM, R.[1]

[1]*Department of Economic Entomology, Kafrelsheikh University, Kafrelsheikh 33516, Egypt*

[2]*Department of Biology, College of Science, Jouf University, Sakaka 2014, Saudi Arabia*

[3]*Department of Biology, College of Science, Taibah University, Al-Madinah 41477, Saudi Arabia*

**Corresponding author*
e-mail: amalbedewy8@gmail.com; phone: +20-106-945-0580

(Received 28th Apr 2025; accepted 26th Jun 2025)

Abstract. This study investigates the effects of two insect growth regulators (IGRs), pyriproxyfen and chlorfluazuron, on mortality, histological structure, haemogram profiles, and physiological responses in larvae of *Spodoptera littoralis*. Larvae were treated with four concentrations (0.01, 0.1, 1, and 10 ppm) of each compound. Both IGRs demonstrated a concentration-dependent increase in mortality, with chlorfluazuron inducing more rapid and higher mortality rates than pyriproxyfen. Severe morphological deformities, including larval-pupal intermediates and disrupted metamorphosis, were observed following treatment with both compounds. Histological analysis revealed extensive integument damage, characterized by cuticle deformation, basement membrane destruction, and vacuole formation, with greater severity at higher concentrations. Differential haemocyte counts (DHC) showed significant alterations as both IGRs reduced prohaemocyte and plasmatocyte populations while increasing granulocyte and oenocytoid populations. The total haemocyte count (THC) initially increased but subsequently declined with prolonged exposure. Haemolymph volume was significantly elevated compared to the control, peaking on the fourth day at 29.44 µL for pyriproxyfen and 27.77 µL for chlorfluazuron at 0.01 ppm. These findings demonstrate the profound physiological and immunological disruptions caused by the IGRs and highlight their potential utility in integrated pest management programs targeting *S. littoralis*.

Keywords: *pyriproxyfen, chlorfluazuron, Spodoptera littoralis, lethal effects, haemocyte dynamics, metamorphosis disruption, histological deformities*

Introduction

Spodoptera littoralis (Lepidoptera: Noctuidae), commonly known as the Egyptian cotton leafworm, is one of the most economically significant pests affecting a wide range of crops, including cotton, maize, soybeans, and vegetables (Kandil et al., 2003). This highly polyphagous insect is notorious for its adaptability to various environmental conditions and agricultural practices, making it a persistent challenge for farmers worldwide. The damage caused by *S. littoralis* includes defoliation as well as direct feeding on leaves, fruits, and seed pods, leading to substantial yield losses and economic harm (Sannino, 2003; Dahi, 2005; Amin, 2007; Lanzoni et al., 2012; Abd El-Razik and Mostafa, 2013).

Traditional control of *S. littoralis* has largely depended on the extensive use of chemical insecticides. While effective, these insecticides have raised serious concerns about environmental pollution, non-target effects, and the rapid development of insecticide resistance (Naqqash et al., 2016). These challenges highlight the urgent need for alternative, more sustainable pest management strategies.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_82058224

Insect Growth Regulators (IGRs) have emerged as promising alternatives due to their selective modes of action and reduced environmental impact. Unlike conventional insecticides, IGRs disrupt key physiological processes such as moulting, metamorphosis, and reproduction, often targeting specific insect species while sparing beneficial organisms (Biddinger et al., 1995; Nicholas et al., 1999). This specificity, coupled with a lower likelihood of resistance development, makes IGRs valuable tools in integrated pest management (IPM) programs (Kai et al., 2009).

Despite their potential, the effects of IGRs on the physiological and immunological aspects of *S. littoralis* remain underexplored. Previous studies have demonstrated IGR-induced disruptions in growth and development in various lepidopteran pests, yet detailed investigations into their impact on haemocyte profiles, histological structures, and haemolymph volume are still limited (Hoffman and Lorenz, 1998; Tunaz and Uygun, 2004; Pfeiffer, 2007). Understanding these effects is crucial for optimizing the use of IGRs and improving pest control strategies.

Although pyriproxyfen and chlorfluazuron are broad-spectrum insect growth regulators with general efficacy against many Lepidopteran species, species-specific evaluation remains critical for *S. littoralis*. This pest is a major agricultural threat in Egypt and the Mediterranean region due to its wide host range, high reproductive potential, and documented resistance to multiple insecticide classes (Salama et al., 1971; Ghoneim, et al., 2015). Additionally, physiological and immunological responses to IGRs—such as haemocyte alterations, tissue damage, and developmental effects—can vary across species and developmental stages. Therefore, targeted assessment of IGR efficacy and mode of action on *S. littoralis* is essential to inform sustainable and region-specific pest management strategies. Therefore, this study aims to evaluate the efficacy of two IGRs, pyriproxyfen (a juvenile hormone analogue) and chlorfluazuron (a chitin synthesis inhibitor), on larval mortality, morphological and histological deformities, haemogram parameters, and haemolymph volume in *S. littoralis*. By providing a comprehensive assessment of these physiological disruptions, this study seeks to enhance the integration of IGRs into sustainable pest management programs.

Materials and methods

Insect culture

A laboratory culture of *S. littoralis* larvae was kindly provided by the plant protection research institute and maintained for further experiments under conditions (temperature: 25 ± 2°C, relative humidity: 60 ± 5%, photoperiod: 16:8 L:D), according to the method described by Eldefrawi et al. (1964). The newly moulted 6th instar larvae were used in this work.

Insect growth regulators (IGRs)

Two insect growth regulators (IGRs) were utilized in this study: chlorfluazuron (Caprice 5% EC) and pyriproxyfen (Muligan10% EC). Four concentrations of each IGR were prepared: 0.01 ppm, 0.1 ppm, 1, and 10 ppm. According to manufacturer guidelines and recent studies, the recommended field application rates are approximately 20–50 g a.i./feddan for chlorfluazuron (Caprice 5% EC) (Abd-Eldaim et al., 2020) and 50 g a.i./feddan for pyriproxyfen (Muligan 10% EC) (Kumar et al., 2014). The concentrations used in this study (0.01, 0.1, 1, and 10 ppm) were selected to encompass sublethal, near-field, and supra-field levels to facilitate a comprehensive dose-response evaluation.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_82058224

(1) Product name: chlorfluazuron 5% EC

Chemical name: (RS)- 1- [2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy) phenyl]-3-(2,6-difluorobenzoyl) urea (IUPAC)
Chemical formula: $C_{17}H_8Cl_2F_8N_2O$

(2) Product name: pyriproxyfen 10% EC

Chemical name: 2-[1-Methyl-2-(4-phenoxyphenoxy) ethoxy] pyridine
Chemical formula $C_{20}H_{19}NO_3$

Treatment procedure

The dipping technique employed to treat newly moulted 6th instar larvae with the IGRs. A stock solution of each IGR concentration was prepared by diluting the respective commercial formulation in an appropriate solvent following the manufacturer's instructions. Castor bean leaf discs were dipped in both compounds and air-dried before feeding the larvae. A control group was given water-treated leaf discs. Three sets of treated and control larvae (10 larvae each) were kept in separate glass jars. Larvae were allowed to feed for 24 h, and their biological parameters were recorded daily.

Haemocytes profile estimation

Following the post-treatment period, haemolymph samples were collected from the treated larvae by amputating one prothoracic leg from each of three individual larvae, using fine scissors. Gentle pressure was applied to the thorax to obtain haemolymph drops. The procedure was replicated three times. The collected haemolymph samples were used to estimate the haemocytes profile.

Differential haemocyte counts (DHC)

Differential haemocyte counts followed Shapiro method (1968). Haemolymph was spread on glass slides, fixed with methyl alcohol, stained with Masy-Grunwald- Giemsa solution, and observed under a light microscope at 400× magnification. Cell types were classified based on shape, cytoplasmic ratio, inclusions, and nucleus shape following Essawy et al. (1985). Percentages of haemocyte types were calculated as follows:

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

Total haemocyte count (THC)

Total haemocyte count was conducted by collecting haemocyte into a Thoma-white blood cell diluting pipette according to Essawy (1991), followed by a 20-fold dilution with a solution containing NaCl, KCl, CaCl2, crystal violet, and acetic acid. After discarding the first three drops, the mixture was dispensed onto a counting slide chamber. Cells in 64 squares at the corners were counted after 3 min, and the number of haemocytes/mm^3 was calculated using Jones' formula (1962):

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

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_82058224

Haemolymph volume

Haemolymph volume was gravimetrically determined by totally bleeding ten larvae (Arnold and Hinks, 1976).

Histological studies

For histological examination, the late 6th instar larvae of *S. littoralis* were treated with IGRs using the leaf dipping technique. After 24 h post-treatment, the larvae were fixed in alcoholic Bouin's solution, dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin wax. Cross sections (5 microns thick) of the prothorax were prepared using a microtome and mounted on clean slides with Mayer's albumin. The sections were stained with haematoxylin and eosin and observed under an Axiophot (Zeiss) light microscope following the methodology of Humason (1979) with modifications, and Bancroft and Gamble (2008).

Malformation and mortality assessment

To evaluate the occurrence of malformations, the treated larvae were carefully examined under a stereomicroscope. Different morphological parameters were assessed, such as abnormal body shape, colour changes, appendage deformities, and wing malformations. The malformation rate was calculated as the percentage of larvae displaying any form of abnormality. The mortality rate of the treated larvae was recorded during the observation period. Larvae that did not exhibit any movement or response to external stimuli were considered dead. The mortality rate was calculated as the percentage of dead larvae relative to the total number of treated larvae.

Experimental replication

The entire experiment was replicated three times to ensure the reliability and reproducibility of the results. Each replicate involved a separate set of larvae and independent treatments with the IGRs and control groups.

Statistical analysis

Data were analysed using Minitab 18 software. The data obtained from haemocyte profile estimation, malformation assessment, and mortality determination were subjected to appropriate statistical analyses. Two-way ANOVA ($p < 0.05$) was used to assess the effects of different concentrations of IGRs and their interaction with treatment groups. To identify significant differences between the control and treatment groups at each concentration, independent samples t-tests were applied.

Results

Lethal effects of pyriproxyfen and chlorfluazuron against S. littoralis larvae

The effects of pyriproxyfen and chlorfluazuron on sixth instar larvae of *S. littoralis* were evaluated over seven consecutive days post-treatment across four concentrations (*Fig. 1*). Both compounds significantly reduced larval survival in a dose-dependent manner (chlorfluazuron: $F = 42.6$, $p < 0.001$; pyriproxyfen: $F = 38.2$, $p < 0.001$). Four days after chlorfluazuron treatment, mortality rates reached 16.7%, 30%, 90%, and 100%

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_82058224

at concentrations of 0.01, 0.1, 1, and 10 ppm, respectively ($p < 0.001$ vs. control, t-test). Pyriproxyfen caused no mortality during this period; however, by the seventh day, mortality reached 26.7%, 33.3%, 86.7%, and 100% across the same concentrations ($p < 0.001$ vs. control). No mortality was recorded in the control group.

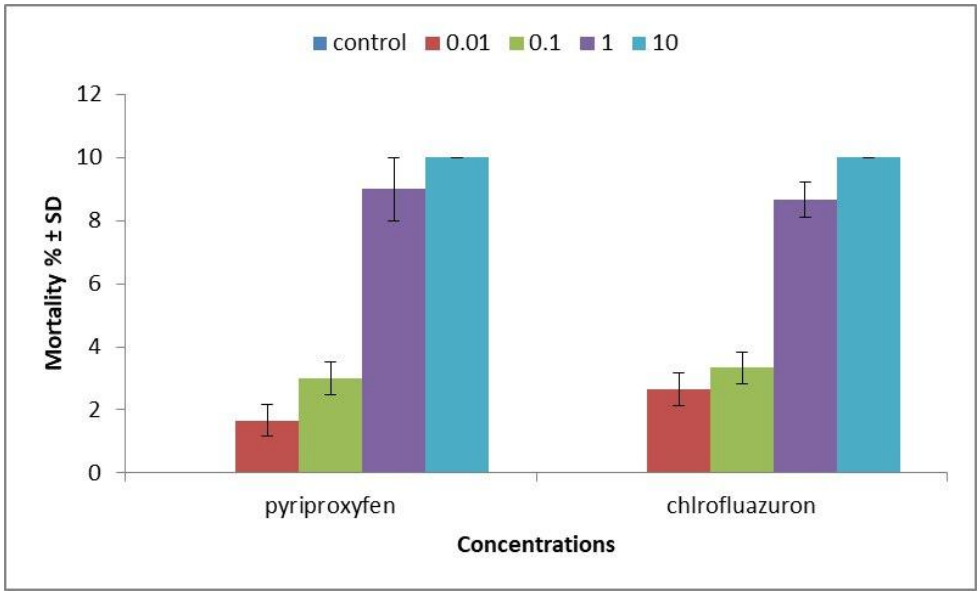

Figure 1. *Lethal effects of pyriproxyfen and chlorfluazuron against S. littoralis larvae*

Morphological deformities

Both compounds induced significant morphological deformities in *S. littoralis* larvae (Chi-square = 19.8, $p < 0.001$). Treated larvae exhibited shrinkage, incomplete moulting, and development of larval-pupal intermediates that failed to complete metamorphosis. Adults from treated groups showed folded wings (*Figs. 2* and *3*). Malformed pupae and pupal-adult intermediates were significantly more frequent in treated groups than in the control ($p < 0.001$, t-test) (*Fig. 4*), with malformation rates ranging from 16% to 100%. Chlorfluazuron induced a greater frequency of malformations than pyriproxyfen ($p < 0.05$).

Histological deformities

The integument of untreated larvae exhibited a well-structured epicuticle, epidermis, and basement membrane (*Fig. 5E*).

Effect of pyriproxyfen

Twenty-four hours post-treatment, larvae exposed to 0.01 ppm pyriproxyfen showed separations between epidermal cells (*Fig. 5A*). At 0.1 ppm, deformation and indentation of the epidermis were observed, with thickening and darkening of the epicuticle (*Fig. 5B*). Severe destruction of the basal membrane and vacuolization occurred at 1 and 10 ppm (*Fig. 5C, D*). Histological damage scores increased significantly at higher concentrations (Chi-square = 21.3, $p < 0.01$ vs. control). By 96 h, deformities worsened, with complete separation of cuticle layers from the hypodermis and severe disintegration (*Fig. 6A–D*; $p < 0.001$ vs. control).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_82058224

Figure 2. *Morphological deformities in larval-pupal intermediates of S. littoralis treated with varying concentrations of pyriproxyfen. (c) normal pupa, and (f) normal larva; (A) 0.01 ppm (B) 0.1 ppm (D) 1 ppm (E) 10 ppm (X = 1)*

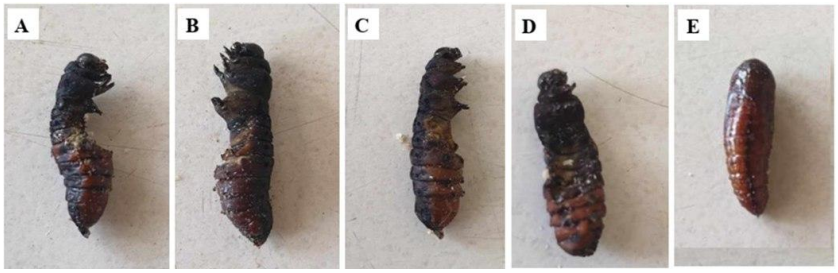

Figure 3. *Morphological deformities in larval-pupal intermediates of S. littoralis treated with varying concentrations of chlorfluazuron. (A) 0.01 ppm; (B) 0.1 ppm; (C) 1 ppm; (D) 10 ppm and (E) control (X = 1)*

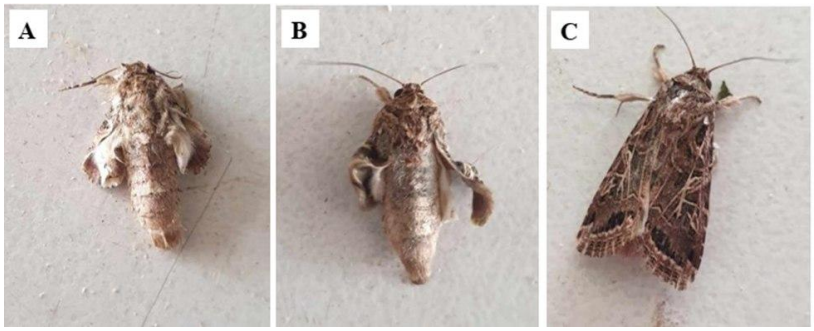

Figure 4. *Malformation in adults of S. litoralis treated with (A) pyriproxyfen and (B) chlorfluazuron (C) Control (X = 1)*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_82058224

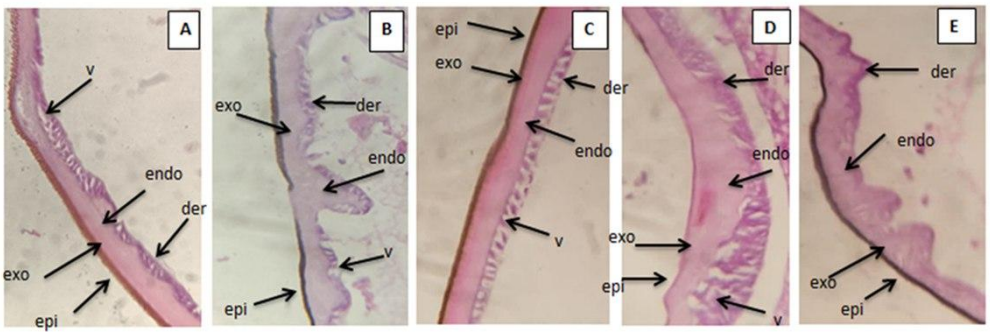

Figure 5. *Histological alterations in S. littoralis larvae 24 h post-treatment with different concentrations of pyriproxyfen. (A) 0.01 ppm, (B) 0.1 ppm, (C) 1 ppm, (D) 10 ppm, and (E) control. (Epi) epicuticle, (Exo) Exocuticle, (Endo) Endocuticle, (Der) Epidermis cells, and (V) Vacuoles. (Stained with hematoxylin; X = 100X)*

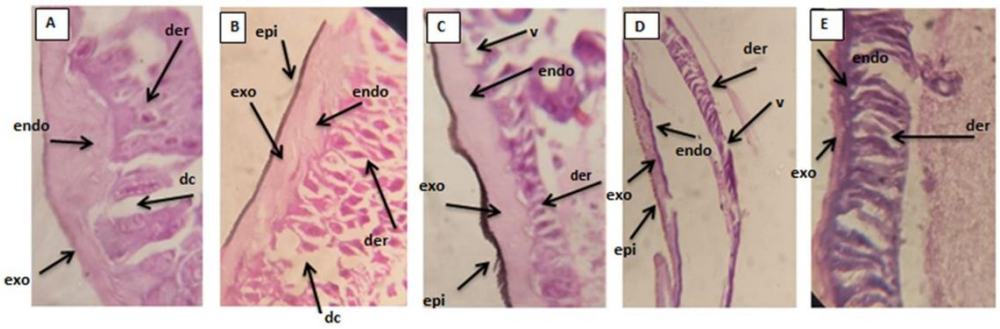

Figure 6. *Histological alterations in S. littoralis larvae, 96 h post-treatment with different concentrations of pyriproxyfen. (A) 0.01 ppm, (B) 0.1 ppm, (C) 1 ppm, (D) 10 ppm, and (E) control. (Epi) Epicuticle, (Exo) Exocuticle, (Endo) Endocuticle, (Der) epidermis cells, and (V) vacuoles; (dc) degraded cells. (Stained with haematoxylin; X = 100X)*

Effect of chlorfluazuron

Chlorfluazuron at 0.01 ppm caused significant abnormalities in the epicuticle and epidermis, progressing to severe collapse at higher doses (*Fig. 7A–E*). By 96 h, all treated larvae had died ($p < 0.001$ vs. control).

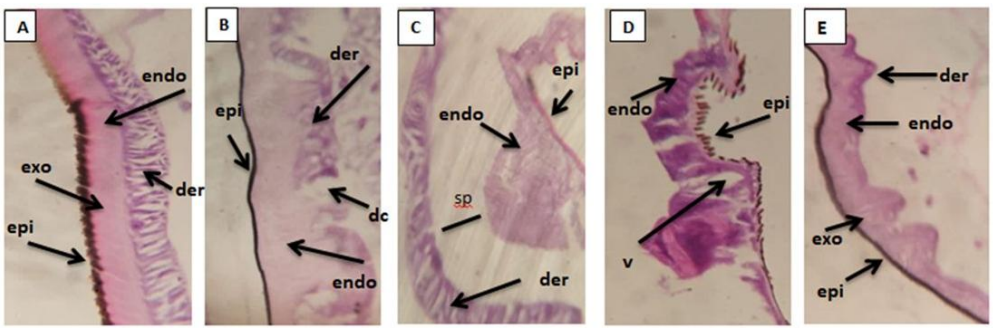

Figure 7. *Histological alterations in S. littoralis larvae 96 h post-treatment with different concentrations of chlorfluazuron (A) 0.01 ppm, (B) 0.1 ppm, (C) 1 ppm, (D) 10 ppm, and (E) control. (Epi) Epicuticle, (Exo) Exocuticle, (Endo) Endocuticle, (Der) Epidermis cells; (V) Vacuoles; (dc) degraded cells and (sp) space. (Stained with haematoxylin; X = 100X)*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_82058224

Toxicity on differential haemocyte counts (DHC)

Pyriproxyfen

Pyriproxyfen significantly reduced prohaemocytes (PRs) and plasmatocytes (PLs) during the first three days compared to the control ($p < 0.01$, t-test). From day 4 to day 7, PRs increased from 8% to 27.6% and PLs from 8.3% to 26% ($p < 0.05$ vs. control). Granulocytes (GRs) and oenocytoids (OEs) increased significantly, reaching 39.3% and 37.3% ($p < 0.01$ vs. control). SPs were significantly reduced ($F = 1.18$, $p = 0.32$ for concentration; $F = 520$, $p < 0.001$ for time) (*Fig. 8*).

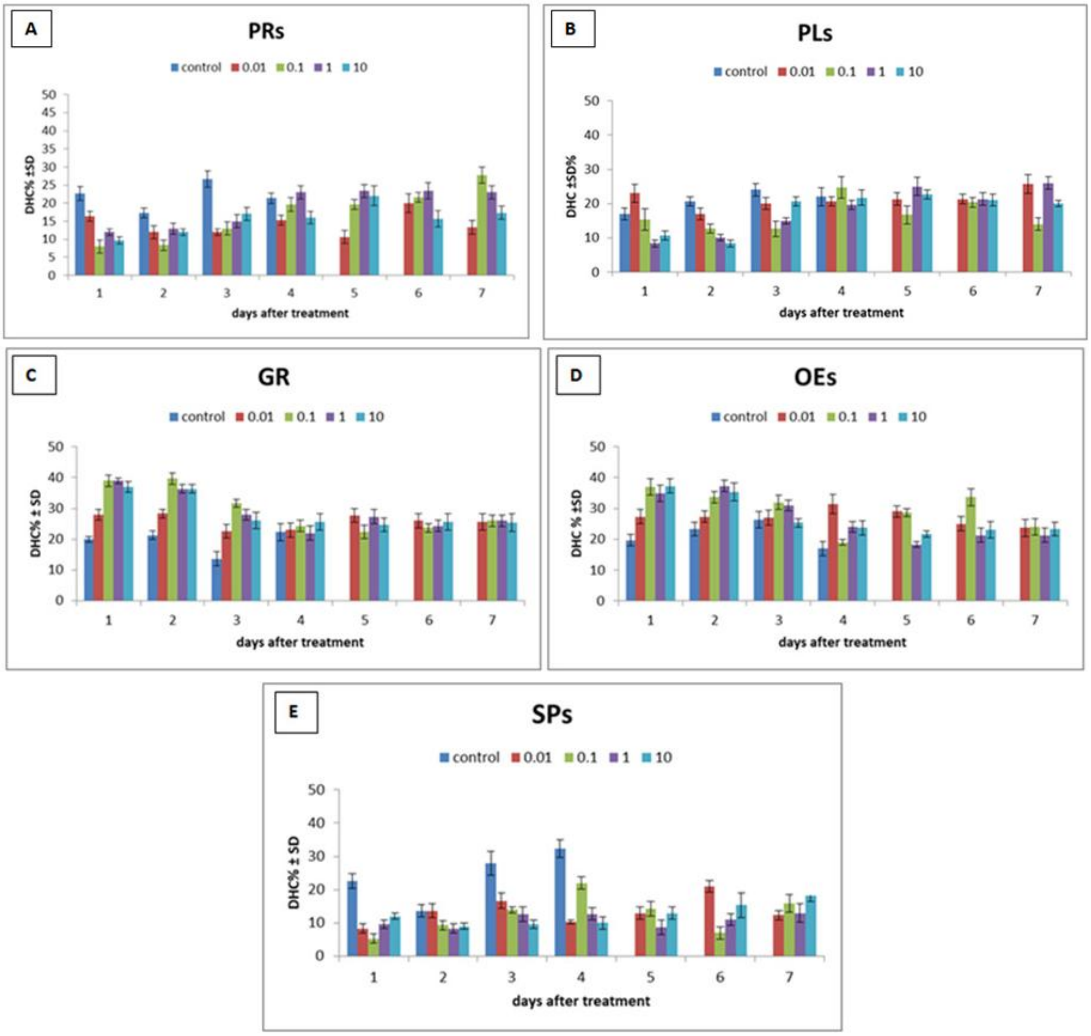

Figure 8. *Differential haemocyte count (DHC) in S. littoralis larvae treated with pyriproxyfen, (A) prohaemocytes, (B) plasmatocytes, (C) granulocytes, (D) oenocytoids, and (E) spherulocytes*

Chlorfluazuron

Chlorfluazuron treatment induced significant decreases in the proportions of prohaemocytes (PRs) and plasmatocytes (PLs), reaching minimum values of 9.3% and 9%, respectively, which were significantly lower than those in the control group ($p < 0.001$, *t*-test; *Fig. 9A, B*). In contrast, the proportions of granulocytes (GRs) and

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_82058224

oenocytoids (OEs) increased significantly in treated larvae, attaining peak values of 38.6% and 39%, respectively ($p < 0.001$ vs. control; *Fig. 9C–D*). Additionally, the number of spherulocytes (SPs) declined significantly across concentrations and time points, as confirmed by ANOVA ($F = 46.53$, $p < 0.001$ for concentration; $F = 32.63$, $p < 0.001$ for time).

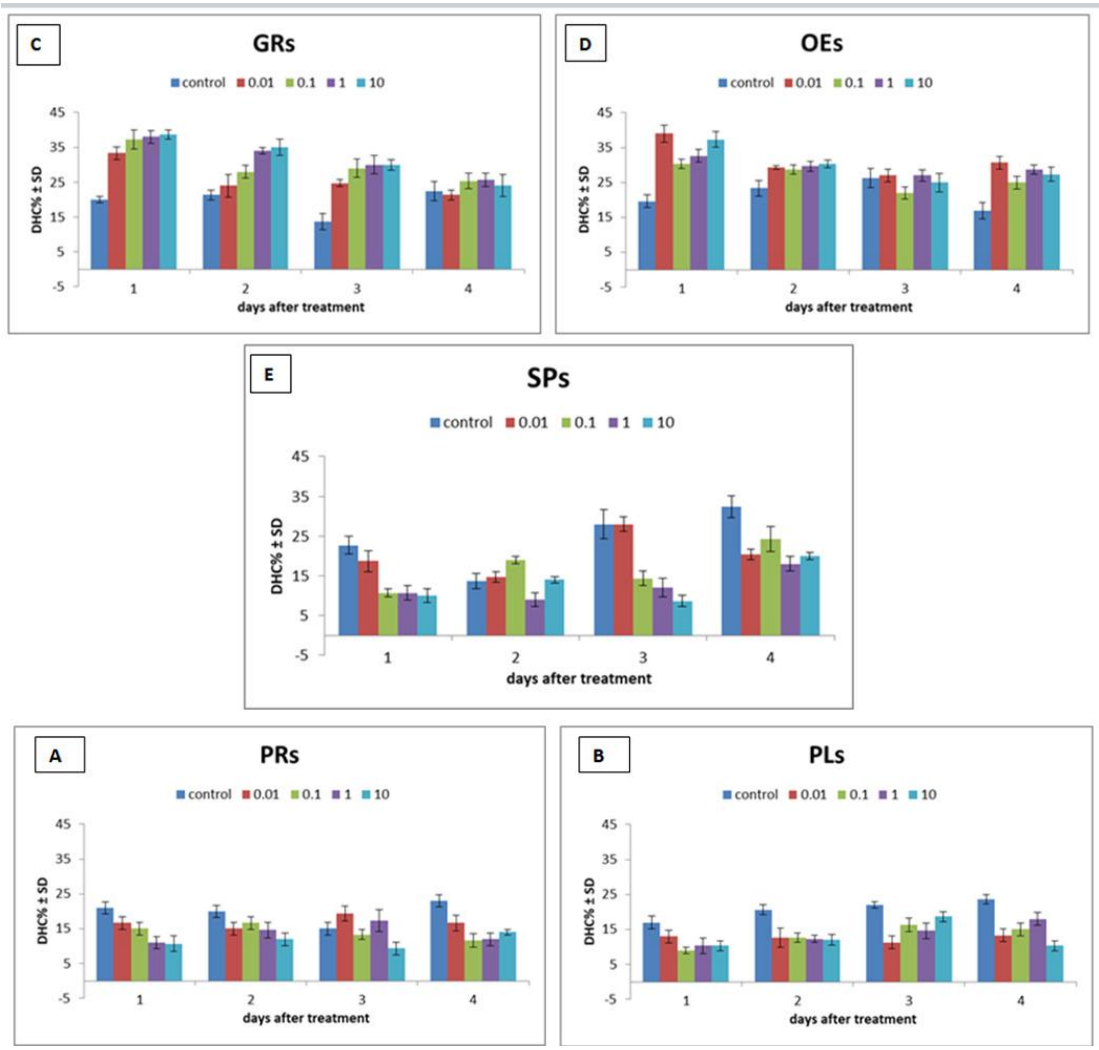

Figure 9. *Differential haemocyte count (DHC) in S. littoralis larvae treated with chlorfluazuron, (A) prohaemocytes, (B plasmatocytes), (C) granulocytes, (D) oenocytoids, and (E) spherulocytes*

Further statistical analyses revealed that GRs were significantly influenced by both pyriproxyfen ($F = 52.25$, $p < 0.001$) and chlorfluazuron ($F = 55.96$, $p < 0.001$). Similarly, PLs displayed a strong concentration-dependent reduction under the influence of both compounds (pyriproxyfen: $F = 18.93$, $p < 0.001$; chlorfluazuron: $F = 35.82$, $p < 0.001$). OEs were also highly elevated in response to both insect growth regulators (pyriproxyfen: $F = 30.89$, $p < 0.001$; chlorfluazuron: $F = 70.60$, $p < 0.001$). PRs showed significant effects due to concentration (pyriproxyfen: $F = 7.64$, $p < 0.001$; chlorfluazuron: $F = 26.4$, $p < 0.001$); however, the effect of time was not significant in chlorfluazuron-treated larvae ($F = 0.47$, $p = 0.75$).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_82058224

Toxicity on total haemocyte count (THC)

Pyriproxyfen significantly increased THC to 15,383.3 ± 35.8 cells/mm³ at 10 ppm, compared to 11,426.7 ± 193.1 cells/mm³ in controls ($p < 0.01$, t-test; $F = 77.83$, $p < 0.001$ for concentration; $F = 14.53$, $p < 0.001$ for time) (*Fig. 10A*). Chlorfluazuron also elevated THC to 15,433.3 ± 157.1 cells/mm³ at 10 ppm ($p < 0.01$ vs. control), with a slight decline to 14,683 ± 291 cells/mm³ by day 4 ($F = 18.60$, $p < 0.001$ for concentration; $F = 140.79$, $p < 0.001$ for time) (*Fig. 10B*).

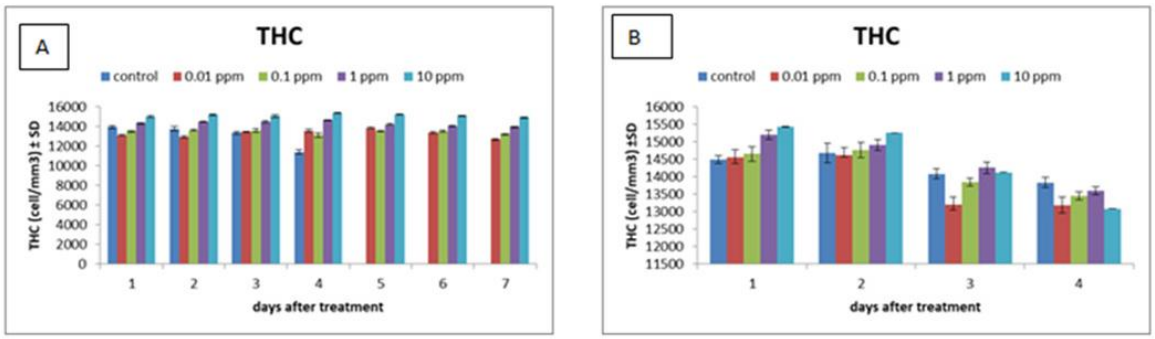

Figure 10. *Total haemocyte count (THC) (cells/mm³) in S. littoralis larvae treated with (A) pyriproxyfen and (B) chlorfluazuron*

Effect on haemolymph volume

Both pyriproxyfen and chlorfluazuron significantly affected haemolymph volume in *S. littoralis* larvae when compared to the control. In pyriproxyfen-treated larvae, haemolymph volume peaked at 29.44 µL at a concentration of 0.01 ppm on the fourth day post-treatment. This value was significantly higher than the 5.55 µL recorded in the control group ($p < 0.001$, *t*-test; $F = 120.13$, $p < 0.001$ for concentration; $F = 76.61$, $p < 0.001$ for time; *Fig. 11A*). A similar pattern was observed in chlorfluazuron-treated larvae, where the haemolymph volume reached a maximum of 27.78 µL at the same concentration and time point. This volume was also significantly higher than that in the control group, which recorded 6.11 µL ($p < 0.001$, *t*-test; $F = 34.38$, $p < 0.001$ for concentration; $F = 26.69$, $p < 0.001$ for time; *Fig. 11B*). These findings suggest that both insect growth regulators disrupt haemolymph regulation mechanisms in treated larvae, possibly reflecting systemic physiological stress responses.

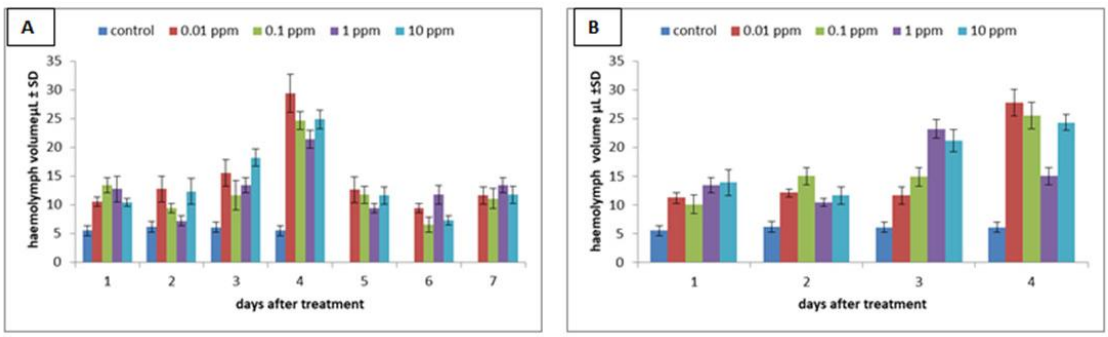

Figure 11. *Haemolymph volume in S. littoralis larvae treated with (A) pyriproxyfen and (B) chlorfluazuron*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_82058224

Discussion

Beyond the environmental pollution, synthetic chemical insecticides also have harmful toxic effects on various taxa, including birds, fish, bees, and mammals (Quarles, 2001; Rose, 2001). Over the past few decades, a new class of comparatively safe insecticides, known as Insect Growth Regulators (IGRs), has been developed (Dhadialla et al., 1998; Khan and Qamar, 2012). In contrast to the traditional chemical insecticides, IGRs do not cause direct toxicity, but act selectively on the development, metamorphosis or reproduction of the target insect species (Martins and Silva, 2004).

Lethal effects of the evaluated IGRs

The obtained data clearly show a dose-dependent increase in larval mortality for both pyriproxyfen and chlorfluazuron. Chlorfluazuron induced significant mortality as early as the 4th day post-treatment, reaching 100% at the highest concentration (10 ppm). This is consistent with previous findings by Mule et al. (2022 and Aly et al. (2024), who observed similar dose-dependent lethality in *Spodoptera frugiperda*. As a chitin synthesis inhibitor, chlorfluazuron disrupts the formation of the insect cuticle during moulting, leading to rapid mortality due to desiccation and failed ecdysis (Rahman et al., 2024). Additionally, the disruption of hormonal regulation as a toxic mechanism has been proposed for certain insect species (Djeghader et al., 2014).

Pyriproxyfen, a juvenile hormone analogue, exhibited a delayed lethal effect, with mortality peaking on the 7th day post-treatment. This delayed action is typical of juvenile hormone analogues, which disrupt the normal development and metamorphosis of insects. Similar results were reported by Golmohammadi et al. (2021), who observed progressive mortality in *Chrysoperla carnea* larvae. This pattern aligns with pyriproxyfen's role in mimicking juvenile hormones, which interferes with larval-pupal metamorphosis, ultimately leading to death. Moreover, the delayed mortality observed in pyriproxyfen treatments could provide ecological benefits by allowing treated larvae to persist longer in the environment. This persistence may facilitate a broader disruption of pest populations, especially when combined with other control measures (Bakr et al., 2021; Martínez et al., 2021; Li et al., 2024).

IGRs, like pyriproxyfen and chlorfluazuron, have distinct modes of action compared to synthetic insecticides. Chitin synthesis inhibitors (CSIs) specifically target chitin formation in the insect exoskeleton and internal structures, such as the peritrophic matrix (Merzendorf, 2005; Merzendorf and Zimoch, 2003). This makes them a valuable component in integrated pest management (IPM) systems. However, the prolonged presence of treated larvae may also affect non-target species and local ecosystem dynamics, emphasizing the need for strategic application to minimize unintended consequences. The rapid action of chlorfluazuron and the delayed effects of pyriproxyfen suggest that these two IGRs could be effectively combined to enhance pest control strategies.

Morphological and histological impacts of IGRs on S. littoralis

The severe morphological deformities observed in *S. littoralis* larvae and pupae following treatment with both compounds included shrunk larvae, malformed pupae, and adults with folded wings. These findings are consistent with those of Noelia et al. (2023), who reported similar deformities in *Eriopis connexa* larvae treated with chlorfluazuron, indicating that chitin synthesis inhibitors compromise the structural integrity of the cuticle

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_82058224

and the moulting process. Pyriproxyfen-treated larvae exhibited a high incidence of larval-pupal intermediates, a hallmark of juvenile hormone disruption, similar to observations by Yusoff et al. (2021) in *Plutella xylostella* larvae, where hormonal interference disrupted metamorphosis, leading to hybrid forms that failed to complete development and eventually died. This highlights pyriproxyfen's role as an effective disruptor of insect development (Hafez and Mobarak, 2024).

Histological analysis of chlorfluazuron-treated larvae revealed significant integument damage, characterized by thickened, darkened cuticle layers and degradation of the procuticle and epidermal cells. Comparable cuticular abnormalities have been observed in previous studies involving chlorfluazuron treatment in *Chrysomya albiceps* (Khater, 2021), *Agrotis ipsilon* (Amein and Mohammad., 2022), and *Musca domestica* (El-Shewy et al., 2024). These structural deformities impair the larvae to maintain homeostasis, ultimately leading to death from desiccation or infection (Tuncsoy and Özalp., 2021).

These findings align with numerous studies showing that various IGRs inhibit growth and development in other insect species, such as cyromazine affecting *Ceratitis capitata* (Viñuela et al., 1993), diofenolan impacting *Papilio demoleus* (Singh and Kumar, 2011), and methoprene and flufenoxuron disrupting *A. ipsilon* (Khatter, 2014; Tanani et al., 2015).

The main histological abnormalities identified in larvae treated with pyriproxyfen were vacuolation and epidermal disintegration. These results are consistent with studies by Xu et al. (2022) on silkworms, Yasmeen and Amir (2022) on *Chrysomya megacephala*, and Rocha et al. (2023) on *Spodoptera cosmioides*, which found that juvenile hormone analogues led to cellular degradation in the integument of treated larvae. Such damage to the cuticle structure likely contributes to the high mortality rates observed in pyriproxyfen-treated insects (Hassan et al., 2017). Given the structural damage noted, it is unsurprising that IGRs have inhibitory effects on the growth and development of *S. littoralis*, with body weight gain serving as a crucial indicator of these effects (Armbruster and Hutchinson, 2002), while developmental duration is viewed as an inverse measure of developmental rate (Richard, 1957). The deformities in *S. littoralis* larvae demonstrate the disruptive effects of IGRs on structural integrity and metamorphosis, supporting the idea that incorporating IGRs into pest management strategies could optimize insect control efforts by targeting developmental processes.

Haematological disruptions in S. littoralis caused by IGRs

The classification of haemocyte types varies, with studies ranging from four to seven types (Gupta, 1979) or between three and nine (Wigglesworth, 1959; Jones, 1962; Arnold, 1972; Al-Khalifa and Siddiqui, 1985). Essawy et al. (1985) classified the haemocytes depending on physiological function into five types. Both pyriproxyfen and chlorfluazuron caused significant alterations in the differential haemocyte counts (DHC) and total haemocyte counts (THC) of treated larvae. Haemocytes are crucial for insect immunity, participating in phagocytosis, encapsulation of pathogens, and wound healing (Lavine and Strand, 2002; Stanley et al., 2023). Initially, both compounds increased THC, indicating an immune response to chemical stress. However, prolonged exposure led to reduced populations, particularly of granulocytes (GRs) and spherulocytes (SPs).

Similar biphasic responses in immunocyte profiles have been reported in sublethal doses of IGRs in *Spodoptera frugiperda* (Duarte et al., 2020), *Agrotis ipsilon* (Shaurub et al., 2022), and *Galleria mellonella* (Ghoneim et al., 2023). These studies showed that sublethal doses initially cause an increase in haemocyte production as a defensive response, followed by immunosuppression with prolonged exposure.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_82058224

The increase in granulocytes and oenocytoids observed in this study is consistent with findings from Abd El-Aziz and Awad (2010) and Shaurub et al. (2022), indicating the immune system's attempt to combat chemical stress. Notably, pyriproxyfen had a significant impact on haemocyte profiles, particularly reducing spherulocyte counts. This immunosuppressive effect may enhance pyriproxyfen's efficacy in integrated pest management (IPM) programs by making larvae more susceptible to microbial infections and natural enemies (Sahayaraj and Hassan, 2023; Bartling et al., 2024).

Both IGRs significantly altered haemocyte dynamics in *S. littoralis*, as supported by multiple studies showing that IGRs affect haemocyte counts and insect immunity. For instance, pyriproxyfen and methoxyfenozide decreased THC in *Eurygaster integriceps* (Zibaee et al., 2012) and *Spodoptera litura* (Irfan et al., 2019), while also increasing pathogen susceptibility in *Spodoptera mauritia* (Resmitha and Kannan, 2017) and altering haemocyte counts in *Galleria mellonella* (Tuncsoy and Özalp, 2021). Chlorfluazuron has been noted to reduce THC in S. littoralis (Sezer and Ozalp, 2015; Sahar et al., 2018), further confirming the immune-suppressive effects observed in other studies involving azadirachtin and diflubenzuron (Shaurub et al., 2014; Ghoneim et al., 2015; Basiouny and Waheeb, 2019).

The fluctuations in THC can be attributed to developmental processes and exposure to IGRs (Gelbic et al., 2006). Pyriproxyfen significantly increased haemocyte production at high concentrations, while chlorfluazuron caused an initial rise in THC during the first two days, followed by a decline. This increase aligns with findings for various chitin synthesis inhibitors (CSIs) such as diflubenzuron (Osman et al., 1984) and others. Notably, the brain-endocrine complex plays a role in haemocyte accumulation in response to stimuli (Nappi, 1974), with ecdysteroids possibly regulating haemocyte numbers (Jones, 1967). Various biological and physiological parameters, including growth (Dean et al., 1980) and haemogram regulation (Essawy, et al., 1985), are regulated by varying levels of hormonal activity. The hormones 20-hydroxy ecdysone and juvenile hormone oversee a wide range of developmental processes in internal tissues such as the epidermis (Riddiford, 1976) and haemocytes (Essawy and Idriss, 1990).

Significant changes in haemocyte counts reveal immune responses triggered by pyriproxyfen and chlorfluazuron, suggesting that IGRs impact insect immunity and potentially increase vulnerability to pathogens. Incorporating these findings into pest management strategies can improve overall efficacy, as understanding these immune dynamics is essential for applied insect control.

Comparative toxicity and changes in haemolymph volume

Various factors can influence an insect's immune responses, including insecticides, hormones, and environmental temperature (Mandato, 1998). Additionally, haemocyte cellular immunity may play a role in the metabolism and detoxification of xenobiotics (Kurihara et al., 1992; Rahimi et al., 2013).

The differential toxicity of pyriproxyfen and chlorfluazuron is reflected in both the timing and extent of their effects. Chlorfluazuron's rapid action can be attributed to its role in disrupting chitin synthesis, a vital process for moulting and cuticle formation (Zhang et al., 2017). In contrast, the interference of pyriproxyfen with hormonal regulation of development leads to a slower but equally lethal effect (Zeng et al., 2023).

The fluctuation in haemolymph volume observed in *S. littoralis* larvae treated with pyriproxyfen and chlorfluazuron may be interpreted in light of findings by Loughton and

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_82058224

Tobe (1969). Pyriproxyfen likely causes hormonal imbalances that delay moulting, resulting in fluid retention, as noted in other lepidopteran species (Etebari et al., 2007; Cremonez et al., 2021). Typically, haemolymph volume fluctuates during the last larval instars, increasing as the larvae approach ecdysis, which aids in inflating and shedding the old cuticle (Loughton and Tobe, 1969). However, the delayed moulting caused by pyriproxyfen disrupts this natural cycle, prolonging the period of increased haemolymph volume, as the larvae fail to properly initiate or complete ecdysis.

Similarly, chlorfluazuron, a chitin synthesis inhibitor, likely disrupts cuticle formation, interfering with this process. This aligns with findings that chlorfluazuron treatment affects fluid regulation, resulting in fluid retention due to impaired cuticle shedding (Zhu et al., 2012; Piri Aliabadi et al., 2016). In a normal larval cycle, haemolymph volume decreases rapidly after ecdysis, either due to water loss or absorption by tissues. However, with cuticle formation compromised by chlorfluazuron, this decrease may not occur as expected, further contributing to the abnormal retention of fluid.

Thus, the fluctuations in haemolymph volume induced by these insect growth regulators may stem from their interference with the larvae's natural moulting cycle. Fluid retention during ecdysis, caused by hormonal disruptions from pyriproxyfen or cuticle defects from chlorfluazuron, extends the period of increased haemolymph volume, disrupting normal physiological balance and contributing to the observed mortality in treated larvae.

Significant changes in haemolymph volume were observed in treated larvae, with both pyriproxyfen and chlorfluazuron leading to increased haemolymph volumes compared to controls. Similarly, Meethal (2023) in *Spodoptera mauritia* and Rolim et al. (2019) in boll weevils discovered that exposure to IGRs affected haemolymph volume and composition. This change may be viewed as a compensatory response to chemical stress, facilitating the distribution of haemocytes and other defence mechanisms throughout the insect's body (Zibaee and Malagoli, 2020; Hasnain et al., 2023).

The varying toxicity and alterations in haemolymph volume caused by pyriproxyfen and chlorfluazuron indicate their complex effects on *S. littoralis* physiology. These changes disrupt natural moulting cycles and contribute to mortality. Insights from these physiological responses can guide effective pest management strategies. Utilizing this knowledge could optimize the application of IGRs in agriculture.

Conclusion

Overall, the findings confirm the multi-faceted impact of pyriproxyfen and chlorfluazuron on *S. littoralis* larvae, ranging from immediate mortality to long-term physiological disruptions. These disruptions not only lead to larval mortality but also reduce the insect's immune system, making them more susceptible to other control agents. The broad-spectrum effect on haemocyte dynamics and fluid regulation highlights the potential of these IGRs to be integrated into IPM programs, either alone or in combination with other biological control agents, enhancing pest management strategies in agricultural ecosystems.

Acknowledgments. This work was funded by the Deanship of Graduate Studies and Scientific Research at Jouf University under grant No. (DGSSR-2024-02-02167).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_82058224

REFERENCES

[1] Abd El-Aziz, N. M., Awad, H. H. (2010): Changes in the haemocytes of *Agrotis ipsilon* larvae (Lepidoptera: Noctuidae) in relation to dimilin and Bacillus thuringiensis infections. – Micron 41(3): 203-209.

[2] Abd El-Razik, M. A. A., Mostafa, Z. M. S. (2013): Joint action of two novel insecticides mixtures with insect growth regulators, synergistic compounds and conventional insecticides against *Spodoptera Littoralis* (Boisd.) larvae. – Am. J. Bioch. Mol. Biol. 3(4): 369-378.

[3] Abd-Eldaim, F. A., Hamaad, R. F., Emam, S. S. (2020): Phytotoxicity effects of some insecticides on cotton plant. – Egy Sci J Pestic 6(4): 52-59.

[4] Al-Khalifa, M., Siddiqui, M. (1985): A comparative study of haemocytes in some coleopterous species. – J. Call. Sci., King Saud Univ. 16: 199-134.

[5] Aly, M. Z., Fangary, H. M., Ibrahim, H. M., Salem, S. A. (2024): comparative biological and biochemical assessment for toxicological profile of some eco-friendly insecticides applied for controlling of the fourth instar larvae of Spodoptera frugiperda. – Egyptian Journal of Zoology.

[6] Amein, N. S., Mohammad, A. M. (2022): The biological and histopathological aspects of the Black Cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) treated with flufenoxuron. – Egyptian Academic Journal of Biological Sciences, F. Toxicology & Pest Control 14(2): 149-156.

[7] Amin, T. R. (2007): The effect of host plants on the susceptibility of the cotton leafworm, *Spodoptera littoralis* (Biosd.) to insecticidal treatments. – Egypt J. Agric. Res. 85(6): 2005-2015.

[8] Armbruster, P., Hutchinson, R. A. (2002): Pupal mass and wing length as indicators of fecundity in *Aedes albopictus* and *Aedes geniculatus* (Diptera: Culicidae). – J. Med. Entomol. 39: 699-704.

[9] Arnold, J. W. (1972): A comparative study of the haemocytes (blood cells) of cockroaches (Insecta, Dictyoptera, Blattari), with a view of their significance in taxonomy. – Can. Ent. 104: 309-348.

[10] Arnold, J. W., Hinks, C. F. (1976): Haemopoiesis in Lepidoptera. I. The multiplication of circulating haemocytes. – Canadian Journal of Zoology. https://doi.org/10.1139/z76-112.

[11] Bakr, N. A., Tanani, M., Hassan, H. A., Ghoneim, K. (2021): Insecticidal activity of pyriproxyfen, a juvenoid, and its suppressive effect on growth and development of the black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae). – Egyptian Academic Journal of Biological Sciences. A, Entomology 14(4).

[12] Bancroft, J. D., Gamble, M. (eds.) (2008): Theory and Practice of Histological Techniques. – Elsevier Health Sciences, Amsterdam.

[13] Bartling, M. T., Brandt, A., Hollert, H., Vilcinskas, A. (2024): Current insights into sublethal effects of pesticides on insects. – International Journal of Molecular Sciences 25(11): 6007.

[14] Basiouny, A., Waheeb, H. (2019): Assessment of the inhibitory impact of novaluron, a recent insect growth regulator, on the reproductive potential of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). – Egyptian Academic Journal of Biological Sciences. A, Entomology 12(1)

[15] Biddinger, D. J., Hull, L. A. (1995): Effect of several types of insecticides on the mite predator *Stethorus punctum* (Coleoptera: Coccinellidae): including insect growth regulators and abamectin. – J Econ Entomol. 88: 358-366.

[16] Cremonez, P. S., Marco, H. G., Andrello, A. C., Neves, P. M., Pinheiro, D. O. (2021): The effect of pyriproxyfen on the concentration of circulating metabolic fuel molecules and chemical elements in the haemolymph of *Acraea horta L*. (Lepidoptera: Nymphalidae): A quantitative analysis. – Pesticide Biochemistry and Physiology 177: 104907.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_82058224

[17] Dahi, H. F. (2005): Egyptian cotton leafworm *Spodoptera littoralis* development on artificial diet in relation to heat unit requirements. – The Third International Conference on IPM Role in Integrated Crop management and Impacts on Environment and Agricultural Products. Plant Protection Research Institute, ARC, Dokki, Giza, Egypt.
[18] Dean, P. N. (1980): A simplified method of DNA distribution analysis. – Cell Proliferation 13(3): 299-308.
[19] Dhadialla, T. S., Carlson, G. R., Le, D. P. (1998): New insecticides with ecdysteroidal and juvenile hormone activity. – Annu. Rev. Entomol. 43: 545-569.
[20] Djeghader, N. E. H., Aïssaoui, L., Amira, K., Boudjelida, H. (2014): Impact of a chitin synthesis inhibitor, Novaluron, on the development and the reproductive performance of mosquito *Culex pipiens*. – World Applied Sciences Journal 29(7): 954-960.
[21] Duarte, J. P., Redaelli, L. R., Silva, C. E., Jahnke, S. M. (2020): Effect of *Azadirachta indica* (Sapindales: Meliaceae) oil on the immune system of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) immatures. – Journal of Insect Science 20(3): 17.
[22] Eldefrawi, M. E., Toppozada, A., Mansour, N., Zeid, M. (1964): Toxicological studies on the Egyptian cotton leafworm, Prodenia litura. I. Susceptibility of different larval instars of Prodenia to insecticides. – Journal of Economic Entomology 57(4): 591-593.
[23] El-Shewy, A. M., Hamouda, S. S., Gharib, A. M., Gad, H. A., Abdelgaleil, S. A. (2024): Potential of insect growth regulators for the control of *Musca domestica* (Diptera: Muscidae) with respect to their biochemical and histological effects. – The Journal of Basic and Applied Zoology 85(1): 1-9.
[24] Essawy M. M. (1991). Haemocytes interrelationship changes during the different periods of development in last larval instar of the silkworm, Bombyx mori (L.). – J Pest Cont & Environ Scie 3(2): 77-89.
[25] Essawy, M. M., Idriss, M. (1990): The juvenile hormone fluctuations and the haemogram during the last larval instar of Philosama ricini Boisduval (Lepidoptera, Saturniidae). – J. Agric. Res. Tanta Univ. 16(3): 536-547.
[26] Essawy, M., Maleville, A., Brehelin, M. (1985): The haemocytes of Heliothis armigera: ultrastructure, functions, and evolution in the course of larval development. – Journal of Morphology 186(3): 255-264.
[27] Etebari, K., Bizhannia, A. R., Sorati, R., Matindoost, L. (2007): Biochemical changes in haemolymph of silkworm larvae due to pyriproxyfen residue. – Pesticide Biochemistry and Physiology 88(1): 14-19.
[28] Gelbic, I., Strbackova, J., Berger, J. (2006): Influence of metyrapone on the morphology of haemocytes of the Egyptian cotton leafworm *Spodopteralittoralis* (Boisd). – Zoological Studies 45: 371-377.
[29] Ghoneim, K., Tanani, M., Hamadah, K., Basiouny, A., Waheeb, H. (2015a): Effects of Novaluron and Cyromazine, chitin synthesis inhibitors, on the larval haemogram of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). – International Journal of Advanced Research 3(1): 554-576.
[30] Ghoneim, K., Tanani, M., Hamadah, K., Basiouny, A., Waheeb, H. (2015b): Bioefficacy of Novaluron, a chitin synthesis inhibitor, on survival and development of Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae). – Journal of Advances in Zoology 1(1).
[31] Ghoneim, K., Hamadah, K., El-Hela, A., Abo Elsoud, A. A. (2023): Quantitatively Disturbed Larval Haemogram of *Galleria mellonellaL*. (Lepidoptera: Pyralidae) by Certain Plant Growth Regulators. – Egyptian Academic Journal of Biological Sciences. A, Entomology 16(1).
[32] Golmohammadi, G. R., Rezaei Trshizi, H. R., Vafaei Shoshtari, R., Faravardeh, L., Rafei-Karehroudi, Z. (2021): Lethal and sublethal effects of three insecticides on green lacewing, *Chrysoperla carnea* (Neuroptera: Chrysopidae) under laboratory conditions. – Journal of Entomological Society of Iran 41(2): 105-121.
[33] Gupta, A. P. (1979): Insect Haemocytes: Development, Forms, Functions, and Techniques. – Cambridge University Press, New York.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_82058224

[34] Hafez, H. S., Mobarak, Y. M. (2024): Possible teratogenic and adults' abnormalities induced by insecticide pyriproxyfen on non-target organisms. – Frontiers in Scientific Research and Technology 8(1).
[35] Hasnain, M., Saeed, S., Naeem-Ullah, U., Ullah, S. (2023): Evaluation of chemosterility effect of different insect growth regulators on *Bactrocera zonata* population. – Science Progress 106(1): 00368504231155388.
[36] Hassan, N. A., Morshedy, M., Shaker, N. (2017): Effect of pre-treatment and histological alterations induced by sub lethal dose of lufenuron on both chlorpyrifos and imidacloprid efficacy on cotton leaf worm, *Spodoptera littoralis* (Bosid.). – Journal of Plant Protection and Pathology 8(9): 437-441.
[37] Hoffman, K. H., Lorenz, M. W. (1998): Recent advances in hormones in insect pest control. – Phytoparasitica 26 (4): 1e8.
[38] Humason, G. L. (1979): Animal Tissue Technique. 4th Ed. – Freeman, San Francisco, CA.
[39] Irfan, M., Sabri, M. A., Abdullah, A., Hussain, M., Ahmdani, M. Y. (2019): Quantitative changes of heamocytes in *Spodoptera litura*Fab. (Lepidoptera: Noctuidae) larvae in response to different insecticides. – Journal of Entomology and Zoology Studies 7(3): 533-537.
[40] Jones, J. C. (1962): Current concepts concerning insect haemocytes. – Amr. Zool. 2: 209-246.
[41] Jones, J. C. (1967): Normal differential count of haemocytes in relation to ecdysis and feeding in *Rhodnius prolixus*. – J. Insect Physiol. 13: 1133-1143.
[42] Kai, Z.-P., Huang, J., Tobe, S. S., Yang, X. l. (2009): A potential insect growth regulator: synthesis and bioactivity of an allatostatin mimic. – Peptides 30: 1249e125.
[43] Kandil, M. A., Abdel-Aziz, N. F., Sammour, E. A. (2003): Comparative toxicity of chlofluazuron and lufenuron against cotton leafworm, *Spodoptera littoralis*. – Egypt. J. Agric. Res. NRC 2: 645-661.
[44] Khan, I., Qamar, A. (2012): Andalin, an insect growth regulator, as reproductive inhibitor for the red cotton stainer, *Dysdercus koenigii* (F.) (Hemiptera: Pyrrhocoridae). – Acad. J. Entomol. 5(2): 113-121.
[45] Khater, K. (2021): Toxicological and ultrastructural effects of chitin synthesis inhibitors (lufenuron and chlorfluazuron) on third larval instars integument of Chrysomyaalbiceps (Diptera: Calliphoridae). – Parasitologists United Journal 14(1): 95-103.
[46] Khatter, N. A. (2014): Effect of two insect growth regulators on the development of Agrotis ipsilon Hufn. (Lepidoptera: Noctuidae). – J. Harmonized Res. Appl. Sci. 2(1): 20-28.
[47] Kumar, V., Dhawan, A. K., Shera, P. S. (2014): Field efficacy of insect growth regulator, pyriproxyfen against sucking insect pests and predatory complex on Bt cotton. – Pesticide Research Journal 26(1): 12-19.
[48] Kurihara, Y., Shimazu, T., Wago, H. (1992): Classification of haemocytes in the common cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae) II. Possible roles of granular plasmatocytes and oenocytoids in the cellular defence reactions. – Applied Entomology and Zoology 27: 237-242.
[49] Lanzoni, A., Bazzocchi, G. G., Reggiori, F., Rama, F., Sannino, L., Maini, S. (2012): *Spodoptera littoralis* male capture suppression in processing spinach using two kinds of synthetic sex-pheromone dispensers. – Bull. Insectol. 65: 311-318.
[50] Lavine, M. D., Strand, M. R. (2002): Insect haemocytes and their role in immunity. – Insect Biochemistry and Molecular Biology 32(10): 1295-1309.
[51] Li, H., Zhang, W., Zhang, Y., Guo, X., Hou, J., Li, H., Li, X. (2024): Effects of pyriproxyfen on development and hormone of the aphis, *Aphis craccivora* (Hemiptera: Aphididae). – Journal of Economic Entomology 117(4): 1493-1502.
[52] Loughton, B. G., Tobe, S. S. (1969): Blood volume in the African migratory locust. – Canadian Journal of Zoology 47(6): 1333-1336.
[53] Mandato, C. A. (1998): Modulators of insect cellular immune response. – PhD dissertation. University of Waterloo, Ontario.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_82058224

[54] Martínez, L. C., Plata-Rueda, A., Serrão, J. E. (2021): Effects of insect growth regulators on mortality, survival, and feeding of *Euprosternaelaeasa* (Lepidoptera: Limacodidae) larvae. – Agronomy 11(10): 2002.
[55] Martins, F., Silva, I. G. (2004): Avaliação da atividade inibidora do diflubenzuron na ecdise das larvas de *Aedes aegypti* (Linnaeus 1762) (Diptera, Culicidae). – Rev. Soc. Bras. Med. Trop. 37: 135-138.
[56] Meethal, K. V. (2023): The level of arylphorin in the haemolymph of *Spodoptera Mauritia* Boisd. (Lepidoptera: Noctuidae) larvae is increased by the insecticide, pyriproxyfen. – International Journal of Tropical Insect Science 43(2): 655-664.
[57] Merzendorf, H. (2005): Insect chitin synthases: a review. – J. Comp. Physiol. B 176: 1-15.
[58] Merzendorf, H., Zimoch L (2003): Chitin metabolism in insects: structure, function and regulation of chitin synthases and chitinases. – J. Exp. Biol. 206: 4393-4412.
[59] Mule, R. S., Naik, K. V., Rajemahadik, V. A. (2022): Larvicidal efficacy of some chitin synthesis inhibitors against *Spodoptera frugiperda* (Je Smith) (Lepidoptera: Noctuidae). – Journal of Experimental Zoology India 25(1).
[60] Nappi, J. A. (1974): Insect Haemocytes and the Problem of Host Recognition of Foreigners. – In: Cooper, E. L. (ed.) Contemporary Topics in Immunology. Vol. IV: Invertebrate Immunity. Plenum Press, New York.
[61] Naqqash, M. N., Gökçe, A., Bakhsh, A., Salim, M. (2016): Insecticide resistance and its molecular basis in urban insect pests. – Parasitol. Res. 115: 1363-1373.
[62] Nicholas, A. H., Thwaite, W. G., Spooner-Hart, R. N. (1999): Arthropod abundance in an disruption and supplementary insecticide treatments for codling moth, *Cydia pomonella* (L) (Lepidopter: Torticidae) Austral. – J Entomol. 38: 23-29.
[63] Noelia, F. M., Clara, S. A., Graciela, M., Inés, S. M. (2023): Toxicity assessment of two IGR insecticides on eggs and larvae of the ladybird *Eriopisconnexa*. – Pest Management Science 79(4): 1316-1323.
[64] Osman, E. E., Rarwash, I., El- Samadisi, M. M. (1984): Effect of the anti-moulting agent "Dimilin" on the blood picture and cuticle formation in *Spodopterea littoralis* (Boisd.) larval. – Bull. Entomol. Soc. Egypt (Econ. Ser.): 14: 3-46.
[65] Pfeiffer, M. (2007): Insect Growth Regulators. the Third-Generation Insecticides Need to be Understood and Used Wisely. – Pesticide Training Resources, Tucson, AZ.
[66] Piri Aliabadi, F., Sahragard, A., Ghadamyari, M. (2016): Lethal and sublethal effects of a chitin synthesis inhibitor, lufenuron, against *Glyphodespyloalis* Walker (Lepidoptera: Pyralidae). – Journal of Crop Protection 5(2): 203-214.
[67] Quarles, W. (2001): Sprays for adult mosquitoes—a failed technology? – Common Sense Pest Control Quart 17(2): 3-7.
[68] Rahimi, V., Zibaee, A., Mojahed, S., Maddahi, K., Zare, D. (2013): Effects of pyriproxyfen and hexaflumuron on cellular immunity of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Romanian Journal of Biology – Zoology 58(2): 151-162.
[69] Rahman, A. U., Khan, I., Usman, A., Khan, H. (2024): Evaluation of Insect Growth Regulators (IGRs) as biological pesticides for control of Aedes aegypti mosquitoes. – Journal of Vector Borne Diseases 61(1): 129-135.
[70] Resmitha, C., Kannan, V. M. (2017): The juvenile hormone mimic, pyriproxyfen, increases the level of the major haemolymph protein in the larvae of *Spodoptera mauritia* Boisd. Lepidoptera: Noctuidae. – International Journal of Entomology Research 2: 2455-4758.
[71] Richard, A. G. (1957): Cumulative Effects of Optimum and Suboptimum Temperatures on Insect Development. – In: Johnson, F. H. (ed.) The Influence of Temperature on Biological Systems. Amr. Physiol. Soc., Washington, DC, pp. 145-162.
[72] Riddiford, L. M. (1976): Hormonal control of insect epidermal cell commitment in vitro. – Nature 259(5539): 115-117.
[73] Rocha, F. A. D., Martinez, L. C., Lima, B. S. A., Farder-Gomes, C. F., Cossolin, J. F. S., Serrão, J. E. (2023): Toxicological and morphological effects of flubendiamide on the

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_82058224

midgut of the *Spodopteracosmioides* (Lepidoptera: Noctuidae) caterpillar. – Research Square. https://doi.org/10.21203/rs.3.rs-3313838/v1.

[74] Rolim, G. G., Barros, E. M., Barbosa, P. R., Arruda, L. S., Torres, J. B. (2019): Sublethal effects of insect growth regulators on boll weevil (Coleoptera: Curculionidae). – Journal of Economic Entomology 112(5): 2222-2228.

[75] Rose, R. I. (2001): Pesticides and public health: integrated methods of mosquito management. – Emerg. Infect. Dis. 7(1): 17-23.

[76] Sahar, E. E., Samah, M. H., Doaa, A. F. (2018): Toxicity of certain IGRs and conventional insecticides against cotton leafworm and their effects on the development and haemocyte counts. – Alexandria Journal of Agricultural Sciences 63(2): 93-103.

[77] Sahayaraj, K., Hassan, E. (2023): Biosafety Assessment of Synthetic Pesticides. – In: Worldwide Predatory Insects in Agroecosystems. Springer Nature, Singapore, pp. 735-783.

[78] Salama, H. S., Dimetry, N. Z., Salem, S. A. (1971): On the host preference and biology of the cotton leaf worm *Spodoptera littoralis* Bois. – Zeitschrift für Angewandte Entomologie 67(1-4): 261-266.

[79] Sannino, L. (2003): *Spodoptera littoralis* in Italy: possible reasons for its increasing spread and control measures. – Inf. Fitopatol. 53: 28-31.

[80] Sezer, B., Ozalp, P. (2015): Effects of pyriproxyfen on haemocyte count and morphology of *Galleria mellonella*. – Fresenius Environmental Bulletin 24(2a): 621-625.

[81] Shapiro, M. (1968): Changes in the haemocyte population of the wax moth, Galleria mellonella, during wound healing. – Journal of Insect Physiology 14(12): 1725-1733.

[82] Shaurub, E. S. H., Abd El-Meguid, A., Abd El-Aziz, N. M. (2014): Quantitative and ultrastructural changes in the haemocytes of *Spodopteralittoralis* (Boisd.) treated individually or in combination with *Spodopteralittoralis*multicapsidnucleopolyhedrovirus (SpliMNPV) and azadirachtin. – Micron 65: 62-68.

[83] Shaurub, E. S. H., Zohdy, N. Z., Abdel-Aal, A. E., Emara, S. A. (2018): Effect of chlorfluazuron and flufenoxuron on development and reproductive performance of the black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae). – Invertebrate Reproduction & Development 62(1): 27-34.

[84] Shaurub, E. S. H., El-Sheikh, T. A., Shukshuk, A. H. (2022): Insect growth regulators and chinaberry (Melia azedarach) fruit acetone extract disrupt intermediary metabolism and alter immunocyte profile in *Agrotisipsilon* larvae. – International Journal of Tropical Insect Science 42(3): 2203-2213.

[85] Singh, S., Kumar, K. (2011): Diofenolan: a novel insect growth regulator in common citrus butterfly, Papilio demoleus. – Phytoparasitica 39: 205-213.

[86] Stanley, D., Haas, E., Kim, Y. (2023): Beyond cellular immunity: on the biological significance of insect haemocytes. – Cells 12(4): 599.

[87] Tanani, M., Hamadah, K., Ghoneim, K., Basiouny, A., Waheeb, H. (2015): Toxicity and Bioefficacy of Cyromazine on Growth and Development of the Cotton Leafworm *Spodoptera Littoralis* (Lepidoptera: Nocuida). – International Journal of Research Studies in Zoology (IJRSZ) 1(2): 1-15.

[88] Tunaz, H., Uygun, N. (2004): Insect growth regulators for insect pest control. – J. Agric. Turk 28: 337e387.

[89] Tuncsoy, B., Özalp, P. (2021): Effects of pyriproxyfen and *Bacillus thuringiensis* Berliner 1915 on enzymatic antioxidant defense system and haemocytes of *Galleria mellonella* (L. 1758) (Lepidoptera: Pyralidae). – Turkish Journal of Entomology 45(2): 159-172.

[90] Viňuela, E., Budia, F., Gacas, J., Adan, A., Marco, M., Del Estal, P. (1993): Differential larval age susceptibility of Medfly *Ceratitis capitata* to cyromazine. – J. Appl. Entomol. 115: 355-361.

[91] Wigglesworth, V. B. (1959): Insect blood cells. – Annu. Rev. Ent. 4: 1-16.

[92] Xu, K., Lan, H., He, C., Wei, Y., Lu, Q., Cai, K., Lv, J. (2022): Toxicological effects of trace amounts of pyriproxyfen on the midgut of non-target insect silkworm. – Pesticide Biochemistry and Physiology 188: 105266.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_82058224

[93] Yasmeen, S., Amir, M. (2022): Ultrastructural, histochemical, and biochemical damage induced by chlorpyrifos in the midgut of *Chrysomya megacephala* larvae after acute exposure. – International Journal of Tropical Insect Science 42(6): 3793-3806.
[94] Yusoff, N., Abd Ghani, I., Othman, N. W., Aizat, W. M., Hassan, M. (2021): Toxicity and sublethal effect of farnesyl acetate on diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). – Insects 12(2): 109.
[95] Zeng, B., Chen, F. R., Liu, Y. T., Guo, D., Zhang, Y. J., Feng, Z. R., Gao, C. F. (2023): A chitin synthase mutation confers widespread resistance to buprofezin, a chitin synthesis inhibitor, in the brown planthopper, *Nilaparvata lugens*. – Journal of Pest Science 96(2): 819-832.
[96] Zhang, P., Zhao, Y. H., Wang, Q. H., Mu, W., Liu, F. (2017): Lethal and sublethal effects of the chitin synthesis inhibitor chlorfluazuron on *Bradysia odoriphaga* Yang and Zhang (Diptera: Sciaridae). – Pesticide Biochemistry and Physiology 136: 80-88.
[97] Zhu, Q., He, Y., Yao, J., Liu, Y., Tao, L., Huang, Q. (2012): Effects of sublethal concentrations of the chitin synthesis inhibitor, hexaflumuron, on the development and haemolymph physiology of the cutworm, *Spodoptera litura*. – Journal of Insect Science 12(1): 27.
[98] Zibaee, A., Malagoli, D. (2020): The potential immune alterations in insect pests and pollinators after insecticide exposure in agroecosystem. – Invertebrate Survival Journal 99-107.
[99] Zibaee, A., Bandani, A. R., Malagoli, D. (2012): Methoxyfenozide and pyriproxyfen alter the cellular immune reactions of *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae) against *Beauveria bassiana*. – Pestic. Biochem. Physiol. 102: 30-37.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_82058224