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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2025.691.699

Research Article
Biochemical Disruption and Lethal Effects of Lufenuron and Hexaflumuron on the Cotton Leafworm (*Spodoptera littoralis* Boisd.)

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Abstract

Background and Objective: The Egyptian cotton leafworm, scientifically named *Spodoptera littoralis*, is a major economically important pest that causes extensive economic losses across Africa, particularly in Egypt. The current study aimed to estimate the effects of lufenuron and hexaflumuron on the survival potential, development, metamorphosis and main metabolites of this economically important insect pest. **Materials and Methods:** Penultimate instar larvae were treated with four concentrations (250, 500, 1000 and 2000 ppm) of lufenuron and hexaflumuron using a leaf-dipping technique on fresh castor bean leaves. Mortality, development time, pupation and metabolite contents were monitored across early-, mid- and late-aged larvae. Major metabolites (proteins, carbohydrates and lipids) were analyzed using standard biochemical assays. Statistical analysis was performed to determine concentration-dependent effects and significance was evaluated at the appropriate confidence level. **Results:** The obtained results revealed that treatment with the highest concentration resulted in complete larval mortality for both compounds. Other concentrations recorded a concentration-dependent increase in larval mortality, with severe mortality observed during the early days after treatment. Both lufenuron and hexaflumuron induced pronounced negative effects on larval growth and developmental rates, regardless of the concentration level or the targeted larval age. Pupation percentages were also affected; higher concentrations induced complete pupation failure. A disturbance in the main body metabolites of the penultimate instar larvae was also recorded across early-, mid- and late-aged larvae. The total main metabolite content was drastically disrupted, regardless of the tested compound or the larval period. **Conclusion:** Lufenuron and hexaflumuron have a potent effect in curbing the cotton leafworm, *S. littoralis* and a strong effect on disrupting its vital and physiological processes.

Key words: *Spodoptera littoralis*, lufenuron, hexaflumuron, larval mortality, metabolites

Citation: Tanani, M.A., A.I. Hasaballah, U.M. Abu El-Ghiet, A.M. Mashlawi and M.A. El-Sakhawy, 2025. Biochemical disruption and lethal effects of lufenuron and hexaflumuron on the cotton leafworm (*Spodoptera littoralis* Boisd.). Pak. J. Biol. Sci., 28: 691-699.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Insects, weeds and diseases are significant agricultural and economic pests that seriously jeopardize human health, economic stability and global food security. Annual yield losses caused by these pests can reach up to 40%[1]. The Egyptian cotton leafworm, scientifically named *Spodoptera littoralis*, is a major defoliating pest of numerous plants throughout the Middle East and much of Africa, regions characterized by semi-arid and subtropical habitats[2,3]. This polyphagous species has a broad host range, attacking over eighty plant species across forty plant families[4].

Substantial effort has been devoted to controlling *S. littoralis* using various classes of synthetic chemical insecticides. Broad-spectrum insecticides have historically been deployed for population management, leading to several issues, including environmental contamination, disruption of natural enemy complexes and the emergence of widespread resistance[5,6]. Recently, compounds with novel modes of action have been developed as a safer generation of insecticides to control *S. littoralis*; these are known as Insect Growth Regulators (IGRs).

The IGR compounds are recognized for their ability to disrupt insect development, making them effective candidates for Integrated Pest Management (IPM) programs[7]. They act by interfering with the endocrine system, adversely affecting development, reproduction, or metamorphosis. This group includes Chitin Synthesis Inhibitors (CSIs) such as lufenuron and hexaflumuron[8]. These benzoylurea-based IGRs primarily work by inhibiting chitin biosynthesis, thereby disrupting the molting process and preventing the formation of a new cuticle[9].

Hexaflumuron and lufenuron are often preferred over traditional neurotoxic insecticides (e.g., organophosphates and pyrethroids) due to their lower mammalian toxicity, favorable environmental safety profiles and targeted action on insect-specific pathways like chitin production. Because vertebrates do not synthesize chitin, CSIs are considered selective and safer for mammals[10,11]. Their effectiveness at sublethal concentrations minimizes impacts on non-target organisms and helps slow resistance development, making them essential for IPM strategies in agriculture[12-14].

Despite belonging to the same class and sharing a similar mode of action, the efficacy of lufenuron and hexaflumuron can vary significantly between and within insect species. Evaluating the toxicity and underlying mechanisms of these CSIs is crucial for developing them as alternatives to conventional insecticides, especially given that *S. littoralis* has developed robust resistance to organophosphates, carbamates and pyrethroids due to intensive overuse[15-18].

Furthermore, although both are CSIs, the sublethal effects of hexaflumuron and lufenuron on biological parameters (e.g., larval development, molting disruptions, mortality rates and reproductive fitness) and biochemical pathways (e.g., chitin synthesis inhibition and alterations in detoxification enzyme activity) remain poorly understood and may differ substantially between species[19,20].

By integrating laboratory bioassays and biochemical analyses, this work aims to provide a comprehensive framework for deploying these IGRs in rotation or combination to delay resistance, enhance crop protection and ensure ecological safety in agricultural ecosystems. Specifically, this work aims to assess the toxic activity of the two tested chitin-inhibiting compounds, lufenuron and hexaflumuron, against the cotton leaf worm larvae, as well as to study some biological and physiological changes resulting from the treatment of such compounds on the ultimate instar larvae of *S. littoralis*.

MATERIALS AND METHODS

Study area: The study was conducted during the spring of 2023 in the Insect Physiology Research Laboratory at the Faculty of Science. Two complete generations were studied under controlled laboratory conditions.

Experimental insect: A sample of the Egyptian cotton leaf worm, *Spodoptera littoralis* pupae, was provided by the Cotton Pesticides Evaluation Department, Plant Protection Research Institute, Agricultural Research Center, El-Sabhia, Alexandria, Egypt. Colony was maintained under laboratory-controlled conditions (27±2°C, 65±5% Relative humidity and 14/10 hrs L/D photoperiod). Rearing procedure was carried out according to the method of Eldefrawi *et al.*[21]. Larvae were fed on fresh castor bean leaves, *Ricinus communis,* daily. Adults who emerged were supplied with a cotton piece soaked in a 10% honey solution. Eggs of moths were laid on *Nerium oleander* branches, then patches of eggs were collected and transferred for the alternate generation. Newly molted 5th instar larvae were segregated from the colony, placed in clean glass petri dishes and starved for 24 hrs, according to the modified method of Hatem *et al.*[22].

Chemicals: Chemicals used here were provided by the Laboratory of Insecticides, Plant Protection Research Institute, Dokki, Giza. Chemical formula of lufenuron: N- [2,5-dichlor o-4-(1,1,2,3, 3-hexafluoro-propoxyl) phenyl amino 2,6 diflubenzamide (CA)] and Chemical formula of hexaflumuron: N-[3,5-dichlo-ro-4-(1,1,2,2 tetrafluoroethoxy) phenyl]3-(2,6 difluorobenzoyl) urea.

The 5th instar larvae of *S. littoralis* were treated with chitin biosynthesis inhibitors: Lufenuron and hexaflumuron. Four concentrations from both compounds were prepared, namely: 250, 500, 1000 and 2000 ppm. Fresh, clean castor bean leaves were treated with each concentration of the tested compound using the dipping technique on discs. Newly molted larvae from each treatment were grouped and fed on treated discs for 24 hrs. Untreated discs of castor bean leaves were used to feed control larvae.

Toxicity assay: Microsoft Office Excel 2007 was used to calculate the (LC_{25}, LC_{50} and LC_{90}) values according to Finney[23]. The toxicity of both treated and untreated (larvae, pupae and adults) insects was recorded daily and corrected according to Abbott's formula[24] as follows:

$$\text{Corrected mortality (\%)} = \frac{\text{Test mortality (\%)} - \text{Control mortaliy (\%)}}{100 - \text{Control mortality (\%)}} \times 100$$

Growth, development and metamorphosis: Treated and untreated larvae were weighed daily (individually) and growth was calculated using the following formula: Growth = initial weight-final weight. Developmental duration was evaluated using Dempster's formula[25]. Developmental rate was evaluated according to Richard's equation[26]. Pupation rate was calculated as successfully developed pupae (%). Deranged metamorphosis was observed and recorded in the larval-pupal intermediates. Pupal deformation was expressed in percentages.

Sample preparation: Tissue homogenate from the 4th instar larvae of the tested insect treated with the tested compounds was homogenized using saline (0.9% 1 g/mL) and kept in sterilized Eppendorf tubes. Then, samples were centrifuged at 5000. r.p.m for about ten minutes and the supernatant was then kept in the freezer -20°C till further investigations. Three replicates were used for each treatment.

Assessment of metabolic aspects: The total carbohydrate contents were determined according to the method of Singh and Sinha[27]. The total protein contents were calculated according to the method of Bradford[28]. Total lipid contents were assessed according to the Knight *et al.*[29] method.

Statistical analysis: All data related to larval mortality, developmental duration, pupation percentage and metabolite contents were subjected to statistical analysis. One-way Analysis of Variance (ANOVA) was used to determine differences among treatments, followed by Tukey's *post-hoc* test to compare means at different concentrations. Data were expressed as Mean±Standard error (SE). Statistical significance was considered at $p<0.05$, representing a 95% confidence level. All analyses were performed using standard statistical software.

RESULTS

Lethality effects of lufenuron and hexaflumuron against *S. littoralis:* Five different concentrations from both tested compounds, ranging from 250 ppm to 2000 ppm, were applied to the 5th instar larvae of *S. littoralis* and their data are reported in Table 1.

Depending on the obtained data, larvae treated with (1000 and 2000 ppm) concentrations completely died in both tested compounds. Larval mortalities exhibited a dose-dependent pattern at lower concentrations, where larval mortality was observed (85 and 55% for 500 and 250 ppm of lufenuron, respectively) and (82.5 and 57.5% for 500 and 250 ppm of hexaflumuron, respectively). Additionally, the obtained results showed that severe mortality was detected through the early days of treatment, while latent effects were shown in the last days of the larval instar. Pupal and adult mortalities in both applied compounds were also high. According to the obtained data, lufenuron showed a more effective pattern of toxicity than the hexaflumuron compound.

The data reported in Table 2 show the sublethal concentrations for the compounds tested. In general, lufenuron showed higher toxicity than hexaflumuron when applied against the *S. littoralis* larvae.

Effect of tested compounds on growth and development: In light of the results obtained, the potent activities of lufenuron on various biological aspects are revealed in Table 3. Lufenuron treatment significantly decreased the mean larval weight, where higher concentrations (2000 and 1000 ppm) showed a high decrease in the mean larval weight with (0.97±0.595 and 1.78±0.30 g), respectively, against the control congeners (2.75±0.17 g).

It is also noticed that the weight gained has significantly decreased when treated with high concentrations of lufenuron. The highest decrease (0.344±0.127 g) was recorded at the highest concentration applied. Also, the growth rate (%) showed a reverse proportion, i.e., it decreased with increasing concentration levels, while the growth inhibition (%) increased with increasing concentration levels. On the other hand, the development process of the tested insect was affected. The duration was prolonged when the

Table 1: Toxic effects of lufenuron and hexaflumuron after treatment of newly moulted 5th instar larvae of *S. littoralis*

		Larval mortality (%)											
Tested CSIs	Conc. (ppm)	1 day	2 day	3 day	4 day	5 day	6 day	7 day	Total mortalities of larvae	Pupal mortality (%)	Adult mortality (%)	Total mortality (%)	Corrected mortality (%)
Lufenuron	2000	82.5^{d}	17.5	0	0	0	0	0	100^{d}	-	-	100	100
	1000	70^{c}	20	0	10	0	0	0	100^{d}	-	-	100	100
	500	65^{c}	0	0	20	0	0	0	85^{c}	57.5	35	97.5	95
	250	45^{b}	0	0	0	0	0	10	55^{b}	27.5	35	75	67.5
Hexaflumuron	2000	90^{d}	6	4	0	0	0	0	100^{d}	-	-	100	87.5
	1000	70^{c}	4	0	6	0	20	0	100^{d}	-	-	100	100
	500	62.5^{c}	10	6	0	0	0	4	82.5^{c}	30	57.5	95	92.5
	250	37.5^{b}	0	0	0	0	20	0	57.5^{b}	17.5	15	62.5	52.5
Control	0	0^{a}	0	0	20	0	0	0	20^{a}	0	0	20	0.00

Conc: Concentration, SE: Standard error, mean values followed by the same letter are not significantly different (p>0.05) and Develop. Rate: Developmental rate

Table 2: Lethal concentrations (LC_{25}, LC_{50}, and LC_{90}) of lufenuron and hexaflumuron against the larvae of *S. littoralis*

Tested CSIs	LC_{25} (Confidence limits mg/L)	LC_{50} (Confidence limits mg/L)	LC_{90} (Confidence limits mg/L)	Slope±standard error	X^2
Lufenuron	45.63 (14.79-79.18)	106.19 (54.07-153.29)	528.534 (386.12-883.69)	1.84±0.347	3.99
Hexaflumuron	68.30 (30.58-106.79)	160.33 (101.10-216.24)	811.13 (510.33-1385.5)	1.82±0.297	3.595

Conc: Concentration, SE: Standard error, mean values followed by the same letter are not significantly different (p>0.05) and Develop. Rate: Developmental rate

Table 3: Lufenuron-induced effects on growth and development of the cotton leaf worm, *S. littoralis*

Tested CSIs	Conc.	Mean weight (gram±SE)	Weight gain (gram±SE)	Growth index (%)	Growth rate (%)	Growth inhibition (%)	Duration (days± SE)	Pupation (%)	Develop. rate
Lufenuron	2000	0.97±0.595^{c}	0.344±0.127^{c}	-	3.79^{e}	64.72^{e}	8.80±0.47^{b}	0	8.80±3.2^{b}
	1000	1.78±0.30^{b}	0.381±0.011^{b}	-	8.27^{d}	35.27^{d}	8.5±0.29^{b}	0	12.19±0.37^{a}
	500	2.00±0.28^{a}	0.413±0.007^{a}	5.42	10.46^{c}	27.27^{c}	7.89±0.57^{a}	14	12.67±0.47^{a}
	250	2.14±0.22^{a}	0.473±0.011^{a}	10.56	12.90^{b}	22.18^{b}	7.77±0.44^{a}	46	12.87±0.53^{a}
Control	-	2.75±0.17^{a}	0.574±0.012^{a}	11.12	21.95^{a}	0.0^{a}	7.19±0.51^{a}	80.0	13.90±0.55^{a}

Conc: Concentration, SE: Standard error, Mean values followed by the same letter are not significantly different (p>0.05) and Develop. Rate: Developmental rate

Table 4: Hexaflumuron-induced effects on growth and development of the cotton leaf worm, *S. littoralis*

Tested CSIs	Conc.	Mean weight (gram±SE)	Weight gain (gram±SE)	Growth index (%)	Growth rate (%)	Growth inhibition (%)	Duration (days±SE)	Pupation (%)	Develop. rate
Hexaflumuron	2000	1.33±0.71^{c}	0.172±0.57^{e}	-	2.66^{e}	51.63^{e}	8.60±1.25^{b}	0	11.62^{c}
	1000	1.90±0.76^{b}	0.224±0.39^{c}	-	4.89^{d}	30.90^{d}	8.70±1.65^{b}	0	11.49^{c}
	500	2.18±0.65^{a}	0.244±0.50^{c}	8.97	6.81^{c}	20.72^{c}	7.89±0.18^{a}	20.0	12.82^{b}
	250	2.12±9.80^{a}	0.354±2.40^{b}	11.73	4.72^{b}	22.18^{b}	7.50±0.42^{a}	50.0	13.33^{a}
Control	-	2.75±0.95^{a}	0.574±1.12^{a}	11.12	21.95^{a}	0.0^{a}	7.19±1.28^{a}	80.0	13.90^{a}

Conc: Concentration, SE: Standard error, mean values followed by the same letter are not significantly different (p>0.05) and Develop. Rate: Developmental rate

Table 5: Total protein (content) of the ultimate instar larvae of *Spodoptera littoralis* treated with lufenuron and hexaflumuron

		Total protein					
Tested CSIs	Conc. (ppm)	Early-aged (mg/g±SE)	Change (%)	Mid-aged (mg/g±SE)	Change (%)	Late-aged (mg/g±SE)	Change (%)
Lufenuron	500	56.40±1.86^{c}	- 21.37	44.50±0.88^{c}	- 31.69	39.63±0.79^{c}	-36.79
	250	66.41±1.08^{b}	-7.84	58.88±0.92^{b}	- 9.62	46.82±0.58^{b}	-25.32
	125	68.63±1.53^{a}	-4.76	67.53±1.8^{a}	3.65	55.6±1.10^{a}	-11.32
Hexaflumuron	500	48.66±1.08^{c}	-32.47	58.88±0.92^{c}	- 9.62	38.12±0.96^{c}	-39.2
	250	57.96±5.4^{b}	-19.57	55. 65±1.17^{b}	-14.58	48.76±0.59^{b}	-22.23
	125	66.63±4.04^{a}	-7.54	58.8±2.69^{a}	-9.74	57.6±1.70^{a}	-8.13
Control	-	72.065±5.69^{a}	-	65.15±1.06^{a}	-	62.70±0.92^{a}	-

Conc: Concentration, SE: Standard error, mean values followed by the same letter are not significantly different (p>0.05) and Develop. rate: Developmental rate

highest concentrations of 2000 and 1000 ppm were applied. It prolonged to (8.80±0.47 and 8.5±0.29 days), respectively, versus control congeners (7.19±0.51 days). However, the pupation (%) and developmental rate decreased sharply, particularly with higher concentrations.

Obtained data in Table 4 revealed that larval growth was restrained as a response to the treatment with different compounds treatment and this effect was concentration dependent. At the lowest concentration applied, larvae gained 0.354±2.4 g versus 0.574±1.12 g for the control;

Table 6: Total carbohydrate content of the ultimate instar larvae of *S. littoralis* treated with lufenuron and hexaflumuron

		Total carbohydrate					
Tested CSIs	Conc. (ppm)	Early-aged (mg/g±SE)	Change (%)	Mid-aged (mg/g±SE)	Change (%)	Late-aged (mg/g±SE)	Change (%)
Lufenuron	500	12.11±0.53^{b}	46.26	9.78±0.52^{b}	111.69	6.82±0.062^{b}	91.57
	250	11.89±0.10^{a}	34.59	8.99±0.12^{a}	94.59	5.68±0.052^{a}	59.55
	125	6.3±0.31^{a}	-23.91	8.78±0.18^{a}	90.04	4.76±0.053^{a}	33.71
Hexaflumuron	500	15.77±0.33^{a}	90.45	12.65±0.06^{b}	173.81	8.76±0.14^{b}	146.07
	250	8.96±0.69^{a}	8.23	10.77±0.22^{a}	133.11	8.12±0.09^{b}	128.09
	125	9.36±0.33^{a}	16.31	7.93±0.30^{a}	71.65	6.5±0.30^{a}	82.58
Control	-	8.28±0.55^{a}	-	4.62±0.06^{a}	-	3.56±0.07^{a}	-

Conc: Concentration, SE: Standard error, mean values followed by the same letter are not significantly different (p>0.05) and Develop. rate: Developmental rate

Table 7: Total lipid content of the ultimate instar larvae of *S. littoralis* treated with lufenuron and hexaflumuron

		Total lipid					
Tested CSIs	Conc. (ppm)	Early-aged (mg/g±SE)	Change (%)	Mid-aged (mg/g±SE)	Change (%)	Late-aged (mg/g±SE)	Change (%)
Lufenuron	500	22.14±0.54^{b}	-4.93	16.80±0.33^{d}	-31.09	26.63±1.03^{a}	-3.89
	250	23.85±1.64^{a}	2.40	17.67±0.43^{c}	-27.52	26.82± 0.84^{a}	-3.28
	125	23.90±2.14^{a}	2.62	-	-	-	-
Hexaflumuron	500	18.90±1.3^{c}	-18.85	20.75±0.45^{b}	-14.89	26.78± 0.52^{a}	-3.35
	250	22.26±0.96^{b}	-4.42	24.64±0.21^{a}	1.11	27.76± 0.98^{a}	0.18
	125	23.16±0.48^{a}	-0.56	24. 75±0.56^{a}	1.52	28.52± 0.67^{a}	2.92
Control	-	23.29±0.71^{a}	-	24.38±0.21^{a}	-	27.71±0.41^{a}	-

Conc: Concentration, SE: Standard error, mean values followed by the same letter are not significantly different (p>0.05) and Develop. rate: Developmental rate

the weight gain decreased to 0.172±0.57 g when the highest concentration was tested. The most potent growth inhibition % (51.63) was obtained by a (2000 ppm) concentration of hexaflumuron. Growth rate (%) was decreased from 2.66 at 2000 ppm (vs 21.95 of control congeners). Hexaflumuron inhibits the larval duration through prolonged time intervals, such as (8.60±1.25, 8.70±1.65 days at 2000, 1000 ppm, respectively) versus 7.19±1.28 days of control congeners. However, the development rate increased with increasing concentration level. It decreased from 13.33 at 250 ppm treatment to 11.62 at the highest concentration, 2000 ppm, versus 13.90 in control congeners.

Biochemical aspects

Effect of tested CSIs on the total protein content: The ultimate larval instar of the cotton leaf worms *S. littoralis* was treated with (500, 250 and 125 ppm) concentrations of lufenuron and hexaflumuron compounds. The total protein content data of the tissue homogenate evaluated at different time intervals (early-day, mid-day, late-day) are shown in Table 5. A remarkable reduction in the total protein (total content) of the ultimate larval instar was observed at different time intervals when 250 and 500 ppm concentrations were applied. Hexaflumuron at 500 ppm significantly reduced the total protein content to 48.66±1.08 with a reduction % of -32.47 in the early-day stage. While for the midday stage, lufenuron reduced the total protein content by about -31.69% when a concentration of 500 ppm was applied. For the late-day stage, a concentration of 500 ppm significantly reduced the total protein content with about (-36.79 and -39.2%) for lufenuron and hexaflumuron, respectively.

Effect of tested CSIs on the total carbohydrate content: The data for total carbohydrate content is given in Table 6. Tested compounds significantly increased carbohydrate content in early-, mid- and late-aged larvae, particularly when high concentrations were applied. The most pronounced increase in percentages was recorded by the 500-ppm concentration of both compounds in each time interval tested. It recorded 46.26, 111.69 and 91.57% for early-, mid- and late-aged larvae treated with lufenuron, respectively, while treatment with the same concentration of hexaflumuron revealed increased percentages of 90.45, 173.81 and 146.07 for early-, mid- and late-aged larvae, respectively.

Effect of tested CSIs on the total lipid content: The data on the effects of tested CSIs lufenuron and hexaflumuron post-treatment of the ultimate larval instar are summarized in Table 7. There were significant differences in total lipid content compared with the untreated congeners. At the early-aged stage, hexaflumuron recorded a significant increase when a concentration of 500 ppm was applied. Contrary, lufenuron induced a significant rise in the total lipid contents when concentrations of 250 and 500 ppm were applied. At the late-aged stage, no remarkable effect was induced.

DISCUSSION

Insect Growth Regulators (IGRs) are considered successful insecticidal agents due to their long-lasting effects in the habitat and low toxicity. Chitin Biosynthesis Inhibitors (CSIs) are considered a class of IGRs, so they inhibit molting or produce insufficient cuticles. These substances are efficient developmental suppressors for the whole life cycle of targeted models[30]. Hexaflumuron and lufenuron possess the same mode of action regardless of their different effects on the same species. The data obtained show that larval mortalities increased significantly with different concentrations tested and lufenuron and hexaflumuron could provide potent larval toxicity against the cotton leaf worm *S. littoralis*. Several CSIs induced a wide range of larval and pupal mortalities, for example, diflubenzuron[31], triflumuron[32], flufenoxuron[33], spinosad and buprofezin[3], chlorfluazuron[34] and lufenuron[35].

The IGRs differ from synthetic insecticides in their mode of action, where they interfere with chitin deposition either on the exoskeleton or in other internal sites[36]. Other factors like bleeding, suffocation, or desiccation due to imperfect failure[37]. Herein, induced larval death may be attributable to the disability of molting larvae to take in enough volume of air to split the cuticle. Additionally, the definite cause of death by lufenuron and hexflumuron may be due to ceasing feeding or starvation due to physiological malformation, leading to death. The death of larvae and pupae is likely caused by the direct prevention of chitin production in the integument or by the breaking of the newly formed cuticle[38]. In addition, the death of adults from high concentrations of lufenuron and hexaflumuron can be explained by the retention and movement of these compounds throughout the insect's body after being quickly transported from the gut to various tissues.

Previous studies revealed that many CSIs induced growth and development inhibitory effects when tested against *S. littoralis*[39]. Buprofezin inhibited the growth of *S. littoralis*[3]. Also, flufenoxuron, lufenuron and novaluron prohibited the growth of *S. littoralis*[33,35,40]. The current study showed different levels of growth inhibition and delayed development of the targeted insect by lufenuron and hexaflumuron-treated penultimate larval instar. Tested compounds also showed a retarded growth effect and this effect was concentration dependent. Also, the duration of larval development was significantly prolonged due to treatment with different compounds applied, revealing slower developmental rates and this effect was not concentration- or time-dependent.

The obtained data agrees with those reported previously about proscribed growth/development effects by various CSIs, for example, *S. litura* by chlorfluazuron[34] and *Agrotis ipsilon* by flufenoxuron[41]. Contrary, other IGRs were not able to affect the growth of insect pests, such as *S. exempta*, *S. exigua* and *Leptinotarsa decemlineata*[37]. However, the inhibited growth reported here may be due to the block of morphogenic peptides release. Additionally, the retarded development of *S. littoralis* may be explicated by a delaying effect of the tested compounds on ecdysis and transformation[42]. Specifically, this chitin synthesis inhibitor acts on the final step of the biosynthetic pathway, preventing the precursor from being converted into chitin[37].

As reported previously, Cohen[30] found that proteins possess multiple functions; they can catalyze metabolic reactions, replicate DNA and also control enzymes and hormones. Adult structure mainly depends on the protein metabolism during the larval/pupal transformation into adults of insects[43]. The results obtained revealed a significant and noticeable decline in the total protein content at different time intervals and this effect was concentration dependent. In the same context, Khedr *et al.*[44] found that the major role during the synthesis of the microsomal detoxifying enzymes is played by proteins. Stress induced by toxins introduced to insects can break down the protein into amino acids, which leads to inhibition of the total proteins[45]. Therefore, protein reduction in the tissues of *S. littoralis* might play a role in compensatory mechanisms under the present toxins' stresses. Djeghader *et al.*[40] stated that "Either CSIs affect the neurosecretory cells that govern endocrine organs, or they affect the hormonal regulation of protein synthesis, degradation and inhibition".

Carbohydrates play a vital role in almost all tissues' structure and function during metamorphosis in insects. Carbohydrates are prerequisite metabolites for the normal functioning of reproductive organs and also during embryonic development[46]. It plays a key role in the physiology of insects exposed to toxins[47]. Herein, lufenuron and hexaflumuron significantly increased carbohydrate content in early-, mid- and late-aged larvae. Both compounds caused an increase in carbohydrate content of larval whole-body weight at any of the measured ages. These effects may be due to disturbance of the hormones induced by these CSIs and this effect could be interpreted by their ability to modify the synthesis of certain metabolites[48]. Other studies state that carbohydrate reserves are associated with the different developmental stages of the insect; they rise during the metamorphosis process and are reduced during the growth periods[49].

Lipids are the main source of energy in insects[50]. In the current study, the tested compounds showed a drastic decrease in the total lipid contents in mid-aged larvae. This result agrees with previous results of reduced lipid content of different insect species post-treatment with various toxic compounds. Bouaziz *et al*.[49] found similar results of reduced lipid contents in different insect species post-larval treatment with some IGRs. To interpret the effect of tested compounds on total lipid content in immature stages of *S. littoralis*, we have to raise a point about the synthesis of lipids in insects, which disruptively affects physiology and other vital functions related to growth and reproduction. However, reduction of the total lipid content might be due to their interference with not only lipid synthesis but also with lipid mobilization.

CONCLUSION

The main implication of the current study is that the tested CSIs, namely lufenuron and hexaflumuron, could be used as control agents to reduce population densities of *S. littoralis*. They act by causing an imbalance and disturbance in key metabolic factors such as proteins, carbohydrates and lipids, which leads to the disruption of the insect's physiological and biological processes. Therefore, these tested CSI compounds are potent control agents against *S. littoralis* and could be included in integrated pest management programs.

SIGNIFICANCE STATEMENT

The cotton leafworm, *Spodoptera littoralis*, is a devastating agricultural pest causing major economic losses. This research demonstrates that two Insect Growth Regulators, lufenuron and hexaflumuron, are highly effective biopesticides against this pest. These compounds not only cause high, concentration-dependent mortality in larvae but also severely disrupt their development, preventing pupation and profoundly altering essential body metabolites. These findings provide a strong scientific basis for incorporating these compounds into integrated pest management strategies, offering a potent and targeted biochemical approach to control this economically significant insect while potentially reducing reliance on broader-spectrum conventional insecticides.

ACKNOWLEDGMENT

This publication was supported by the Deanship of Scientific Research at Prince Sattam bin Abdulaziz University, Al-Kharj 11942, Saudi Arabia.

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