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Effective use of two chitin synthesis inhibitors against cotton leaf worm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) based on toxicity, biological and biochemical studies

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Abstract
The present study aimed to the evaluate the effect of two Chitin Synthesis Inhibitors, Lufenuron (Kafroseil® 5%) and Chlorfluazuron (Caprice® 5%) on 2nd and 4th instar larvae of the cotton leafworm, *Spodoptera littoralis* (Boisd.). Results showed that the 2nd instar larvae were more susceptible than 4th instar larvae, as denoted by the determined low LC_{50} obtained for 2nd instar larvae. Results also showed that larval and pupal duration, and pupation and the adult emergence percentage, of survived larvae were significantly decreased compared to control. Moreover, adult longevity for male and female moths were also affected by treatment. In addition, adult fecundity and fertility of survived-treatment larvae was also reduced. Results also revealed that treatment with tested compounds has dramatically affected certain enzyme activities.

Keywords: chitin synthesis inhibitors, cotton leafworm, enzyme activity, biological aspects, biochemical aspects

1. Introduction
Cotton plants are liable to be attacked by a great number of insect species, several of which cause serious damage to this plant. The cotton leafworm *S. littoralis* is one of the most destructive pests in the tropical and subtropical areas of the world [1]. It attacks at least 175 species of plants of varying economic importance [2]. Over the last few decades, the intensive use of broad-spectrum insecticides against the Egyptian cotton leafworm, *S. littoralis* has led to the development of resistance to many registered pesticides making their control even more difficult [3,4]. Insect growth regulators (IGR's) is considered as the possible alternative way of conventional synthetic insecticides for controlling this pest [5]**.** They have novel mode of action which disrupt the physiology and development of the target pest. Such compounds tend to be selective and generally less toxic to non-target organisms than conventional insecticides [6]. Many IGR's have shown potentiality against lepidopterous insect including *S. littoralis* (Boisd) [7, 8, 9]. The present study was conducted to evaluate the effect of "Lufenuron and Chlorfluazuron" an insect growth regulator on the biotic potential of *S. littoralis* (Boisd).

2. Materials and methods
2.1 Rearing technique of the Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisd)
A laboratory susceptible strain of the cotton leaf worm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) which had been reared in the laboratory for more than ten generations (without any exposure to chemicals) and was obtained as egg masses from the Research Division of the cotton leaf worm, Plant Protection Research Institute. These eggs were kept in plastic cups covered with gauze under laboratory condition of 27±2°C and 65±5% R.H. until hatching. The larvae were reared on fresh leaves of castor bean *Ricinus commanis* till the 2nd and 4th larval instar described by [10].

2.2 Insect growth regulator (chitin synthesis inhibitors):
a. Common name: Lufenuron. Trade name: Kafroseil® 5%. This chemical was obtained from Kafr El Zayat Pesticides and Chemicals Co. S.A.E.
b. Common name: Chlorfluazuron. Trade name: Caprice® 5%. This chemical was obtained from ElHelb Pesticides and Chemicals Co. S.A.E.

2.3 Susceptibility test
Insecticidal activity of Lufenuron, and Chlorfluazuron. Was assessed against 2nd and 4th instar larvae of *Spodoptera littoralis*. A series of aqueous concentration of Lufenuron were prepared, the concentrations were 0.2, 0.1, 0.05, 0.025, 0.0125, and 0.00625 ppm. A series of aqueous concentration of Chlorfluazuron were prepared, the concentrations were 0.2, 0.1, 0.05, 0.025, 0.0125, and 0.00625 ppm. The leaf dipping technique was used, where castor oil leaves, *Ricinus communis*, were dipped in each concentration. Leaves were then left to dry at room temperature and were then offered to the newly molted 2nd and 4th larval instars, the larvae were starved for 2-4 hours before offered treated leaves [11]. Treated larvae were allowed to feed on treated leaves for 24 hr., then provided with fresh, clean and untreated castor oil leaves for the duration of the following larval stage and until pupation. Five replicates, each containing 20 larvae, were used for each concentration. As a control castor oil leaves were dipped in distilled water and offered to larvae of the same larval instars. Cumulative larval mortalities were determined and corrected by Abbott's formula [12]. The data were subjected to probit analysis [13] for determining the LC_{50} value for the insect growth regulator "Lufenuron and Chlorfluazuron."

2.4 Biological studies
From the maintained insect culture, 2nd and 4th instar larvae were collected, these larvae were offered castor oil leaves treated with the determined the LC_{50} value of Lufenuron and

Chlorfluazuron. Larvae were examined daily and the following parameters studied: larval and pupal duration of each instar, percentage of pupation and weight of pupae. Pupae were sexed and then placed in pairs in the glass globes. Subsequently, percentage of adult emergence, longevity of moths and the fecundity and fertility of eggs/female, were determined.

2.5 Biochemical studies

The following biochemical studies were carried out for the 4th instar larvae of *S. littoralis* following their treated with the LC_{50} of each of the tested chitin synthesis inhibitors.

2.5.1 Preparation of insects for analysis

The insects were prepared according to [14]. They were homogenized in distilled water (50 mg /1 ml). Homogenates were centrifuged at 8000 r.p.m. for 15 min at 2 ºC in a refrigerated centrifuge. The deposits were discarded and the supernatants, was stored at least one week without appreciable loss of activity when stored at 5°C**.**

a. Total carbohydrates were determined according to [15].
b. Total proteins were determined according to [16].
c. Total lipids were determined according to [17].
d. Chitinase activity was determined according to [18].
e. α- and β-esterases were determined according to [19].

3. Results & Discussion

3.1 Bioassay

The LC_{50} of Lufenuron to 2nd and 4th instars of *S. littoralis* was found to be 0.0116 ppm and 0.0188 ppm, respectively, Meanwhile the LC_{50} of Chlorfluazuron was found to be ppm. 0.0110 And 0.0138 ppm. To 2nd and 4th instars, respectively (Table. 1 and Fig. 1&2). Our results are confirmed with those obtained by Gad Allah *et al.* [20], who worked on *Hiliothis armigra* larvae treated with IGR Pyriproxyfen. Haga *et al.* [21] showed that Chlorfluazuron is very toxic to insects because it metabolizes slowly inside the insect body. The toxicity of Flufenoxuron against *S. littoralis* larvae was slightly similar to that of the Chlorfluazuron against *H. armigra* [22] and *A. ipsilon* [23]. Also, Abdel-Al *et al.* [24] reported that chitin synthesis inhibitors caused high mortalities to 2nd and 4th instars larval of *S. littoralis*.

Table 1: Toxicity of the tested Insect growth regulators Lufenuron and Chlorfluazuron against the 2nd and 4th instar larvae of cotton leafworm, *S. littoralis*

Tested compounds	Larval instar	LC_{25} (Ppm.)	LC_{50} (Ppm.)	LC_{90} (Ppm.)	Slope ± S.E.
Lufenuron	2nd	0.0035	0.0116	0.1123	1.298±0.1155
	4th	0.0057	0.0188	0.1811	1.304±0.1177
Chlorfluazuron	2nd	0.0033	0.0110	0.111	1.2766±0.1211
	4th	0.0034	0.0138	0.1919	0.8639±0.1133

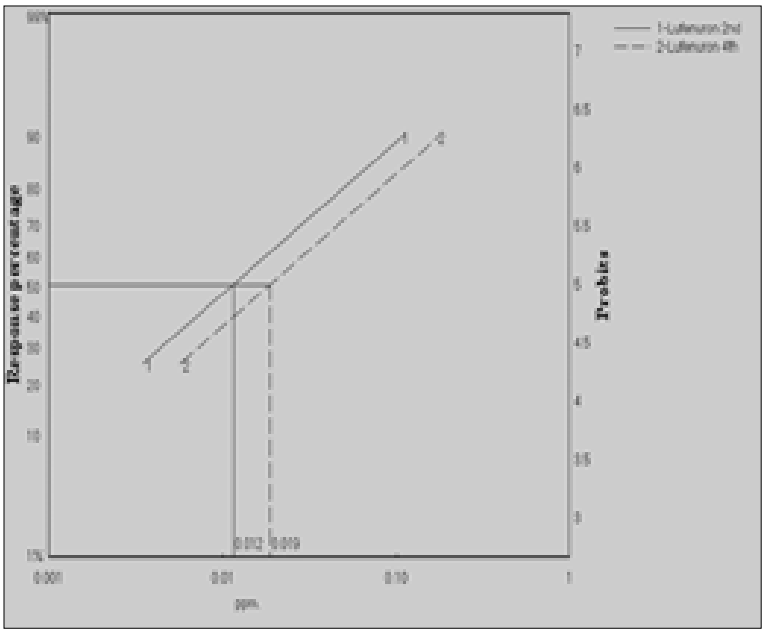

Fig 1: Toxicity of Lufenuron on 2nd and 4th instar larvae of *S. littoralis*

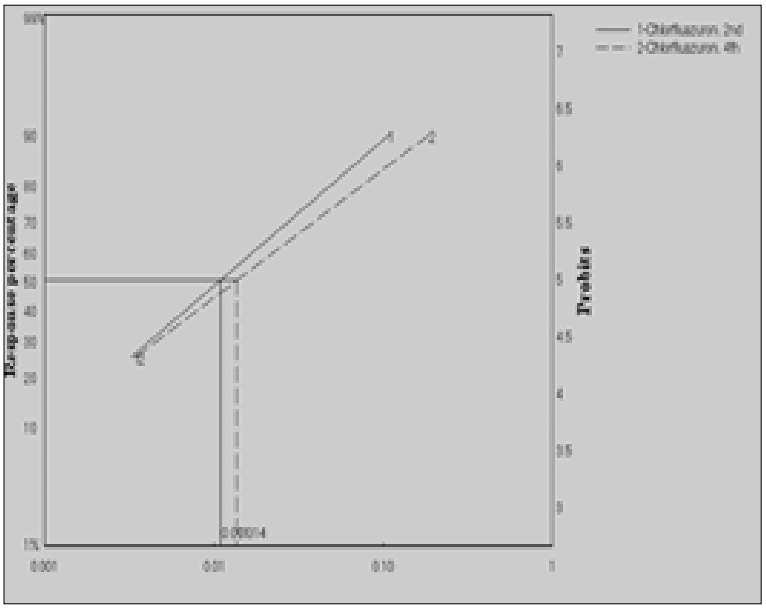

Fig 2: Toxicity of Chlorfluazuron on 2nd and 4th instar larvae of *S. littoralis*.

3.2 Biological studies

The duration of larvae treated with Lufenuron and Chlorfluazuron as 2nd instars lasted 13.0 and 12 days for Lufenuron and Chlorfluazuron respectively, up to pupation which was less than the control by nearly two days (Table 2). Meanwhile pupal stage was 12.6 and 13.2 days for Lufenuron and Chlorfluazuron respectively, as compared to 14.6 days in the control, i.e. nearly a day less. Treatment of 4th instars larvae with LC_{50} of Lufenuron and Chlorfluazuron also increased the remaining larval instars duration to 10.3 and 9 days respectively as compared to 11.3 days in the control (Table 2). The percentage of larvae entering pupation was markedly less in treated 2nd and 4th instars larvae which was 46% and 48 % for Lufenuron, respectively, Chlorfluazuron also scored 48% and 49% less than the control (Table 2). These results agreed with Abdel-Aziz [25] who found a reduction in larval duration of *S. littoralis* treated by Lufenuron.

Table 2: Effect of Lufenuron and Chlorfluazuron on larval duration, pupation% and pupal duration of 2nd and 4th instar larvae of *S. littoralis*

Tested Compounds	Mean larval duration (days) ± S. E.		%Pupation		Mean pupal duration (days) ± S. E.	
	2nd	4th	2nd	4th	2nd	4th
Lufenuron	13±0.4b	10. ±0.58b	46	48	12.6±0.6b	12.0±1.6b
Chlorfluazuron	12±0.3b	9.6± 0.3b	48	49	13±0.1b	12.3±0.1b
Control	15±0.2a	11.6±1.1a	100	100	14.6±0.5a	14.0±1.7a

Means followed by the same small letter in a column are not significantly different at the 5% level of probability (Duncan's Multiple Range Test).

Meanwhile, percentage of adult emergence was decreased than the control to 80% and 83% for Lufenuron the treated 2nd and 4th respectively, and 82% and 87% for Chlorfluazuron respectively. Moth's emerging from treated 2nd or 4th instar larvae with LC_{50} of Lufenuron and Chlorfluazuron showed a shorter life span than the untreated insects (Table 3). Schneidermann [26] suggested that decrease in adult emergence in insects treated with IGR could be due to the fact that the toxin block the differentiation of imaginal discs at metamorphosis. Also, Atwa *et al.* [27] showed that latent effects of IGRs on treated insects were manifested as decrease of pupation and adult emergence. Gamil [28] and Abdel-Aziz [29] also found that the development time of larvae and pupae were extended as well as adult emergence after treatment with IGR.

Table 3: Effect of Lufenuron and Chlorfluazuron on adult emergence percentage and adult longevity of 2nd and 4th instar larvae of *S. littoralis*

Tested compounds	% Adult emergence		Mean adult longevity (days) ± S. E.			
	2nd	4th	2nd		4th	
			♂	♀	♂	♀
Lufenuron	80.00	83.10	11.3±0.58a	14.6±1.15a	13.0±1.0b	14.0±1.7a
Chlorfluazuron	82.00	87.00	12.3±0.4a	12±0.28b	12.6±0.28b	11.3±0.1b
Control	100.00	100.00	13.6±1.15a	15.6±0.58a	16.0±1.0a	15.3±0.57a

Means followed by the same small letter in a column are not significantly different at the 5% level of probability (Duncan's Multiple Range Test).

Table (4) showed the latent effect of treatment of 2nd and 4th instar larvae with the LC_{50} level of Lufenuron and Chlorfluazuron on the mean number of laid and hatched eggs/female. Both of used IGRs significantly decreased the mean number of eggs laid/female and mean number of hatched eggs/females. Lufenuron was the most effective of

Chlorfluazuron, for 2nd and 4th instar larvae as compared the control. Many researchers reported a reduced reproductive capacity in the cotton leafworm moths treated with IGRs [25, 30]. Moreover, Abdel-Aal and Abdel-Khalek [31] found that Teflubenzuron significantly decreased the fecundity percentage of *S. littoralis* especially when treated females mated with treated males.

Table 4: Effect of Lufenuron and Chlorfluazuron on fecundity and fertility of 2nd and 4th instar larvae of *S. littoralis*

Tested compounds	Mean no. of eggs/female ± S.E.		Mean no. hatched eggs/female ± S.E.	
	2nd	4th	2nd	4th
Lufenuron	712±10.2^{b}	880±15.7^{b}	399±4.9^{c}	653±6.11^{b}
Chlorfluazuron	663±9.1^{c}	789±13.38^{c}	415±3.6^{b}	519±21.3^{c}
Control	2135±60.6^{a}	1875±15.1^{a}	2103±4.04^{a}	1857±12.11^{a}

Means followed by the same small letter in a column are not significantly different at the 5% level of probability (Duncan's Multiple Range Test).

3.3 Biochemical studies

Results given in Table (5) indicated that two tested IGRs led to decrease in total protein compared with control. Total protein content were 41.6 and 44.3 (mg/g.b.wt) for Lufenuron and Chlorfluazuron, respectively, while it was 45.6 (mg/g.b.wt) with control. Similar results were obtained by [32] stated that total proteins significantly decreased using spinetoram on *S.littoralis*. The protein pool of the heamolymph functions as a reserve source of protein synthesis need for growth and development of the adult stage during pupal stage [33]. Different results were obtained by Mostafa [34] and Sokar [35] for the total protein of the same species treated with triflubenuron and hexaflumuron, respectively.

Results revealed significant decrease in assayed total Carbohydrates which were 51.3 and 53 (mg/g.b.wt) for Lufenuron and Chlorfluazuron, respectively, as compared with control 72.6 (mg/g.b.wt). These observations agreed with Assar *et al*., [9] with hexaflumuron and teflubenzuronon the same insect.

The both tested IGRs led to decrease in total lipids It was 40.3 and 44.3 (mg/g.b.wt) for Lufenuron and Chlorfluazuron, respectively, as compared with control 46.66 (mg/g.b.wt). These results agree El-Sheikh *et al*. [36] and Abdel Aziz, [37].

Table 5: Effect of Lufenuron and Chlorfluazuron on total proteins, total carbohydrates and total lipids activity in 4th instar larvae of S. *littoralis* after treatment with LC_{50}

Tested compounds	Total proteins (gm. /g b.w.) (Mean a ±S.E)	Total carbohydrates (gm. /g b.w.) (Mean ±S.E)	Total Lipids (gm./g b.w.) (Mean ±S.E)
Lufenuron	41.6 ± 0.89 ^{a}	51.3 ± 1.3 ^{b}	40.3 ± 0.9 ^{b}
Chlorfluazuron	44.3 ± 1.2 ^{a}	53 ± 1.5 ^{b}	44.3 ± 2.4 ^{ab}
Control	45.6 ± 1.4 ^{a}	72.6 ± 1. 5 ^{a}	46. 6 ± 1.2 ^{a}

Means followed by the same small letter in a column are not significantly different at the 5% level of probability (Duncan's Multiple Range Test).

Chitinase activity was tabulated in Table (6) Chitinase values was increased in both tested IGRs were 230.3 and 266.3 (µg NAGA/min/g.b.wt) with Lufenuron and Chlorfluazuron, respectively, while it was 204.33 with control (µg NAGA/min/g.b.wt). Similar results were obtained by Abd El-Mageed and Shalaby [38] and Abdel Aziz, [37] they found that chitinase activity was increased when *S. littoralis* was treated with mixtures of IGRs with insecticides.

The tested IGRs caused an increase in alpha esterase activity, Chlorfluazuron recorded the highest of activation 325.6 (µg α-naphthol/min./g.b.wt) followed by Lufenuron 217.6 (µg α-naphthol/min./g.b.wt) compared with control 196.3 (µg α-naphthol/min./g.b.wt).

The beta esterase in our results show that two tested IGRs have an observed significantly increase in were 221.6 and 215.3 (µg β-naphthol/min./g.b.wt) for Lufenuron and Chlorfluazuron, respectively, as compared with control 208 (µg β-naphthol/min./g.b.wt), It is clearly noticed that IGR's may be cause different levels of significant changes in alpha and beta esterases on *S.littoralis*. These observations agreed with Assar *et al*., [9] with hexaflumuron and teflubenzuronon on *S.littoralis*. Abdel-Mageed *et al*., [39] and Abdel Aziz, [37] also found increased the activity of both alpha & beta esterases when treatment *S. littoralis* with Chitin Synthesis Inhibitors.

Table 6: Effect of Lufenuron and Chlorfluazuron on Chitinase activity, α and β - esterasesin 4th instar larvae of *S. littoralis* after treatment with LC_{50}

Tested compounds	Chitinase activity (µg NAGA/min/g.b.wt) ± SE	A-esterase (µg α-naphthol/min./g.b.wt) (Mean ±S.E.)	B-esterase (µg β-naphthol/min./g.b.wt) (Mean ±S.E.)
Lufenuron	230.3 ± 14.8 ^{ab}	217.6 ± 6.3 ^{b}	221.6± 14.6 ^{a}
Chlorfluazuron	266.3 ±18.6 ^{a}	325.6± 10.1 ^{a}	215.3 ± 3.9 ^{a}
Control	2.4.33 ±5.3 ^{b}	196.3± 2.6 ^{b}	208± 10 ^{a}

Means followed by the same small letter in a column are not significantly different at the 5% level of probability (Duncan's Multiple Range Test).

4. Conclusions

According to obtained results, it was found that tested IGRs were very effective against *S. littoralis* 2nd and 4th instar larvae. In addition, the effectiveness of the tested IGRs was noticed from the latent effect on some biological and biochemical aspects. This suggests that the IGRs are safe and efficient pest control method not only for *S. littoralis* but against many insect pests. Moreover, IGRs represent valid alternatives for conventional synthetic insecticides.

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