

Direct and Latent Effects of Two Chitin Synthesis Inhibitors to *Spodoptera littoralis* Larvae (Boisd.)

¹S.M. Abdel Rahman, ²E.M. Hegazy and ¹A.E. Elwey

¹Central Laboratory of Pesticides, Cairo, Egypt

²Department of Economic Entomology, Faculty of Agriculture,
Alexandria University, Alexandria, Egypt

Abstract: The direct and latent effects of the growth inhibitor Lefenuron EC₅₀ [N-{2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)-phenylaminocarbonyl}-2, 6-difluorobenzamide] and the combination of Lefenuron/Deltanet EC₂₃₅ [O-n-butyl-O-(2, 2-dimethyl-2, 3-dihydrobenzofuran-7-yl)-N-N'-dimethyl-N-N'-thiodicarbamate] on the development of *Spodoptera littoralis* larvae were tested. Different concentrations of each compound were incorporated into the meridic diet of *S. littoralis* larvae. The newly moulted 3rd larval instars were fed for 24 or 48 hours on the treated diet. Both compounds proved to be toxic to the test insect larvae. Lefenuron proved to be more toxic than Lefenuron/Deltanet. *S. littoralis* larvae suffered from more mortality when they were fed for a longer period on the treated diet. The affected larvae ceased feeding within 48 hours and most deaths occurred during moulting to the fourth instars. Incorporating 0.18 ppm of Lefenuron into the diet induced 100% mortality. No significant differences were detected in periods of larvae, prepupae or pupae of survived individuals of both compounds. However, both compounds had delayed effects on survived treated larvae. Some larvae failed to pupate successfully. In some cases, the delayed effects were manifested in the pupal or adult stages. This was expressed by pupal or adult deaths and significant reduction in the reproductive potential of apparently unaffected moths resulting from treated larvae. The effect was a concentration-dependent. Several insect parasitoids are associated with *S. littoralis* larvae. So, when insect growth inhibitors should be used for *Spodoptera* larvae control, dosages and timing of application should be carefully considered.

Key words: *Spodoptera littoralis* · insect growth inhibitors · delayed effects

INTRODUCTION

The cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera : Noctuidae) is one of the key pests that cause great damage to cotton plants as well as other field and vegetable crops in Egypt [1-3]. The widespread and continuously increasing use of different types of nonselective pesticides in cotton fields in Egypt and elsewhere disturb the biological balance and cause outbreaks of insect and mite pests.

The effect of pesticides on the non-target organisms was represented by the destruction of entomophagous agents associated with the cotton leafworm and primary or secondary pest-upsets were recorded in Egypt in areas treated extensively with insecticides [4, 5]. For instance, the rate of parasitism in the cotton leafworm, especially in

the fall generation, was about 75% during the years 1968-1972 [6], before the excessive use of pesticides, while it has now dropped to 1.9-6.2% in 1977. The American bollworm *Heliothis armigera* (Hubner) was observed as a serious pest in Egypt, although it was recorded by Willcocks and Bahgat [1] as a minor pest. The other minor pests that have risen to pest status on cotton plants in recent years are the white fly, *Bemisia tabaci*, stink bugs, *Nezara viridula* and the leafhopper, *Empoasca lylica* beside different species of tetranychid mites.

Recent reports indicate that cotton fields are the main areas where large-scale aerial and ground applications of pesticides are used. This has led to an increasing concern over both the immediate and long-term effects of such very toxic chemicals on the non-target organisms in the cotton fields [5].

The pest control strategies for the future are directed towards the wise and carefully monitored use of compounds that are non or less toxic to man, plants and all classes of existing beneficial creatures. Therefore, the integrated control concepts has been developed through the integration of biological and chemical methods. This concept was broadened to include all control methods [7]. The concept of pest management has now been extended to cover all classes of pests and it is commonly referred to as Integrated Pest Management (IPM).

In an integrated pest management programs, one should try to use selective pesticides or those which have less adverse impact on non-target organisms. In general, insect growth regulators which act as chitin synthesis inhibitors or juvenile hormone analogs have been regarded as excellent integrated control insecticides because of their specificity to target pests and their general safety to vertebrates, molluscs and plants [8, 9].

Two insect growth regulators, namely Lefenuron EC_{50} and the combination of Lefenuron/Deltanet EC_{235} (Furathiocarb) have shown promise for the control of insect pests on cotton, soya beans, vegetables, deciduous fruits, grapes and citrus. These chemicals induce their effect by inhibiting chitin deposition, which results in molting failure or rupture of the new cuticle. The effect of these chemicals on *S. littoralis* larvae comprises the object of the present study.

MATERIALS AND METHODS

Rearing of the cotton leafworm: The culture of cotton leafworm, *S. littoralis* was obtained from a laboratory colony established at Department of Entomology, Faculty of Agriculture, Alexandria University. The colony of *S. littoralis* was originated from individuals collected from field crops including cotton at Alexandria. However, feral individuals were added to the colonies twice a year to maintain genetic diversity. Larvae of *S. littoralis* were reared using the medium and methods of Hegazi *et al.* [10] at $27\pm 1^\circ C$, 60-65% RH and a 14:10 photoperiod. Eggs of *S. littoralis* were sterilized in 1% sodium hypochloride for five minutes and rinsed with tap water for a similar period. All rearing processes of experiments were conducted in incubators maintained under 14:10 (L:D) photoperiod regiment at $25\pm 1^\circ C$ and about 60% RH. Prior to any experiment, individual larvae were examined daily and the instars were determined by counting the number of head capsules shed.

Mortality curves of the larval stage: The insect growth inhibitor Lefenuron EC_{50} [N-{2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)-phenylaminocarbonyl}-2,6-difluorobenzamide] and the combination of Lefenuron/Deltanet EC_{235} [O-n-butyl-O-(2,2-dimethyl-2,3-dihydrobenzofuran-7-yl)-N,N'-dimethyl-N,N'-thiodicarbamate] were tested. The two compounds were discovered and are being developed by CIBA-GEIGY Ltd., in Basel [11, 12].

Preliminary tests were carried out to determine the suitable series of concentrations for each test material. Concentrations-response experiments with *S. littoralis* larvae were then conducted. These materials were formulated by suspending each in distilled water and mixing with the artificial diet to obtain an end volume of 50 ml diet before the agar solidified. Methylene blue dye (1%) was added to the water as a visual guide to ensure thorough mixing of the toxicants with artificial diet. Laboratory tests proved that the dye was nontoxic at that dose. The diet was poured, while still warm, into diet cups, refrigerated and tested within 2 d. By this way, four diet concentrations of Lefenuron of 0.04, 0.06, 0.08, 0.10, 0.20 ppm were prepared for experiment 1. Five concentrations of the compound Lefenuron/Deltanet (Furathiocarb) of 0.02, 0.20, 0.40, 0.80, 2.0, 6.0 ppm were selected for experiment 2. Each diet in a cup which was a treatment, was divided into small cubical portions ca. 2 cm³ and seeded each in a small plastic Petri-dish with five *S. littoralis* larvae at the beginning of zero day of the 2nd moult. Each concentration was tested against 75-100 larvae. In this way, every treatment included 15-20 Petri-dishes. For each compound, the test larvae were left to feed for 24 or 48 h and then transferred to untreated diet until the achievement of mortality counts. A control experiment was set up in the same manner but with distilled water only. The concentration-response curves of *S. littoralis* larvae were calculated from the data of larval mortality. Expected mortality frequency was determined on the basis of observed mortality frequencies produced by each chemical alone. All results were analyzed and corrected according to Abbott's formula [13].

Effects on the development of the cotton leafworm: The effects of two compounds on the developmental stages of the insect were tested. For each compound four concentrations of 0.04, 0.08, 0.12, 0.18 ppm were used. Each concentration was tested against 35-40 newly moulted 3rd instar larvae. The same procedure used in both experiments 1 and 2 was followed in this test. After

treatments, *S. littoralis* larvae were reared individually in disposable small plastic Petri dishes (3.5x1.4 cm) to obtain daily detailed records of the effect of the test duration of the last four larval instars, prepupal and pupal stages and longevity of the emerging adults.

The morphogenetically, unaffected externally, normal adults obtained in different experimental variants of the last experiment were grouped in pairs. This was achieved by pairing and confining a female resulting from treated larvae with two males from normal laboratory culture, with the intention of obtaining daily records of the effect of the test compounds on the oviposition period and egg-laying capacity of the survived adults. The caged adults were sexed in the pupal stage and kept as isolates in plastic cups (7x5.8 cm) covered with muslin. After emergence, the adults were fed daily on 10% sugar solution contained in suspended cotton plugs and provided with a piece of paper as an oviposition site. Daily records on the oviposition period and egg-laying capacity (wt. in mg) and other biological records were carefully collected.

Statistical analysis: Analysis of variance was carried out to determine if there were significant differences among results of different treatments. In case of dealing with results of two treatments, the existence of a significant difference was determined by t-test. The L.S.D method was used for comparison between the means in certain results [14].

RESULTS AND DISCUSSION

Mortality curves of the larval stage: Figure 1 shows the concentration-mortality curves after feeding newly moulted 3rd instar of *S. littoralis* larvae for 24 or 48 h on diet treated with Lefenuron. The larvae that were fed for 24 h on diets with one concentration of 0.04, 0.06, 0.08, 0.10 or 0.20 ppm produced ca. 16, 54, 65, 80 and 99% mortality, respectively. On testing other group of larvae for longer period (48 h), the same concentrations gave mortality ranged from 24 to 99%. In both, the percentage mortality of the larvae that were fed on IGR-free diet did not exceed 6.0%.

Following the bioassay statistical analysis by Litchfield and Wilcoxon [15], when the Lc-p-lines of these results were plotted (Fig. 1), they proved to be a good fit as the differences between the experimental and tabulated χ^2 values were always insignificant. The slope function of the resulted curve for the larvae fed for 24 h on the treated diet with the IGR was 1.58, while it decreased to 1.36 when the larvae were allowed to feed on the treated

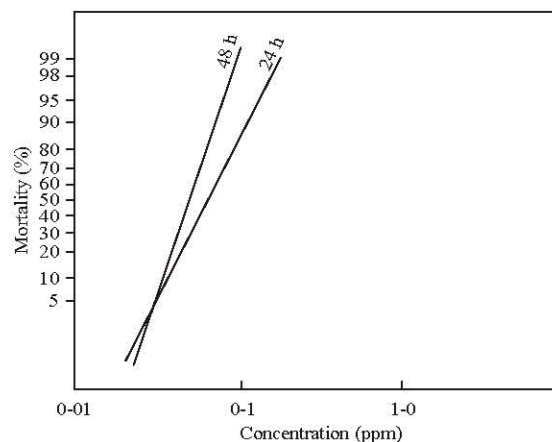


Fig. 1: Concentration-mortality curves after feeding *S. littoralis* 3rd-instar larvae for 24 or 48 h on the semi-meridic diet treated with Lefenuron

diet for 48 h. This further indicates that the cotton leafworm larvae suffered more mortality when they were fed for a longer period on the treated diet. The LC_{50} value also reached 0.06 ppm with fiducial limits from 0.04 to 0.08 for the larvae treated for 24 h. However, a slight decrease in the LC_{50} value (0.05 ppm) and a narrower range for the fiducial limits (0.04 to 0.05) were observed when the larvae were fed for 48 h on treated diets.

The mortality in the larval stage was clearly due to the moulting-disturbing effect of the Lefenuron. The *S. littoralis* larvae ceased feeding within 48 h and most deaths occurred while the larvae were moulting, usually between the third and fourth instar. Generally, the *S. littoralis* larvae died within the old cuticle and the newly formed cuticle was extremely thin.

Figure 2 shows the effect of exposing newly moulted 3rd instar larvae to other emulsifiable concentrates of the IGR Lefenuron/Deltanet EC₂₃₅. The larvae that were fed for 24 h on concentrations of 0.2, 0.4, 0.8, 2.0 and 0.6 ppm induced ca. 3, 11, 22, 49 and 91% mortality, respectively, while death value for the larvae fed on normal diet (control) was 6.67%. The LC_{50} value was 1.7 ppm with fiducial limits of 1.41-2.04 ppm, while the slope value of the line was 2.59. The effect was nearly similar when *S. littoralis* larvae were left to feed on the same concentrations for 48 h. The percentage mortalities obtained were ca. 8, 16, 35, 63 and 100%, respectively. The LC_{50} value was 1.3 ppm with fiducial limits of 1.01 to 1.66 ppm, while the slope value of the line was 3.56. At the low concentrations of 0.2 and 0.4 ppm and the intermediate ones of 0.4 and 0.8 ppm, there were no clear moulting-disturbing effects from the Lefenuron/Deltanet as most of

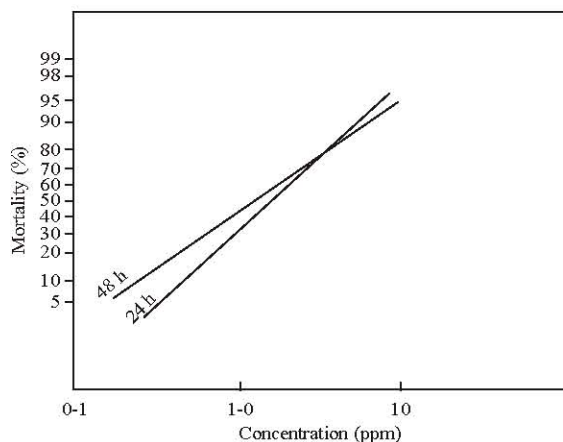


Fig. 2: Concentration-mortality curves after feeding *S. littoralis* 3rd-instar larvae for 24 or 48 h on the semi-meridic diet treated with Lefenuron/Deltanet

the tested larvae survived after treatments. However, the highest concentration (6.0 ppm) induced serious effects within the first two days after treatments in both feeding periods and many larvae suffered from moulting failure. Although the range of concentrations tested for Lefenuron were lower than those used for Lefenuron/Deltanet, the former IGR was more effective than the other. Whereas, *S. litura*, the oriental leafworm, which is a pest of cotton and tobacco in Asia and Australia, was susceptible to diflubenzuron [16, 17]. *S. exigua*, the beet armyworm, was not susceptible to

diflubenzuron, but penfluron and chlorfluazuron were very effective. This species is able to metabolize or detoxify or eliminate diflubenzuron and escape its insecticidal effects [18]. It seems that *S. littoralis* larvae were able to escape the insecticidal effects of the formulation Lefenuron/Deltanet if it is used at the same concentration of Lefenuron alone.

The present work showed that the mortality was clearly caused by moulting failure of *S. littoralis* larvae. This effect is mainly induced by inhibiting chitin formation [19, 20], thereby causing abnormal endocuticular deposition and abortive moulting [21]. Other effects of the chitin inhibitor-compounds were reported. They are known to act on the peritrophic membrane by affecting its chitin-protein structure, hindering its role in protecting the secreting cells from any damage [22].

Development of survived treated *S. littoralis* larvae: The results of the effect of Lefenuron and Lefenuron/Deltanet on the development of survived treated *S. littoralis* larvae are shown in Table 1. For Lefenuron-treated larvae, all larvae treated with 0.18 ppm died during moulting from third to fourth instar. However, some of larvae treated with lower concentrations survived treatments. The duration of the last four larval instars in the control ranged from 8 to 11 days with an average of 9.18±0.15 days. This range lasted for 8 and 10 days in treated larvae with an average of 8.34±0.01, 8.52±0.51 and 9.0±0.0 days for those fed on diets containing 0.04, 0.08

Table 1: Duration (in days) of survived *S. littoralis* stages (mean±SE) after treatment of newly 3rd-instar larvae by Lefenuron and Lefenuron/Deltanet

Con. (ppm)	Duration (in days)					
	Larvae	Pre-pupae	Pupae		Adult longevity (in days)	
			♂	♀	♂	♀
Lefenuron						
0.00	9.18a±0.15	2.85a±0.10	8.50a±0.15	9.44a±0.16	7.45a±0.21	5.56a±0.18
0.04	8.34a±0.10	2.52a±0.10	8.33a±0.15	5.58a±0.15	7.33a±0.19	5.50a±0.21
0.08	8.52a±0.51	2.42a±0.51	8.38a±0.74	8.38a±0.52	7.50a±0.55	5.14a±0.69
0.12	9.00a±0.00	2.50a±0.52	7.80a±0.84	8.60a±0.53	6.30a±0.96	3.80b±0.50
0.18	All larvae died					
Lefenuron/Deltanet						
0.00	10.05a±0.08	2.68a±0.08	8.50a±0.12	9.10a±0.16	7.94a±0.22	5.90a±0.16
0.04	10.29a±0.08	2.50a±0.09	8.30a±0.12	9.00a±0.16	7.53a±0.15	5.77a±0.20
0.08	10.09a±0.11	2.88a±0.12	8.31a±0.11	9.08a±0.15	6.88a±0.27	5.75a±0.18
0.12	9.65a±0.14	2.80a±0.13	7.35a±0.12	7.55a±0.16	6.41a±0.27	5.09a±0.21
0.18	9.47a±0.11	2.50a±0.10	7.31a±0.13	7.87a±0.19	6.46a±0.33	5.20a±0.20

For each set of *S. littoralis* stage, figures marked by the same letter are not significantly different (p>0.05)

and 0.12 ppm, respectively. However, no significant differences were found between the larval periods in all treatments.

On reaching the prepupal stage, some larvae suffered from partial moult inhibition and died during their attempt to shed the old cuticle. The prepupal duration in the control ranged from 2 to 4 days with an average of 2.85 ± 0.10 days, while those of surviving prepupae of treated larvae varied from 2 to 3 days in all treatments. The analysis of variance proved no significant differences between the durations of the prepupal period of all the tested larvae. On reaching the pupal stage, some of the pupae resulting from the treated larvae were apparently morphologically perfect individuals. The duration of these pupae ranged from 7 to 10 days for males and 8 to 9 days for females. There were no significant differences between the pupal period of all resulting pupae including those of the control. However, delayed effects were observed among some of treated larvae. Some of the latter developed into malformed pupae and some failed to pupate successfully instead they formed larval pupal intermediate. All these larvae died while attempting to moult. The adults with eclosion problems were, by far, one of the most serious effects that faced some adults resulting from the treatments with intermediate concentrations (0.08 and 0.12 ppm). This phenomenon was noted when the affected adults attempted to extricate themselves from the pupal skin. In other cases, some adults freed the abdomen successfully from their pupal exuvia, but the thorax and head remained bound to the pupal skin and others having vestigial wings.

The effect of Lefenuron/Deltanet on the development of *S. littoralis* larvae is shown in Table 1. Of the treated larvae 77.78% survived the highest tested concentration of 0.18 ppm and reached their adult stage. The affected larvae died as larval-pupal, pupae, pupal-adult intermediates or dead adults inside their pupal skin or emerged as imperfect adults. Most of these symptoms are similar to the Lefenuron treatments.

The development of apparently non-affected larvae with higher concentrations (0.12 and 0.18 ppm) was one day faster in the duration of the larval or pupal stages than those larvae which survived lower concentration or ones that were fed on Lefenuron/Deltanet diet-free. However, the differences between either of larval, prepupal or pupal stages of the surviving larvae and the controls was insignificant.

Figure 3 shows percentages of *S. littoralis* moths resulting from larvae treated with Lefenuron and Lefenuron/Deltanet. The effect of Lefenuron treatments

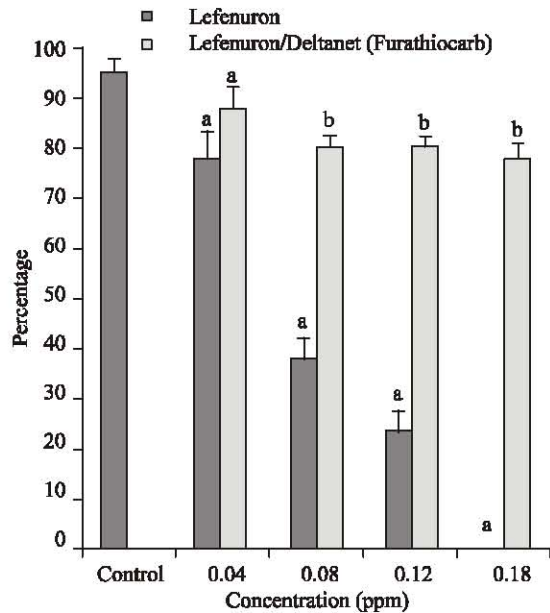


Fig. 3: Percentage of *S. littoralis* moths (mean±SE) resulting from larvae treated with different concentrations of IGR's. Mean values followed by different letter above each bar within each set are significantly different at $p=0.05$

were dose-dependent. The percentage of the formed adults was 92.50% when the larvae were fed on Lefenuron dite-free. This figure dropped significantly with increasing the concentration of the compound in the diet. The doses of 0.04, 0.08, 0.12 and 0.18 ppm provided 77.78, 37.80, 23.33 and 0.0% adults, respectively. However, the same doses of Lefenuron/Deltanet were sublethal and did not adversely affect larval or adult survival. There was a significant effect on the percentage of the adults resulting from the larvae fed on diets containing 0.08, 0.12 or 0.18 ppm, when compared with either those produced from the control larvae or those treated with the lowest concentration (0.04 ppm). These data further indicate that Lefenuron/Deltanet at concentrations not lethal to the treated 3rd-instar larvae or the pupae caused changes in emerging adults (Fig. 3). These results of Lefenuron/Deltanet were similar to those reported by Madore *et al.* [23] on the molt-inhibiting IGR, uc-62644 when the 6th - instar larvae of *Choristoneura funiferana* (Clemens) were treated with sublethal concentrations of the compound. Madore *et al.* [23] also reported that where chemicals such as uc-62644 are used as larvicides, workers should be aware that delayed effects may occur in later stages of the survivors, a case which can be applied for the Lefenuron/Deltanet too.

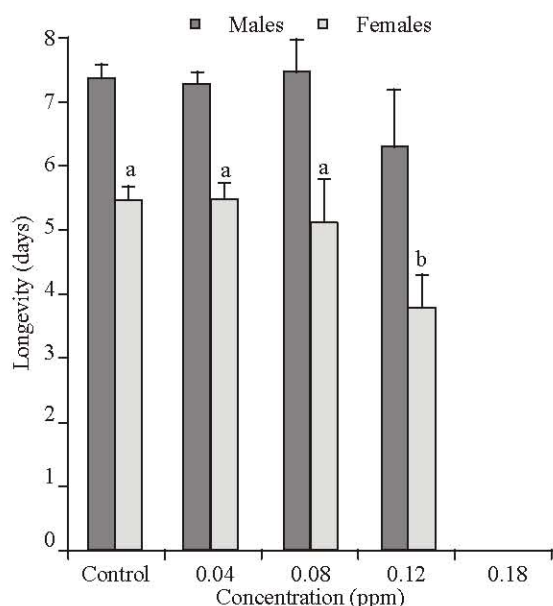


Fig. 4: Longevity (in days) of *S. littoralis* moths (mean±SE) resulting from 3rd instar larvae treated with Lefenuron. Mean values followed by different letter above each bar among female moths are significantly different at p=0.05

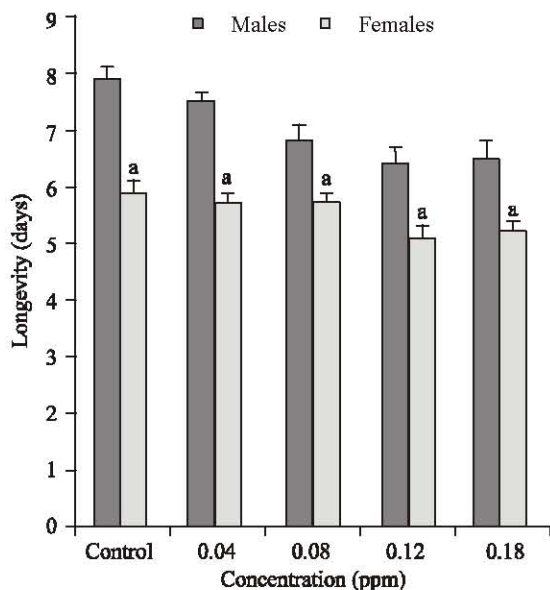


Fig. 5: Longevity (in days) of *S. littoralis* moths (mean±SE) resulting from 3rd instar larvae treated with Lefenuron/Deltanet (Furathiocarb). Mean values followed by different letter above each bar among female moths are significantly different at p=0.05

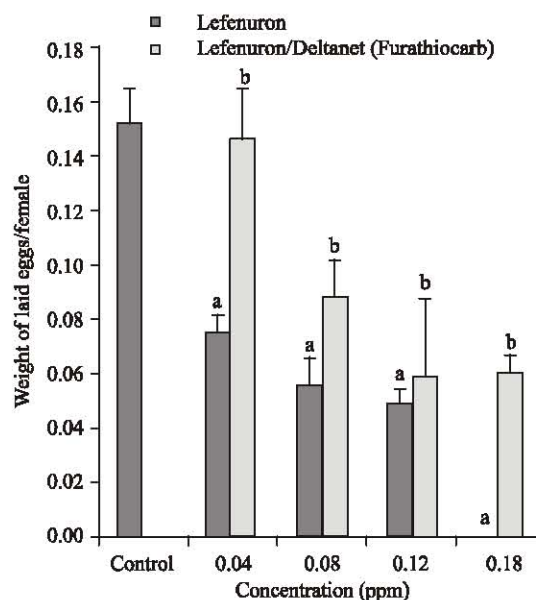


Fig. 6: Egg laying capacity (in mg) of *S. littoralis* moths (mean±SE) resulting from larvae treated with different concentrations of IGR's. Mean values followed by different letter above each bar within each concentration set are significantly different at p=0.05

Other delayed effects of the tested compounds can be seen in the longevity of apparently perfect adults that resulted from treated 3rd-instar *S. littoralis* larvae (Fig. 4 and 5). The first two doses (0.04 and 0.08 ppm) of Lefenuron incorporated into the test diets did not adversely affect the longevity of adults derived from treated larvae. The resulting adults in the control lived for an average of 7.45 and 5.56 days for male and females, respectively. These figures were not significantly greater than those recorded in the treatments (Table 1). However, the longevity of females produced by larvae fed on 0.12 ppm of the same compound incorporated into the diet decreased significantly to an average of 3.8 days (Fig. 4). On the contrary, the adults developed from larvae fed on the tested doses of Lefenuron/Deltanet lived almost as long as control adults (Fig. 5 and Table 1).

Egg-laying capacity of survivor female moths: The adult fecundity of externally normal female moths of *S. littoralis* resulting from treated larvae is shown in Fig. 6. The effect was concentration-dependent reduction in the reproductive potential of the emerging adults. The average weight of laid eggs/control female reached

0.152 mg. This figure was significantly reduced for female resulting from Lefenuron-treated larvae (Fig. 6).

In case of *S. littoralis* females formed from Lefenuron/Deltanet treated larvae, the lower concentration of 0.04 ppm did not adversely affect the egg-laying capacity of the female moths. However, by increasing the incorporated concentration of the same compound into the larval diet, the weight of the deposited eggs by the surviving adults significantly decreased and was concentration-dependent.

The adult fecundity of *S. littoralis* was disrupted by both Lefenuron or Lefenuron/Deltanet treatments. Surviving females from treated larvae oviposited significantly fewer eggs. Madore *et al.* [23], working on the spruce budworm, *C. fumiferana* demonstrated similar results. The experimental insect growth regulator uc-62644 fed at sublethal concentration to 6th-instar larvae caused a dose-dependent reduction in reproductive potential of the emerging adults.

Finally, as with all IGR's [24], the present tested compounds have a prolonged knockdown time on the target insect (*S. littoralis* larvae). However, results of the present work indicated that many *S. littoralis* larvae cease feeding within 48 h after treatment. The prolonged delayed effects should not be a limiting consideration for including these IGR's in management programs. Nevertheless, the effects of these compounds on the non-target insects are some of important concepts which should be investigated in detail.

REFERENCES

1. Willcocks, F.C. and S. Bahgat, 1937. The insect and related pests of Egypt. Vol. 1, Part 2, Insect and mites injurious to the cotton plant. Royal Agric. Soc. Entomological Section, Cairo, Egypt.
2. Bishara, I., 1954. The cotton worm, *Prodenia litura* F., in Egypt. Soc. Ent. Egypt. Bull., 18: 288-420.
3. Moussa, M.A., M.A. Zaher and F. Kotby, 1960. Abundance of the cotton leafworm *Prodenia litura* (F.), their effect on biology. Bull. Soc. Ent. Egypt, 44: 241-251.
4. Kamal, M., 1951. The biological control of the cotton leafworm *Spodoptera littoralis* F. in Egypt. Bull. Soc. Ent. Egypt, 35: 221-270.
5. Hafez, M., 1972. Certain factors influencing mortality of natural enemies of cotton pests. Agric. Res. Rev., Cairo, 50: 1-16.
6. Attia, H.H., 1977. Ecological assessment of pesticide management on terrestrial ecosystem in Egypt. Proceedings of the VC. AID University of Alexandria, A.R.E. Seminar Workshop in Pesticide Management, March 5-10.
7. Smith, R.F., 1970. Pesticides. Their use and limitation in management. (in concept of pest management. Robb, R.L. and F.E. Guthrie, (Eds.). N.C. State University, Raleigh).
8. Wilkinson, J.D., K.D. Bieveri, C.M. Ignoffo, W.J. Pons, R.K. Morrison and R.S. Seay, 1978. Evaluation of diflubenzuron formulations. J. Ga. Entomol. Soc., 13: 227-236.
9. Deakle, J.P. and J.R. Bradly, 1982. Effects of early season applications of diflubenzuron and azinphosmethyl on population levels of certain arthropods in cotton fields. J. Ga. Entomol. Soc., 17: 200-204.
10. Hegazi, E.M., A.M. El-Minshawy and S.M. Hamed, 1977. Suitability of *Spodoptera littoralis* larvae for development of *Microplitis rufiventris*. J. Agric. Sci. Camb., 89: 659-662.
11. Anonymous, 1988. Deltanet 235EC insecticide against foliar and soil pests. Ciba-Geigy Limited, Basle, Switzerland, Agricultural Division, C: 11-4002.
12. Anonymous, 1989. CGA 184699 insect growth inhibitor for cotton, soya, vegetables, potatoes, deciduous fruits, grapes and citrus. Ciba-Geigy Limited, Basle, Switzerland.
13. Abbotts, W.S., 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.
14. Bishr, M.A. and M.M. El-Robi, 1976. Introduction on statistical methods and experiments design, in Arabic. Textbook, Dar El-Maarif, Alexandria.
15. Litchfield, J.I. and F. Wilcoxon, 1949. Staniford research laboratories. Entomologie, 89: 193-198.
16. Sundaramurthy, V.T. and M. Balasubramiam, 1978. Effect of an inhibitor of chitin deposition in tobacco caterpillar (*Spodoptera litura* Fb.) under induced hyper hormone condition. Z. Ang. Ent., 85: 317-321.
17. Santharam, G. and M. Balasubramianiam, 1980. Note on the control of *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) on tobacco with a nuclear polyhedrosis virus and diflubenzuron. Indian J. Agric. Sci., 50: 726-727.
18. Granett, J., B. Bisabri-Ershadi and M.J. Hegazi, 1983. Some parameters of benzoylphenyl urea toxicity to beet armyworm. J. Econ. Entomol., 13: 133.

19. Ishaaya, I. and J.E. Casida, 1974. Dietary TH 6040 alters composition and enzyme activity of house fly larval cuticle. *Pestic. Biochem. Physiol.*, 4: 484-490.
20. Post, L.C., B.J. de Jong and W.R. Vincent, 1974. 1-(2,6-disubstituted benzoyl)-3-phenylurea insecticides: Inhibitors of chitin synthesis. *Pestic. Biochem. Physiol.*, 4: 473-483.
21. Mulder, R. and M.J. Gijswijt, 1973. The laboratory evaluation of two promising new insecticides which interfere with cuticle deposition. *Pestic. Sci.*, 4: 737-745.
22. Clark, L., G.H.R. Temple and J.F.V. Vincent, 1977. The effects of chitin inhibitor-Dimilin on the production of peritrophic membrane in the locust, *Locusta migratoria*. *J. Insect. Physiol.*, 23: 241-246.
23. Madore, D.C., B.G. Drion and B.J. Dimond, 1983. Reduction of reproductive potential in spruce budworm (Lepidoptera: Tortricidae) by a chitin inhibiting insect growth regulator. *J. Econ. Entomol.*, 76: 708-710.
24. Chandler, L.D., S.D. Pair and W.E. Horison, 1992. RH 5992, a new insect growth regulator active against corn earworm and fall armyworm (Lepidoptera: Noctuidae). *J. Econ. Entomol.*, 85: 1099-1103.