Enhancement of *Bacillus Thuringiensis* var. *Kurstaki*, an Entomopatholgenic Bacterium, and Flufenoxuron, an Insect Growth Regulator, to Control *Spodoptera Littoralis* (Boisd.), a Cotton Leafworm

El Shaimaa N. Ibrahim [1], Nashwa S. Amein [1], Aziza E. Abdelal [1] and Amal Th. Hussien [2]

ABSTRACT

The study examined the effects of two compounds, *Bacillus thuringiensis* var. *kurstaki* and Flufenoxuron (CSI) on the histology, physiology, and toxicity of *Spodoptera littoralis* (Boisd.), an Egyptian cotton leafworm, in its second and fourth instars respectively. The experiments were conducted in a laboratory setting. According to the findings, the LC_{10} and LC_{50} values of *B. thuringiensis* compound treatments on larvae in their second instar were found to be, respectively, 0.0027 and 0.2331 gm/ml. Flufenoxuron-treated larvae in their fourth instar had corresponding values of 1.4716 ppm and 0.0025 ppm. Interaction effects were noted when LC_{10} of flufenoxuron was applied in conjunction with LC_{50} of *B. thuringiensis* either immediately or 48H after *B. thuringiensis* treatment of the second instar larvae. After applying the IGRs, zero time and 48H, respectively, the average mortality rate of larvae treated with *B. thuringiensis* alone rose from 50.001% to 51.22 and 65.03%. In addition, 52.32% of LC_{10} Flufenoxuron mortality occurred after LC_{50} *B. thuringiensis* therapy. Ultimately, the results showed that the two tested *B. thuringiensis* and Flufenoxuron had longer mean larval and pupal durations for larvae in their second or fourth instar who survived treatment, compared to their LC_{50} values. Moreover, the studied chemical decreased all other biological effects, including pupation, pupal weight, adult emergence, and adult longevity in both sexes. After six days of treatment, the histological structure in the midgut of larvae that survived treatment of either *B. thuringiensis* or flufenoxuron, respectively, showed many alternations. These larvae were in their second and fourth in stars. There were signs of peritrophic membrane breakdown, microvilli disruption, and epithelial layer vacuolation. The addition of flufenxuron (LC_{10}) to *B. thuringiensis* (LC_{50}) resulted in the exfoliation of columnar cells, vacuolation, and loss of the compact look of the muscularis layer. When the LC_{50} of flufenoxuron was administered to 4th instars, the fat body of the surviving late 6th instars collapsed and there was minor vacuolization.

Key words: *B. thuringiensis*, Flufenoxuron, *Spodoptera littoralis*, biological aspects, histology.

INTRODUCTION

Due to its wide host plant range, *Spodoptera littoralis* (Boisd.) the Egyptian cotton leafworm is regarded as one of the harmful and eradicative insects. In many field crops, vegetables, and fruits, this insect results in significant financial loss. As such, it necessitates the management of several tactics. In Egypt, the management of *S. littoralis* was limited to the extensive use of conventional insecticides leading to the rise of high resistance to many chemical pesticides, resurgence, and residues of chemical pesticides in the environment (Metayi *et al*., 2015 and Eldesouky *et al*., 2019). As a result, finding new alternative, effective, safer for humans and less toxic to our ecosystem, is requisite (Assar *et al*., 2016). Of those groups, chitin synthesis inhibitors (CSIs) and bio-pesticides that showed high selectivity and low toxicity to human and environment is highly appreciated. Chitin synthesis inhibitors (CSI's) such as Flufenoxuron (Novo) act by interfering with chitin biosynthesis during moulting period in insects, which confers a remarkable action specificity with low harm to beneficial arthropods and humans. The blocking of chitin synthesis occurs by disruption of the function of connecting N-acetylglucose amine moities to the chitin chain. *Bacillus thuringiensis* (Bt) such as endoxine are most important microbial insecticides used in the world (BenFarhat-Touzri *et al*., 2013) as an alternative or supplement to chemical insecticides. (Bt) endotoxins are effective in controlling different cotton pests including *S. littoralis* but not their natural enemies (Mhalla *et al*., 2018). Nevertheless, many drawbacks restrict its application, including its limited range of operation and brief field persistence (Sleem *et al*., 2012). In an effort to maximize the effectiveness of the bio-agent, reduce the need for chemical insecticides, and lessen the potentiation of Bt through the inclusion of toxic (CSIs) compounds, the combination of bio-agent and chemical insecticides was studied (Khalique and Ahmed, 2005). Consequently, the purpose of the current investigation was to assess the relative effectiveness of this chemical against the second larval instar of *S. littoralis* as well as the compatibility of *B. thuringiensis* with chitin synthesis inhibitors (CSIs) under laboratory conditions. In order to effectively control *S. littoralis*, the best time to apply CSIs was either with or after larval exposure to *B. thuringiensis*. It was also determined how this treatment

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[1]Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.

[2] Faculty of graduate studies and environmental Research,

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would affect some features of this insect's biology and histology.

MATERIAL AND METHODS

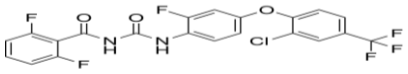
Tested Insects:

According to El-Defrawi *et al.* (1964), a laboratory strain of *Spodoptera littoralis* (Lepidoptera: Noctuidae), the cotton leafworm, was collected and raised in the cotton leafworm department of the Plant Protection Research Institute, Dokki, Giza, under constant laboratory conditions. A 12-hour photoperiod at 25±1% relative humidity (RH) and 25±1% temperature was used for rearing.

Cmpounds:

a- **Protecto® WP 9.4% (*Bacillus thuringiensis* var.*kurstaki*):** *Bacillus thuringiensis* (Kingdom: Eubacteria; Order: Bacillales; Family: Bacillaceae) is a gram-positive sporeforming bacterium that produces crystalline proteins called deltaendotoxins during its stationary phase of growth. This product is used at rate of 300 gm/feddan with LC50 value is 3.2. 102 Iu/ml. Feddan is equal to 4200 square meter. Was obtained from Organic Biotechnology Co., LTd.

b- **Novo 10% DC:** A commercial insect growth regulators product Flufenoxuron (Chitin Synthesis Inhibitors). Inhibit the production of chitin, a major component of the insect exoskeleton. Insects treated with CSIs become unable to synthesize new cuticle, and therefore unable to successfully molt into the next stage, from Kemet Co.



Bioassay experiments:

Effect of *Bacillus thuringiensis* var. *kurstaki* and Flufenoxuron against the larval of *S. littoralis*:

The insecticidal activity of *B. thuringiensis var. kurstaki* and Flufenoxuron were assessed on newly ecdysed 2nd and 4th instar *S. littoralis* larvae, respectively.

***B. thuringiensis*:** A series of dilution were prepared from 1 gm. of the product obtained as a wettable powder, (0.5, 0.25, 0.125, 0.06 and 0.031 gm/ml).

A series of aqueous concentrations of Flufenoxuron which was (1, 0.5, 0.25, 0.125 and 0.0625 ppm.).

Lab-based bioassay experiments were conducted utilizing the leaf-dipping method, as previously reported by Tabashnik *et al.* (1990). Before being given to the larvae, fresh castor bean leaves were dipped in a series of concentrations for each constituent made in distilled water, allowed to dry at room temperature, and then delivered to the larvae. For every concentration, three replicates containing 10 larvae each were examined, and every bioassay was conducted three times. Water-treated leaves were fed to control larvae. For 48H, the larvae were left exposed and fed on treated leaves. Then, they were provided with fresh, clean and untreated castor oil leaves until pupation. The mortality percentages of treated larvae were corrected against those of the control by Abbott's formula (Abbott, 1925). To estimate LC_{10}, LC_{50} and slope values, the corrected mortality percentages were subjected to Probit analysis using Ldp-line software according to Finney (1971).

Interaction between *B. thuringiensis* and Flufenoxuron:

The purpose of this experiment is to determine when *B. thuringiensis* and flufenoxuron should be applied. The aim behind this was to determine the best time of exposure that might show high efficacy when *B. thuringiensis* and Flufenoxuron were applied together at the same time or in different times. Joint toxic action between the *B. thuringiensis* and Flufenoxuron were evaluated against the 2nd larval instar of *S. littoralis* at two different times. The larvae were treated with LC_{50} of *B. thuringiensis* or LC_{10} of Flufenoxuron (The castor was treated with LC_{50} of *B. thuringiensis*, and after the paper dried in the laboratory conditions, the castor paper was dipped in LC_{10} of Flufenoxuron (Zero time). The larvae were exposed to LC_{50} of *B. thuringiensis* only for 48H then, the same larvae were treated with LC_{10} of Flufenoxuron (48H). Alternatively, the larvae were treated with LC_{50} of *B. thuringiensis* after only 48H of exposure to LC_{10} of flufenoxuron. Three replicates with ten 2nd instar larvae per replicate were used for each treatment. The larvae were exposed and fed on treated leaves for 48H. Then, they were provided with fresh.

Biological investigation:

Newly molted 2nd and 4th instar larvae were treated with *B. thuringiensis* and Flufenoxuron, respectively, and larvae living after Interaction between *B. thuringiensis* and Flufenoxuron zero time and 24H). Treated larvae were examined daily to determine the post treatment effects on those insects survived the treatments, (e.g., the larval and pupal durations, pupal weight, adult emergence Percentage and longevity). These parameters were compared with the untreated control larvae.

Histopathological study:

The study focused on the histopathology of the midgut of late sixth-instar larvae that had survived treatments as second instars with *B. thuringiensis* and treatments as fourth instars with flufenoxuron, zero time, and 24H. The tested tissues were fixed in aqueous Bouin's solution for 24H. The normal paraffin wax

embedding procedure was followed. The sections were cut 6µ thick and stained with heamatoxylin and eosin for microscopic examination. Control sections of non-treated larvae were also carried out (Junqueira and Carneiro, 1980).

Statistical analysis:

Statistical analysis using student t-test of the obtained data was performed by using COSTAT program, for Windows.

RESULTS AND DISCUSSION

Toxicological studies:

Efficacy of *Bacillus thuringiensis var. kurstaki* and Flufenoxuron against the 2nd and 4th instar *S. littoralis* larvae:

Table (1) displayed the toxicity of the chemicals evaluated against *S. littoralis* larvae in their second and fourth in stars, respectively, using *B. thuringiensis* var. *kurstaki* and flufenoxuron.

Results showed that both compounds exhibited nearly the same toxic effect against the 2nd and 4th instar larvae as determined from LC_{10} and LC_{50} values obtained. The percentage mortality of treated larvae was increased by increasing the concentration. These results were agreed with El-Sabagh *et al***.** (2017) who reported the emamectin benzoate and *Bacillus thuringiensis* as bioinsecticides under laboratory conditions. The LC_{50} values for *S. littoralis* larvae treated with various concentrations of lufenuron were 0.0921 ppm, and the results were also consistent by Amein *et al.* (2021). These substances don't harm the environment and can be applied to pesticide resistance management programs as well as integrated pest management (IPM) (Ishtiaq *et al.*, 2012).

Interaction between *Bacillus thuringiensis var. kurstaki* and Flufenoxuron:

Potentiation, antagonistic and additive interaction effects were observed upon application of LC_{10} of Flufenoxuron in combination with LC_{50} of *B. thuringiensis* zero time or after 48H from treating the 2nd inster larvae with *B. thuringiensis* as shown in Table (2). The average mortality among larvae treated with *B. thuringiensis* alone was 50.001 % and increased to 51.22 and 65.03% by adding the IGR's, Zero time and 48H, respectively (Table 2). Conversely, mortality was 52.32% following treatment with LC_{10} of flufenoxuron and 48 hours following treatment with LC_{50} *B. thuringiensis*.

A similar finding was reported by Khalifa *et al*. (2015) investigated the effect of applying mixtures of chlorantraniliprole ($LC_{12.5,}$ LC_{25} and LC_{50}) with *B. thuringiensis* ($LC_{12.5,}$ LC_{25} and LC_{50}) against the 4th larval instar of *S. littoralis*. They reported that the mixture of chlorantraniliprole (at LC_{25} and LC_{50}) with *B. thuringiensis* (at LC_{25} and LC_{50}) resulted in an antagonistic effect while, with the mixture of chlorantraniliprole (at $LC_{12.5}$) with *B. thuringiensis* (at $LC_{12.5}$) resulted in additive effect. According to El-Saadany *et al*. (2019), when tested as a bait against the fourth larval instar of *S. littoralis*, treatment of the tested insecticides after 24 hours of larval exposure to *B. thuringiensis* was more effective than when paired with *B. thuringiensis* at zero time.

Table 1. Susceptibility of the 2nd to *Bacillus thuringiensis var. kurstaki* and 4th instars to Flufenoxuron *S. littoralis* (Boisd.)

Tested Compound	Larval mortality %		ethal concentration (L.C.)		Slope ± S. E.
	LC_{10}	LC_{50}	LC_{10}	LC_{50}	
Bacillus thuringiensis (gm/ml)	26.92	50.001	0.0027	0.2331	1.661± 0.246
Flufenoxuron (ppm)	25.93	48.15	0.0025	1.4716	1.464±0.249

Table 2. Interaction between LC50 of B. thuringiensis with LC10 of Flufenoxuron on 2nd larval instar of S. littoralis

Treatment	Intervals (hours)	Larval mortality %
B. thuringiensis + Flufenoxuron	0	51.22
B. thuringiensis + Flufenoxuron	48	65.03
Flufenoxuron + *B. thuringiensis*	48	52.32

Biological Studies:

Results presented in Table (3) showed the effect of compounds at LC_{50} on the mean larval duration, pupation percentage, pupal duration and pupal weight. When *B. thuringiensis* was administered to larvae in their second instar, the mean larval duration rose in contrast to the untreated control group. The percentage of larvae treated as 2nd instar with LC_{50} of the *B thuringiensis* metamorphosing to pupae was markedly reduced to approximately half the value of their control (Table 3). 56.67% of pupations were observed. Additionally, a mean weight of 0.24 gm was obtained for newly developed pupae, compared to 0.35 g for untreated pupa, indicating a substantial decrease in weight compared to control. The pupal stage of *S. littoralis* was 15 days, this stage was lengthened to 17 days in pupal treated as 2nd instar larvae with LC_{50} of *B thuringiensis*. The results agreed with El-Sabagh *et al.* (2017) and Amein (2023).

Table (3) stated the results of treating *S. littoralis* larvae in their fourth instar with LC_{50} of Flufenoxuron. It was determined that the larval stage from the first treated instar was 13 days, which was 3 days longer than their control. The pupation percentage of larvae treated as 4th instar with LC_{50} of Flufenoxuron was 49.0 %. Furthermore, the weight of newly formed pupae was significantly increased to 0.26 gm. which was only a 0.05 gm. increase than those of the control (Table 3). Under conditions of the present work, in untreated insects the duration of the pupal stage was 13.1 days. The pupal stage of larvae treated with LC_{50} value as fourth instars increased to 15.0 days, according to the larvae. Prior research using El-Banna *et al.* (2020) has demonstrated these.

After treating 2nd instar larvae with *B. thuringiensis* LC_{50}, the percentage of adult eclosion remained largely unchanged at 76.51%, a notable decrease from the control group (Table 4). Moreover, the mean adult life span of both males and females treated with *B thuringiensis* has been markedly reduced. The results agreed with María *et al.* (2022), showed high toxicity towards the fall armyworm, *Spodoptera frugiperda* (J.E. Smith), 4th instar larvae in bioassays using the microdroplet ingestion technique of *B. thuringiensis.*

Table (4) demonstrated the impact of the applied LC_{50} of Flufenoxuron against *S. littoralis* larvae in their fourth instar. Results showed that treatment with tested compound highly decreased adult emergence percentage as they treated as pupae. The adult emergence percentage was 83.33% compared to untreated group. Moreover, results presented in Table (4) revealed the effect of tested compound on the mean adult life span of both male and female moths. Comparing with the untreated group, the treatment has decreased the mean male and female longevity. These results agreed with El-sayed *et al.* (2017) and Shaurub *et al.* (2020) who reported a significantly decreased in adult emergence percentage and adult life span due to treatment of *S. littoralis* with different types of Insect growth regulators.

Table 3. Larval duration from initial instar treated, pupation percentage, weight and duration of pupae of *S. littoralis* treated with LC_{50} as 2nd instar larvae of *B thuringiensis kurstuki* and as 4th instar larvae of Flufenoxuron

Treated Instar	Mean larval duration post treatment (days ± S.E.)	Pupation %	Mean pupal weight (gm ± S.E.)	Mean pupal stage duration (days ± S.E.)
B. thuringiensis	18.7** ± 0.41	56.67	0.24* ± .012	17.0** ± 0.29
Control	15.3± 0.18	86.67	0.35 ± .029	15.0 ± 0.18
Flufenoxuron	13.0**± 0.12	49.0	0.26*± .012	15.0**± 0.24
Control	10 ± 0.17	88.0	0.31 ± .022	13.1± 0.06

**: moderately significant ($p < 0.01$) and *: significant ($p < 0.05$), (student-t test).

Table 4. The percentage of adult emergence and adult longevity of *S. littoralis* treated with LC_{50} as 2nd instar larvae of *B. thuringiensis kurstuki* and as 4th instar larvae of Flufenoxuron

Treated Instar	Adult emergence %	Mean of adult life span (days ± S.E.)	
		♀	♂
B. thuringiensis	76.51	8.67** ± 0.07	7.33** ± 0.11
Control	92.31	12.3 ± 0.18	11.33± 0.19
Flufenoxuron	83.33	10.0** ± 0.12	7.63** ± 0.18
Control	96.29	12.8 ± 0.24	10.60±0.12

**: moderately significant ($p < 0.01$), (student-t test).

Histological Studies:

McFarlane (1985) and Chapman (1988) provided extensive documentation on the histological anatomy of the midgut in untreated *S. littoralis* larvae. A single layer of three different types of cells that lie on a basement membrane makes up the epithelial layer that lines the midgut, as depicted in Fig. (1). The majority of which were expressed by columnar cells which contain a large coarse nucleus that occupies a middle position within the cell and bears a striated or brush-like border (microvilli). Also, several calyx-shaped goblet cells were scattered randomly between the columnar cells, and each has a large ampulla opening by a narrow neck in the lumen of the midgut inner surface. Regenerative cells were also detected that appear small in size and rest on the basement membrane between the bases of the epithelial cells. The cells have an elongated or spherical appearance, with a big nucleus encircled by a tiny amount of cytoplasm that is extremely basophilic.

The histological structure in larvae after 6 days surviving their treatment with LC_{50} of Flufenoxuron (Fig. 2) as 4th instar larvae appear somewhere impaired. The columnar cells grow considerably enlarged with damaged microvilli and lose their compact look. Additionally, while the cells are intact in certain regions, the epithelial cells lose their strong link with the basement membrane in other areas. The peritrophic membrane appears detached or broken down in some places. Lysis of muscle layer. On the other hand, treatment of 4th instars of *S. littoralis* with the LC_{50} of *B. thuringiensis* (Fig. 3) exfoliation of the midgut epithelium from the underlying circular muscle fibers occurred. There was clear peritrophic membrane rupture as well as vacuolization of the midgut epithelium and striated boundaries. Towards the end of the sixth late instar, some of the degenerated columnar cells fuse with the damaged peritrophic membrane.

Both the muscle layer and the epithelium layer separated, losing their compact look. When LC_{50} of *B. thuringiensis* zero time and LC_{10} of flufenoxuron were evaluated, this effect was more noticeable (Fig. 4). On the other hand, that treatment of *S. littoralis* 4th instars with the LC_{10} of Flufenoxuron in combination with LC_{50} of *B. thuringiensis* after 48H (Fig. 5) caused exfoliation of the midgut epithelium of surviving 6th instars from the underlying circular muscle fibers, leaving a large vacuole or space. Vacuolization of the midgut epithelium and disappearance of peritrofic membrane. The findings are consistent with what Iman (2018) and Saleh *et al.* (2021) have indicated.

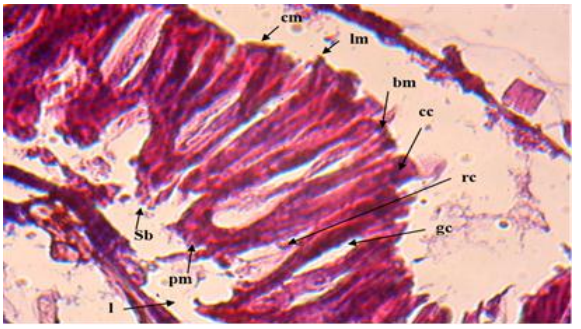


Fig. 1: T. S. in the mid gut of untreated late 6th instar larvae of *S. littoralis* (40 X)

lm: longitudinal muscle layer, **cm:** circular muscle layer, **bm:** basement membrane, **rc:** regenerative cell, **cc:** columnar cell**, Sb:** striated border, **pm:** peritrophic membrane, **gc:** goblet cell and **l:** lumen.

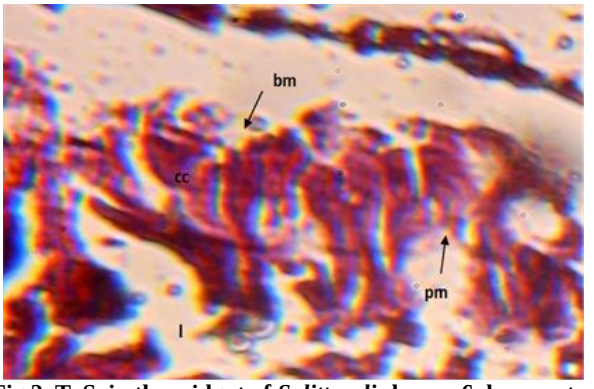


Fig 2. T. S. in the midgut of *S. littoralis* larvae 6 days post treatment with LC_{50} of Flufenoxuron as 4th larvae instar (40 X)

bm: basement membrane, **rc:** regenerative cell, **cc:** columnar cell**, pm:** peritrophic membrane and **l:** lumen.

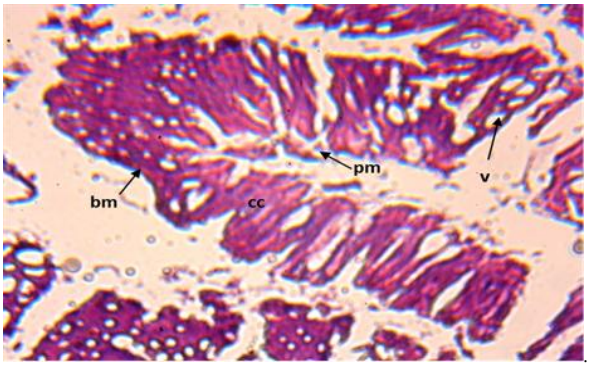


Fig 3. T. S. in the midgut of *S. littoralis* larvae 6 days post treatment with LC_{50} of *B. thuringiensis* as 4th larvae instar (40 X)

bm: basement membrane, **cc:** columnar cell, **v:** vacuoles and **pm :** peritrophic membrane.

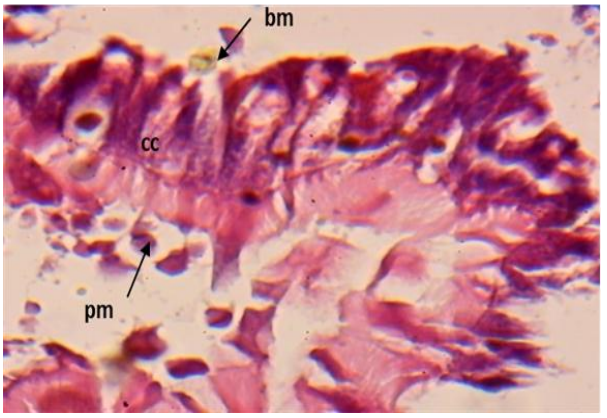


Fig 4. T. S. in the midgut of *S. littoralis* larvae 6 days' post treatment with LC_{10} of Flufenoxuron pretreated with LC_{50} of *B. thuringiensis* as 4th larvae instar (20 X)

bm: basement membrane, **cc:** columnar cell and **pm:** peritrophic membrane.

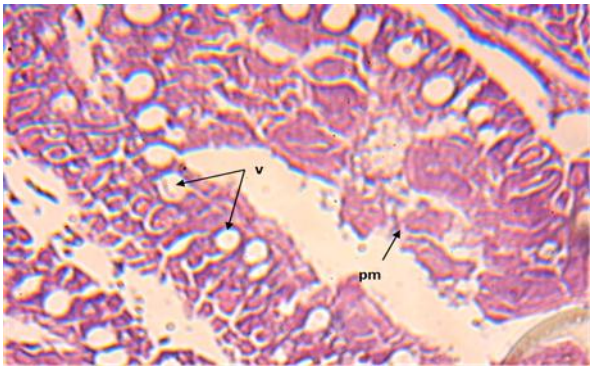


Fig 5. T. S. in the midgut of *S. littoralis* larvae 6 days post treatment with LC_{50} of *B. thuringiensis* pretreated with LC_{10} of Flufenoxuron as 4th larvae instar (40 X)

Fat body:

Normally, the late sixth instars of *S. littoralis* have a fat body that resembles a ribbon. An intricate membranous coating covers the cell masses' exterior surfaces, and the fat cells are intimately attached. Fig. (6) revealed that their cytoplasm is inclusion-free and uniform. When fourth instars were treated with Flufenoxuron at the LC_{50}, the fat bodies of the surviving late sixth instars collapsed, and minor vacuolization occurred (Fig. 7). But in fat cells of *S. littoralis* sixth instars, treatment with the LC_{50} of *B. thuringiensis* had no effect (Fig. 8). In earlier research, Badr and Darwish (2024) demonstrated these.

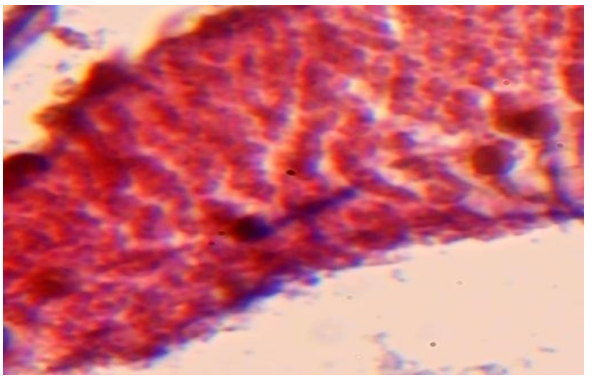


Fig 6. T. S. in the fat body of normal late 6th larval instar of *S. littoralis* (X 40)

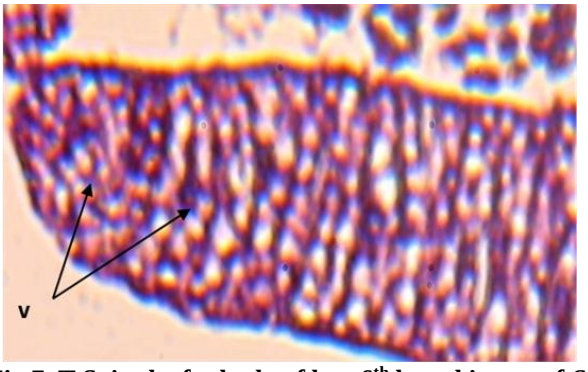


Fig 7. T.S. in the fat body of late 6th larval instar of *S. littoralis* treated with LC_{50} of Flufenoxuron as 4th larvae instar (40 X)

V: Vacuole

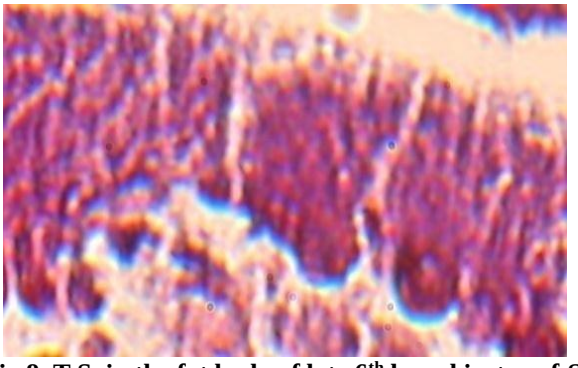


Fig 8. T.S. in the fat body of late 6th larval instar of *S. littoralis* treated with LC_{50} of *B. thuringiensis* as 4th larvae instar (40 X)

CONCLUSION

In the current study, use two compounds *B. thuringeinsis var. kurstaki* and Flufenoxuron (CSI's) on the 2nd and 4th instar Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.) to detect the toxicological, biological and histological changes. The

use of LC_{10} and LC_{50} values of *B. thuringiensis* were effect on the intended insect. Furthermore, interaction effects were observed upon application of LC_{10} of Flufenoxuron in combination with LC_{50} of *B. thuringiensis*. The histological studies on the 2nd and 4th instar larvae with LC_{50} either *B. thuringiensis* or Flufenoxuron showed vacuolation in the epithelial layer, disruption of the peritrophic membrane as well as in the microvilli. Addition of Flufenxuron (LC_{10}) to *B. thuringiensis* (LC_{50}) showed vacuolation and exfoliation of the columnar cells. These results suggested the potential utilization of *B. thuringeinsis var. kurstaki* and Flufenoxuron (CSI's) for the effective control of *S. littoralis* population in Egypt.

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الملخص العربي

تحفيز المركب البكتيري باثيلس ثورانجينسس بمنظم نمو الحشري الفلوفينوكسيورون لمكافحة يرقات دودة ورق القطن

الشيماء نجيب إبراهيم، نشوي سعيد أمين، عزيزه السيد عبد العال، أمال ثابت ثابت حسين

تمت دراسة الأثار السمية والبيولوجية والهستولوجية للمركب البكتيري باثيلس ثورانجينسس ومنظم نمو الحشري الفلوفينوكسيورون (المثبط لنخليق الكيتين) ضد يرقات العمر الثاني والرابع لدودة ورق القطن علي التوالي، تحت ظروف معملية. أشارت النتائج إلى أن قيم LC_{10} وLC_{50} ليرقات العمر الثاني المعاملة بمركب باثيلس ثورانجينسس حيث بلغت ٠,٠٠٢٧ و٠,٢٣٣١ جم/مل، على التوالي. وفي الوقت نفسه، كانت هذه القيم ٠,٠٠٢٥ و١,٤٧١٦ جزء في المليون ليرقات العمر الرابع المعاملة بمركب لفلوفينوكسورون. وقد لوحظ التأثير المشترك عند استخدام التركيز السام LC_{10} لمركب لفلوفينوكسورون مع التركيز النصفي مميت LC_{50} لمركب باثيلس ثورانجينسس في وقتين مختلفين (Zero time و بعد ٤٨ ساعة) من معاملة يرقات العمر الثانية بمركب باثيلس ثورانجينسس. حيث أظهرت النتائج ارتفع معدل الوفيات لليرقات المعاملة بمركب باثيلس ثورانجينسس وحده بنسبة ٥٠,٠٠١% إلى ٥١,٢٢ و٦٥,٠٣% عند إضافة منظم النمو الحشري في وقتي Zero time و٤٨ ساعة على التوالي. كما سجلت نسبة الوفيات ٥٢,٣٢% للتركيز السام LC_{10} لمركب الفلوفينوكسورون بعد المعاملة بالتركيز النصف مميت LC_{50} للباثيلس ثورانجينسس. وأدت المعاملة بصفة عامة إلي إطالة متوسط فترة الطور اليرقي والعذري لليرقات الباقية على قيد الحياة من معاملة العمر اليرقي الثاني أو الرابع بالتركيز النصفي المميت LC_{50} للمركبين باثيلس ثورانجينسس والفلوفينوكسورون على التوالي. كما أدى المعاملة إلى انخفاض جميع التأثيرات البيولوجية الأخرى (نسبة التعذر، وزن العذراء، ونسبة خروج الفراشات متوسط عمر الطور البالغ لكلا الجنسين) مقارنة باليرقات غير المعاملة. كما أوضحت هذه الدراسة التغيرات النسيجية التى تحدث فى طبقات المعى الأوسط فى اليرقات بعد ٦ أيام من معاملة العمر الرابع بالتركيز القاتل لـ٥٠% من اليرقات المعاملة بمركب باثيلس ثورانجينسس والفلوفينوكسورون فقد كانت التغيرات السائدة هي تكوين فجوات في الخلايا الطلائية مع أحداث تلف في الغشاء المحيط بالغذاء.