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BIOLOGICAL AND HISTOLOGICAL EFFECTS OF SPINOSAD ON *spodoptera littoralis* (BOISD.).

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ABSTRACT

The efficiency of Spinosad was evaluated on larvae of the Egyption cotton leaf worm, *Spodoptera littoralis* (Boisd): (Lepidoptera: Noctuidae). Second instar larvae were more sensitive to Spinosad than 4th instar larvae, as denoted by the determined LC_{50} which was 5.73 and 7.83 ppm for 2nd and 4th instars, respectively. Meanwhile, LC_{90} was 37.15 and 43.49 ppm for the respectively mentioned instars compared to their control. The duration of treated larvae was lengthened when either instars larvae were treated as compared to their control. Furthermore, percentage of pupation was reduced than the control by 51.03 and 49.5% when 2nd and 4th instar larvae were treated with LC_{50} of Spinosad, respectively. Adult emergence was insignificantly affected as it was less than the control by 5.3 and 4.1% to the respective mentioned treated larval instars. The number of laid eggs was insignificantly affected; however, percentage hatchability was markedly reduced. The total protein content in larvae treated as 4th instar showed a gradual increase from 2nd and 6th days after treatment as it was 34.79- 35.18 mg respectively, compared with 25.8 to 26.5 mg in control. The midgut tissue of 5th instar larvae treated in the 4th instar larvae with LC_{50} of Spinosad showed some alternations and several aberration in some cell organelle such mitochondria, nucleolus, microvilli and golgi apparatus as observed by light and electron microscope.

Keywords: *Spodoptera littoralis*, Spinosad, biological, total protein and histological.

INTRODUCTION

Spinosad is a bioproduct from the naturally occurring soil actinomycete, *Saccharopolyspora spinosa* which was found to have a high insecticidal activity. It is now commercially formulated under the name Spinosad (Thompson *et al.* 1997 and Sparks *et al* 1998). This formulation acts primarily on the insect's nervous system at the nicotinic acetylcholine receptor and also exhibits activity on the gamma-amino butyric acid receptor GABA (Salgado, 1997; Thompson and Hutchins 1999; Sparks *et al.* 2001). Spinosad shows a low toxicity to mammals and beneficial insects (Liu *et al.*, 1999 and Dutton *et al*., 2003), which attributes its ideal use in an IPM programme and to be used carefully within well planned resistance management strategy .The objective of the present research was to evaluate the susceptibility of 2nd and 4th instars larvae of the Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisd.) to Spinosad, and its effects on some biological and histological aspects of this insect.

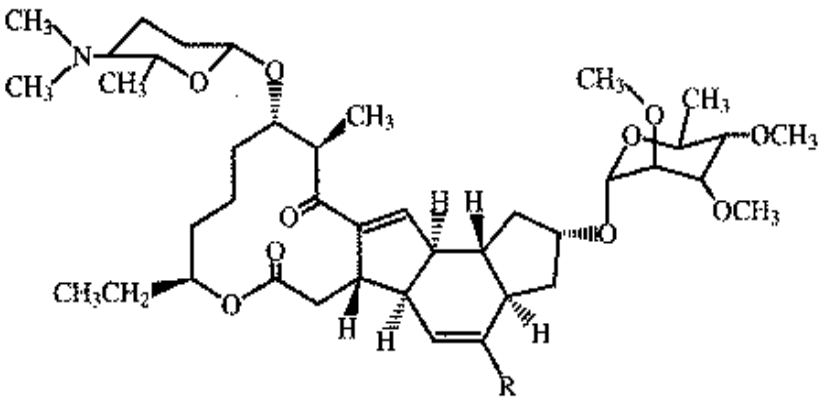
MATERIALS AND METHODS

-Rearing technique of *Spodoptera littoralis*:

The rearing of *S. littoralis* was conduced under laboratory conditions at 27±2^{O}C and 60 ± 5% RH. Egg masses of a susceptible strain of *S. littoralis* were obtained from a well establish culture at the Research Division of the Cotton Leafworm, Plant Protection Research Institute, ARC. Newly hatched larvae were transferred to clean glass jars covered with muslin held in position with rubber bands. Larvae were fed on fresh castor bean leaves, *Ricinus communis* (L.) which was supplied daily in sufficient amounts. Upon pupation, pupae were collected; sexed and emerged moths were placed in pairs in breeding glass globes and supplied with branches of, *Nerium oleander* (L.) containing several leaves as an oviposition site. Each globe was provided with 10 % honey solution soaked in cotton wool which was placed in small plastic cup for moths feeding. The honey solution was renewed daily to avoid fermentation and growth of microorganisms.

-Chemical structure of Spinosad :-

Spinosad was obtained from Dow Agroscience Co. and has the following chemical structure:



spinosyn A: R = H, MW = 731.98
spinosyn D: R = CH_3, MW = 746.00

- Bioassay Technique :-

- Susceptibility test:

Insecticidal activity of Spinosad was assessed against 2nd and 4th instar larvae of *S. littoralis*. A series of aqueous concentration of Spinosad were prepared, (50, 25, 12.5, 6.125 and 3.062 ppm.)

The leaf dipping technique was applied as described by Abo El-Ghar *et al*., (1994), clean fresh castor bean leaves were dipped in one of the prepared concentrations. Leaves were left to dry at room temperature and offered to the newly ecdysed 2nd and 4th instar larvae for 24 hours after which larvae

were fed on untreated leaves till the end of the larval stage. Four replicates, each containing 20 larvae, were used for each concentration. As a control, castor bean leaves were dipped in distilled water and offered to larvae of the same instars. Cumulative larval mortalities were determined and corrected by Abbott's formula (Abbott, 1925). The data were subjected to probit analysis (Finney, 1971) for determining the LC_{90} and LC_{50} values for Spinosad.

- Biological studies:

From the maintained insect culture, newly ecdysed 2nd and 4th instar larvae were collected and fed on castor bean leaves treated with the determined LC_{50} value of Spinosad. Larvae were examined daily and the following parameters were studied ,duration of each larval instar, pupation percentage, pupal stage duration and percentage adult eclosion. Eventually, the reproductive potential of moths emerging from the treatment of 2nd or 4th instars larvae of *S. littoralis* larvae with LC_{50} value was determined. Upon pupation, pupae were sexed then placed in pairs in glass globes, allowed to mate and laying their eggs. The number of oviposited egg masses, number of eggs per each egg mass as well as egg hatchability were determined. A control was set comprising a similar number of untreated moths.

- Biochemical studies:

Total protein content in larvae treated withLC_{50} was determined after 2nd, 4th and 6th days post treatment according to the method described by Bradford (1976).

- Histological studies :

The histology of the midgut in 5th instar *S. littoralis* larvae, surviving the treatment of 4th instar larvae with the determined LC_{50} of Spinosad was studied. Similarly, investigation of untreated same instar larvae was considered as a control. Larvae were dissected in Ringer solution and their mid gut were removed and quickly fixed in aqueous Bouin's. Midgut samples were then prepared for paraffin sectioning according to Junqueira and Carneiro (1980), cross section was carried out at a 6µ thickness by a microtome and mounted on glass slides. Sections were stained with Haematoxylene and counterstained in alcoholic Eosin and prepared for observation by light microscope: To study the ultrastructure of the larval midgut tissues, the midgut was removed from 5th larvae instar previously treated as 4th instars with the LC_{50} value of Spinosad, the fixed in cacodylate buffered glutaraedhyde, and post fixed in 2% osmium tetroxide and prepared for electron microscope investigation according to Reese *et al.,* (1972); McLaughlin and Sikorowski, (1978)

RESULTS AND DISCUSSION

The younger 2nd instar larvae of *S. littoralis* were found to be more sensitive to Spinosad as depicted by the determined LC values, the LC_{50} and LC_{90} were 5.73 and 37.15 ppm, respectively. Meanwhile, these values were 7.83 and 43.49 ppm when the older 4th instar larval were treated, results are

shown in [Table 1]. The slope values were 0.92 and 1.87 for 2nd and 4th instars, respectively, showing the homogeneity of the larvae.

The accumulative percentage mortality at the termination of the larval stage [Table 1] were relatively similar when either 2nd or 4th instar larvae were treated with the determined LC_{50} giving 52.5 and 51.0%, respectively. Meanwhile, these percentages reached 90% when LC_{90} was administered to either 2nd or 4th instar larvae, but none of these few surviving larvae pupated and eventually died. Generally, treated larvae were observed to be less active in their movement with obvious muscle contractions and prior to their death larvae exhibited severe tremors followed by paralysis. Meanwhile, other larvae treated with LC_{50} values as 2nd or 4thinstars showed lesser Effects, leading to their recovery.

Table 1. Susceptibility of *S littoralis* 2nd and 4th instar larvae to Spinosad.

				Accumulative % mortality (at the end of larval stage)	
Larval Instars	**LC_{50} (ppm)**	**LC_{90} (ppm)**	**Slope**	**LC_{50} (ppm)**	**LC_{90} (ppm)**
2nd	5.73	37.15	0.92	52.5	90.0
4th	7.83	43.49	1.87	51.0	90.5

The duration of the subsequent larval instars treated as 2nd instar with LC_{50} of Spinosad was 16.0 days which was more than that of the control by 2 days, i.e. an increase of 14.3% than the control [Table 2]. The percentage of larvae metamorphosing into pupae following treatment of 2nd instar larvae was low (47.5%) which was a reduction by nearly half its value in untreated insects [Table 2]. Meanwhile, the duration of the pupal stage was 6.9 days as compared to 9.0 days in the control, i.e. less than the control by 2.5 days making a 23.9% reduction. Meanwhile, percentage of adult emergence was a high of 93.5% which was only a slight decrease than their control by 5.3%. Emerged moths exhibited a non significant shorter life span [Table 2]. Effect of Spinosad on 4th instar larvae was restively similar to the treatment of 2nd instar larvae. The larval instars following treatment of 4th instars with LC_{50} value of Spinosad was lengthened by 2 day, i.e. 12.0 days as compared to 10.0 days in the control making a 20.0% increase. Percentage of larvae entering the pupal stage was reduced to 49.0%. Meanwhile, adult eclosion was not affected as 95.9% of pupated insects emerged as moths. The life span of these emerged moths was 9.7 days as compared to 12.0 days in the control, i.e. a decrease in their life span by 19.6%.

The reproductive potential of mated moths emerging from larvae treated as 2nd instar with Spinosad at LC_{50} value showed that their reproductive potential was slightly significantly affected [Table 3]. The number of laid egg masses was 7 per female, comprising a total of 1335.6 eggs as compared to 5 egg masses/female with a total of 1476.3 eggs in the control. However, egg fertility impaired as percentage hatchability was 42.3% in moths emerging from treated 2nd instar larvae.

Table 2. Larval duration, pupation percentage, pupal duration and moth emergence of *S. littoralis* treated as 2nd or 4th instar larvae with LC_{50} of Spinosad.

Treated Instar	Mean larval duration (days± S.E.)	Pupation %	Mean pupal stage (days± S.E.)	Adult emergence %	Mean adult longevity (days± S.E.)
2nd instars	16.0 ± 0.3** (14.3)	47.5 (51.03)	6.9 ± 0.5* (23.9)	93.5 (5.3)	12.7 ± 0.4 ns (4.5)
Control	14.0 ± 0.2	97.0	9.0 ± 0.3	100	13.3 ± 0.2
4th instars	12.0 ± 0.3** (20.0)	49.0 (49.5)	8.3 ± 0.2** (22.2)	95.9 (4.1)	9.7 ± 0.2** (19.6)
Control	10.0 ± 0.3	97.0	10.7 ± 0.2	100	12.0

Numbers between brackets are percentages of reduction than the control.
ns: Not significant. *: Significant at P< 0.05
****: highly significant at P< 0.01.**

Similarly, when LC_{50} of Spinosad was administered to 4th instar larvae, emerged moths oviposited 5.3 egg masses patches /female with a total sum of 1267.6 eggs. Fertility was 66.5% which was less than those obtained from 2nd instars were treated, but lower than their control by 32%.

Table 3. Reproductive potential of *S. littoralis* moths emerging from larvae treated as 2nd or 4th instars with LC_{50} of Spinosad.

Instars Treated	No. of egg masses/female ± S.E	Mean No. of Eggs/female ± S.E	Egg hatchability %
2nd instars	7.0 ± 0.5**	1355.6 ± 31.7* (8.2)	42.3 (56.6)
Control	5.0 ± 0.2	1476.3 ± 26.6	97.5
4th instars	5.3 ± 0.2*	1267.7 ± 28.9* (8.2)	66.5 (32.0)
Control	4.3 ± 0.3	1380.3 ± 23.0	97.8

Numbers between brackets are percentage of reduction than the control.
ns: not significant. *: Significant at P< 0.05
****: highly significant at P< 0.01.**

(1) Total protein content in *S. littoralis* larvae:

In untreated larvae, total protein contents ranged approximately between 25.8 to 26.5 mg, [Table 4].

When total protein content was determined on the 2nd day post treatment in larvae treated with LC_{50} of Spinosad, it was found to be 34.79 mg which was an increase than their control by approximately 8.91mg. This value then dropped to nearly it's value in untreated insects on the 4th day post treatment only to rise again on the 6th day to 35.18mg, as compared to 26.49mg in the control.

Table 4. Total protein content in larvae treated as 4th instars larvae with LC_{50} Spinosad.

Experimental larvae	Days after treatment		
	2nd	4th	6th
Treated	34.79** ± 1.9	24.6ns ± 0.6	35.18** ± 0.8
Untreated (Control)	25.88 ± 1.6	26.35 ± 1.3	26.49 ± 1.4

**: highly significant a P< 0.01. ns: not significant.

- Histology of midgut in 5th instars larvae treated with LC_{50} of Spinosad as 4th instar:

The histological structure of the midgut in untreated 5th instars of *S littoralis* larvae is composed of an epithelial layer composed of closely arranged granulated columnar epithelial cells, bearing an apical microvilli border. Also, observed are small goblet cells and regenerative cells are observed, these cells rest on a basement membrane. The epithelial cells are shielded from the midgut lumen content by a peritrophic membrane. Encircling the epithelial layer is muscular layer, formed of longitudinal fibers to the outside and circular layer of muscles adjacent to the epithelial layer (Fig. 1). Treatment of *S littoralis* 4th instars larvae with LC_{50} of Spinosad, caused detachment of the musclosa. The columnar cells loose their compact appearance and become some what distended with disrupted microvilli. Also, the epithelial cells lose their close association with the basement membrane, in other areas the cells remain intact and in some areas vacuoles are apparent. The peritrophic membrane appears detached or broken down in some places (Fig. 2). The midgut ultrastructure of untreated *S. littoralis* 5th instar larvae show that the basal labyrinth of the columnar cells consists of numerous basal infoldings. These infoldings are perpendicular to the basal lamina, and incompletely divide the basal cytoplasm into compartments of varying sizes. These compartments are characterized by the presence of mitochondria with well-developed dense cristae (Fig. 3). Goblet cell cavity contains large numerous cytoplasmic projections towards the base of the cell, but towards the apical region the cytoplasmic projections were smaller (Fig. 5). The conspicuous brush border seen with the light microscope is resolved as a regular array of long and thin microvilli (Fig 7). The nucleus are large and occupy a middle position within the cell (Fig 9). The midgut in 5th instar larvae treated as 4th instar with LC_{50} of Spinosad shows that the mitochondria were partly destroyed (Fig. 4). The goblet cell of treated larvae lost its cytoplasmic projections and amorphous granules can be seen within its cavity (Fig. 6). The microvilli in treated larvae were shorter, broken and prominent gaps were seen among them. Peritrophic membrane was also disintegrated (Fig. 8). The chromatin material of the nucleus was disorganized (Fig. 10).

The LC_{90} or LC_{50} of Spinosad was reached to the termination of the larval stage, which proves the slow action of this chemical to *S. littoralis* larvae. This observation is supported by the fact that mortality was relate wily low soon after treatment but increased gradually with larval development.

Furthermore, the larval stage was extended and longer than that of untreated larvae. Salgado, (1998) reported that Spinosad causes an excitation of the insect's nervous system and alters the function of nicotinic and GABA-gated ion channels. This might explain the symptoms observed in treated larvae leading to the insects paralysis, furthermore, according to Wanner *et al.*, (2000) paralysis was a primary effect of Spinosad with mortality as a secondary result.

The reduced number of larvae entering pupation or moth emergence could be a result of accumulation of toxic material in the insects body as suggested by Adan *et al*., (1996), Scott (1998) and Wanner *et al.*, (2000).

The fecundity of moths emerging from treated larvae was not greatly affected. This fact is some what supported by the high level of protein content in treated insects suggesting that production extraovarion yolk was not impaired. However, egg fertility was lower in moths emerging from treated larvae with LC_{50} values, especially when 2^{nd} instar larvae were considered, presumably a factor affected egg viability and further physiological studies are needed to verify this observation. The present study shows that Spinosad caused some histological symptoms to the midgut of *S. littoralis* larvae treated with LC_{50} of Spinosad. In the available literature little information explains the histological changes in the midgut of lepidopterous larvae as a result of feeding on Spinosad. Vacuolation and sloughing in midgut of treated insects may suggest that some metabolic disturbance has been done. This suggestion was offered by Hussien (2003) and Fahmy (2005). In this study, one of the most common ultrastructural changes observed with LC_{50} of Spinosad caused enhancement of lysosomal organelles in the midgut columnar epithelium, disruption of microvilli, and fragmentation of the nuclear chromatin materials. Similar observations were reported by Refaie and El-Shazly (2001) and Abdel-Aal (2003) in insects treated with some insect growth regulators.

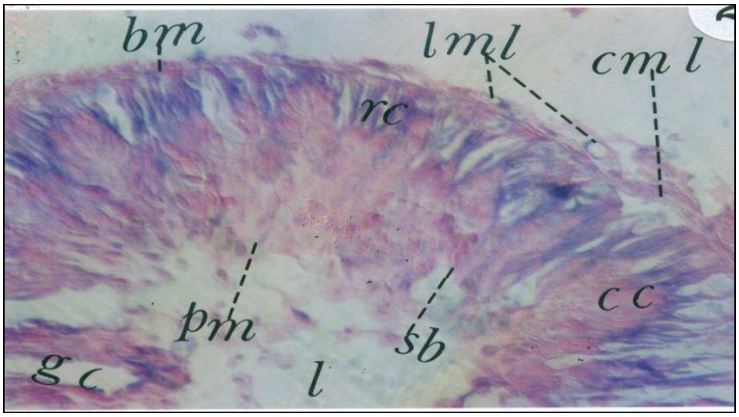


Fig. 1. T.S.in the midgut of untreated 5th instar larvae of *S. littoralis*(20 X).

lml: longitudinal muscle layer.
cml: circular muscle layer.
bm: Basement membrane.
rc: regenerative cell.
cc: columnar cell.
Sb: striated border.
pm : peritrophic membrane.
gc: goblet cell.
L: lumen.

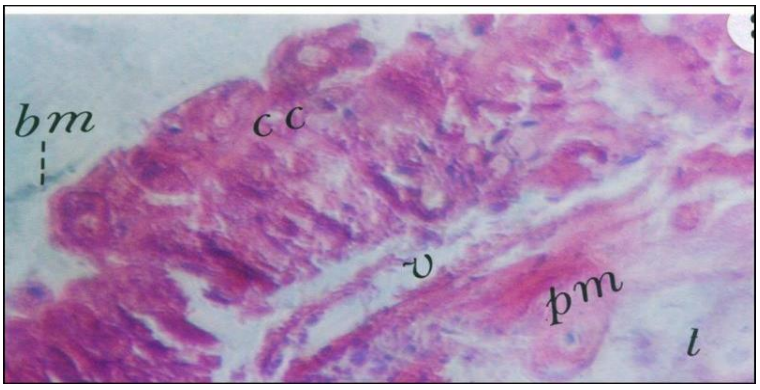


Fig. 2 T. S. in the midgut of 5th instar larvae of *S. littoralis* treated 4th instar with LC_{50} of Spinosad as (20 X).

bm: basement membrane [broken up in some areas].
cc: Columnar cell were compacted and lost their appearance and become detached from the basement membrane.
V : Vacuoles appear between pm and cc].
pm : peritrophic membrane.[appear detachment].
L: lumen.

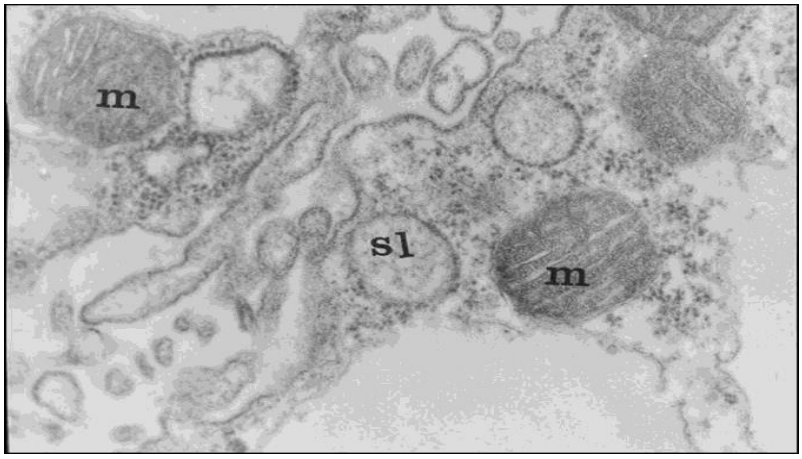


Fig.3. Micrograph showing mitochondria and lysosomes bodies in the midgut columnar cells in untreated 5th instar of *S. littoralis* larvae *(*X 14000).

m: mitochondria.
sl: secondary lysosomes

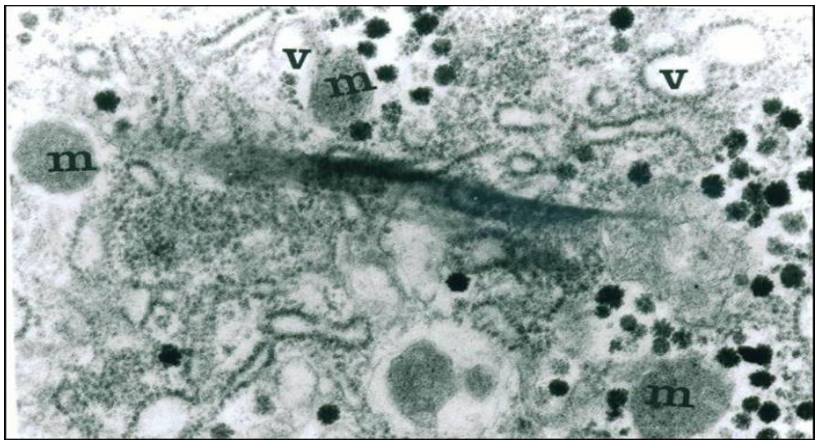


Fig. 4. Micrograph showing mitochondria of the midgut columnar cells of 5th instar larvae of *S. littoralis* treated as 4th instar with LC_{50} of Spinosad (X 14000).

m: broken mitochondria cristae
v: vacuoles appears in the cytoplasm of the columnar cells].

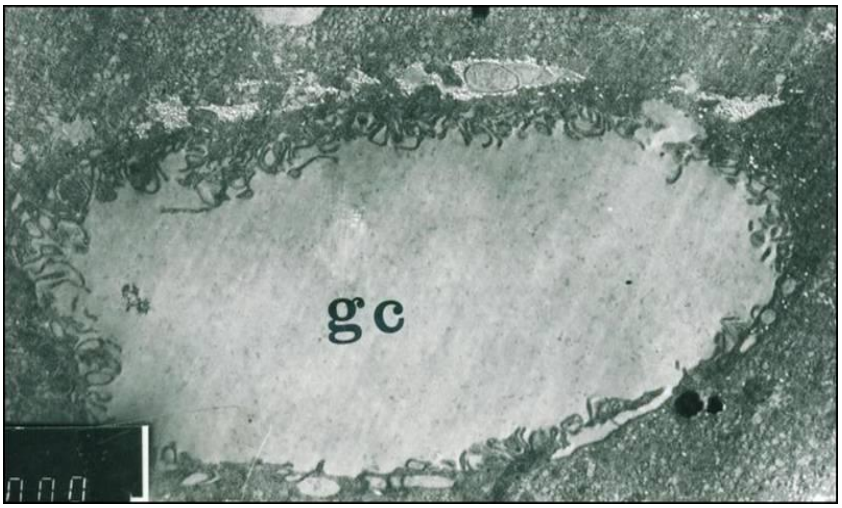


Fig. 5 . Micrograph of the goblet cell cavity in the midgut of untreated 5th instar *S. littoralis* larvae (X 50000).
gc :goblet cell cavity.

Fig. 6. Micrograph of the goblet cell cavity in the midgut of 5th instar larva of *S. littoralis* treated as 4th instar with LC_{50} of Spinosad (X 4000).

gc :goblet cell cavity showing destruction of cytoplasmic projection and appearance of amorphous granules within the cavity.

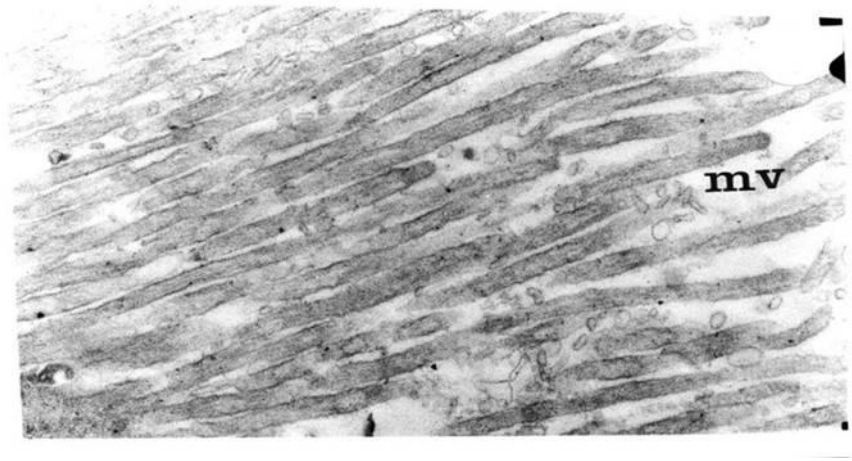


Fig. 7. Micrograph in a small portion of the apical region of the midgut columnar cell in untreated 5th instar *S. littoralis* larvae showing the microvilli and fine filamens with the cytoplasm, many of which project into the microvilli (arrow) (X 3000).
mv: microvilli

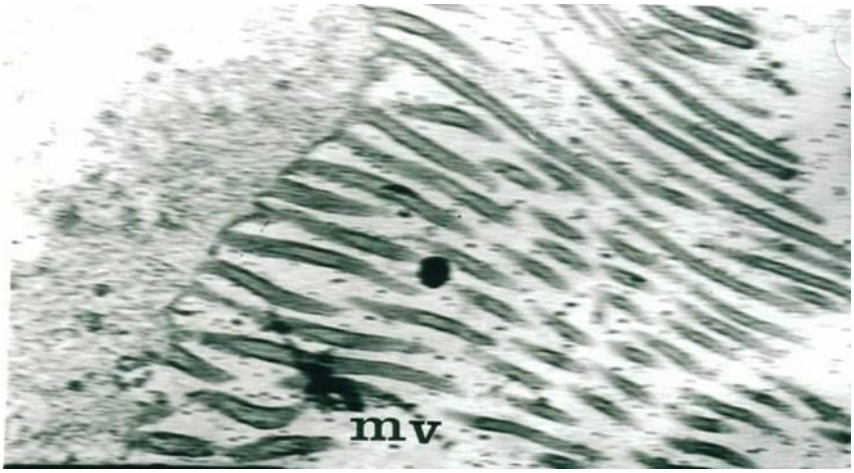


Fig. 8. Micrograph in the apical region of the midgut columar cell of 5th instar of *S. littoralis* larvae treated as 4th instar with the LC_{50} of Spinosad (X 3000).

mv: microvilli [showing destruction of microvilli].

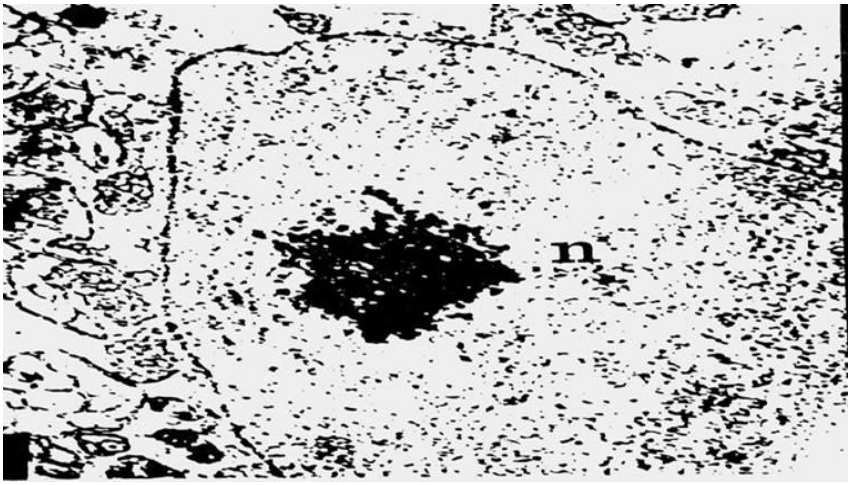


Fig. 9. Micrograph of the midgut columnar cell nucleus of untreated 5th instar *S. littoralis* larvae (X 3000).
n: nucleus

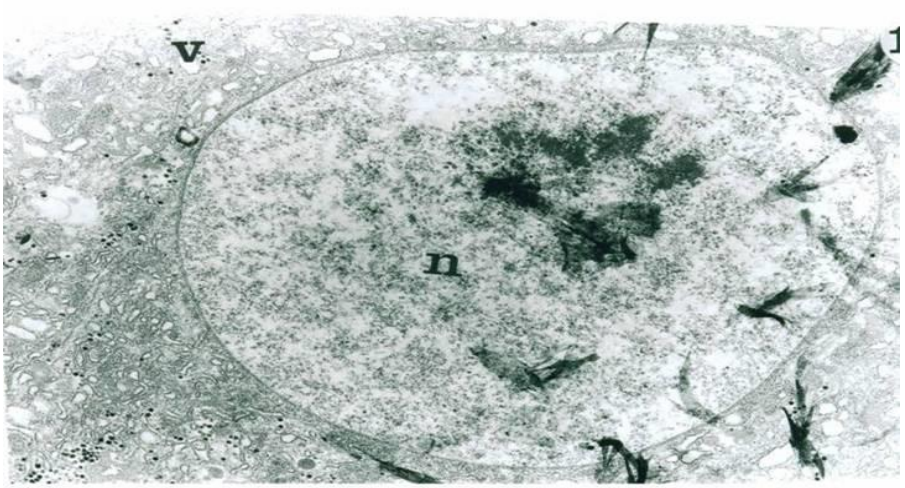


Fig.10. Micrograph of the midgut columnar cell of late 5th instar larvae of *S. littoralis* treated as 4th instar with the LC_{50} of Spinosad (X 3000).
n: nucleus showing disorganized the chromatin material.
v: appearance of vacuoles in the cytoplasm of midgut cells.

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التأثيرات البيولوجية والنسيجية لمركب السبينوساد على دودة ورق القطن

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2- قسم وقاية النبات - كلية الزراعة - جامعة عين شمس – شبرا الخيمة- القاهرة- مصر.

أجريت الدراسة لتقييم فاعلية عدة تركيزات من مركب سبينوساد علي العمرين الثاني والرابع ليرقات دودة ورق القطن (رتبة حرشفية الأجنحة). وقد أظهرت النتائج أن العمر اليرقي الثاني كان أكثر تأثرا بالمركب من العمر اليرقي الرابع وقد أتضح ذلك عند تقدير التركيز النصف مميت LC_{50} حيث بلغ 5.73، 7.83 جزء في المليون للعمرين الثاني والرابع علي التوالي. كما زاد طول العمر اليرقي بالمقارنة باليرقات غير المعاملة و أدت المعاملة إلى نقص في نسبة التعذر بحوالي 51.03 و49.5 % و نقص غير معنوي في معدل خروج الفراشات بنسبة 5.3 و4.1عند معاملة العمرين الثاني والرابع علي التوالي. كما لوحظ عدم وجود تأثير معنوي للمعاملة علي عدد البيض الموضوع ولكن انخفضت نسبة الفقس و كان للمعاملة تأثير علي المحتوي الكلي للبروتين في يرقات العمر الرابع المعاملة بالتركيز نصف المميت (LC_{50}) بزيادة تدريجية من اليوم الثاني إلى اليوم السادس للمعاملة حيث سجلت 34.79 ، 35.18 ملجم، مقارنة بالكنترول الذي سجل 25.88، 26.49 ملجم من اليوم الثاني إلى اليوم السادس. كما أدت معاملة يرقات العمر الرابع بالتركيز النصف المميت LC_{50} لمركب سبينوساد إلي حدوث تغييرات نسيجية في المعي الأوسط في عضيات الخلايا مثل الأجسام السبحية (الميتوكوندريا)، النواة، الخملات الدقيقة و جهاز جولجي. وقد تم فحص تلك المكونات الخلوية بواسطة المجهر الضوئي ودعّم بالمجهر الإلكتروني.