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Biological and Histological Effects of Certain Insecticides on *Spodoptera littoralis* (Bosid)

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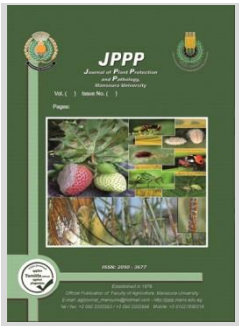

ABSTRACT

This work aims at throw light on the efficiency of Lufenuron (Owner 5%), diflubenzuron (Dimilin48%), Emamectin benzoate (Emafel4%) and Indoxacarb (Strong 30%) on the 2nd and 4th larval instar of the cotton leaf warm, (Owner) is the most potent insecticide against *S. littoralis* larvae ,which caused the shortest larval, pupal and adult durations being 11.26,10.80 and 6.33 days respectively. Current study was conducted on the insecticidal effect of Lufenuron on percentages of larval mortality (80%), pupation 20% and adult 53.7%. The tested dose level of all treatments showed highly histopathological disturbanecs in the midgut of this pest including distruction of the muscle layers, disorgarization in the epitheliul cells, separation of the peritrophic membrane as well as detachment of the basement membrane and appearance of vaculaizations . This observation might explain high mortality in treated larvae as compared to the control.

Keywords: *Spodoptera littoralis*, Biological aspects, histological aspects, insecticides.

INTRODUCTION

Cottons leaf-worm**,** *Spodoptera littoralis***,** Boisd., Lepidoptera: Noctuidae, well known as most one of important devastating insects of cotton and other vegetables in Egypt. As one of the most important global economic problems, the protection of crops from pests is urgently needed. Many different countries search for natural alternatives to chemical dangerous pesticides using safe compound with minimal costs and ecological side effects (Matloub *et al*. 2021). This insect is attacking more than 112 host plants mainly on vegetable, field, ornamental crops and many other economic plants. Larvae are causing a great damage to leaves, buds, flowers, bolls and can seriously retard growth or reduce crop production (Pineda *et al*., 2007 and Rawi *et al*., 2011).

There are two important methods of insect control, biological and chemical methods. Use of insecticides has several disadvantages such as reduces population of natural enemies, leads to environmental pollution and development of pesticides resistant of insects (Salahuddin *et al*., 2004 and Abo Elghar *et al*., 2005). New control methods are needed to diminish reliance on conventional insecticides as part of IPM programs (Hamama *et al* 2015).

The bio-chemical controlling for some insect-pests is effected with insecticides for example synthetic-insect-growth-regulator (IGRs), especially; for essential agent action on insect-developments and insect-growthing. Insect-growth-regulator "IGRs" disruption cause regulation hormone association with insect-metamorphosis can ultimately cause death of insect (Gholami *et al*. 2013). Emamectin benzoate (1.9% EC) (Methylamin :Avermactin) belongs to A vermactin group of chemicals which block the post synaptic potential of neuro muscular junction leading to paralysis and finally to the death. (Fritz *et al*., 1979) Indoxacarb has a good toxin effect as a new class of oxidiazine insecticide against Lepidoptera pest with nearly no effect on non-target insects by blocking the movement of sodiumion and cause stop feeding and paralysis (Dinter and Wiles, 2000). These compounds interfere with the normal growth for development of insects and their effect could extended to affect the insects reproduction potential as well as other effects on the physiology of treated insects (Abdel-Aziz, 2012).

Current work was conducted to evaluation and comparison efficacy of Owner 5% EC, Dimilin 48%, Emafel 4% ME and Strong 30% SC., on some biology and histology on *S. littoralis* larvae.

MATERIALS AND METHODS

Insect Culture:

Laboratory-strain of cottons leaf-worm S. *littoralis* was rear to several generations in the lab. of Plant, Prot., Res., Inst.; Agric., Res., Center; Dokki-Giza-Egypt. Tested insect was reared on fresh castor-bean-leaves, *Ricinus communis* L., under controlled condition in an incubator at 27±2˚C and 65±2% RH according to El-Sawaf (1971).

Bioassay:

The chosen 2nd larval-instar for *S. littoralis*, and starved about 4 hours before feeding on leaves of castor bean which were treated by the followed compounds by using leaf dipping technique castor leaves were dipped in each concentration for 2 minutes and placed after complete dry in glass jars (250 ml capacity). Larvae which remaining living were allowed to feed on castor-bean-leaves until, pupation period and emergence. Each concentration of insecticides included 3replicates (20 larvae for each) and 3replicates contains larvae fed on

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untreated leaves were used as a control. All experiments were kept at 27±2°C and 65±2% R.H.

Tested compounds:

Table 1. The tested pesticides applied on the 2nd larval-instar of *S. littoralis*.

Trade name	Common name	Concentration used	produced by
Owner 5% EC	Lufenuron	160 cm^3/fed.	El-moneer for Agricultural
Dimilin 48%Sc	Diflubenzuron	125 cm^3/fed.	Eristia life co.
Emafel 4% ME	Emamectin benzoate	35 cm3/100L	Al-Qawafel Technical Ind. Agr.Co.
Strong 30% SC	Indoxacarb	12.5 cm3/100L	Al-Qawafel Technical Ind. Agr.Co.

Biological Aspects:

To evaluate efficacy of the tested pesticides on *S. littoralis* biology, at the end of the experiments, survival larvae of the treatments and control were kept individually into glass jars containing untreated fresh castor leaves and maintained at 27 ± 2ºC and 60 ± 2 % R. H. and checked daily until pupation to identify the larval duration. The newly formed pupae were carefully individually weighted before transferring to a glass vial (4 × 10 cm) and then covered with muslin fitted with a rubber band. All vials were kept in conditions at 27 ± 2ºC and 60 ± 2 % R. H., and they were daily-checked until, the adults emerged and estimate the pupal-duration, percentage pupation, pupal weight (mg), and newly emerged adults of the previous pupae were recorded

Also individually transferred into new glass vials and fed on a 10% honey solution. The vials were also covered with muslin using rubber band and examined daily to record the adult longevity.

Statistical Analysis:

The obtained data were analysed statistically by using variance analysis "ANOVA", (F) test according to Fisher (1954), F. value test using COSTAT computing program were adopted to determine the significance between treatments.

Histological preparation of mid gut for light microscopy

The histological effects of the Four insecticides against 4th instars larvae of *S. littoralis* was carried out by using feeding application for 48 h on treated leaves with 1/16 of concentration field of Lufenuron, Diflubenzuron, Emamectin benzoate and Indoxacarb. After 24h of treatment, approximately 10 specimens of each insecticides and control larvae were individually dissected in petri dish containing in alcoholic solution "Bouin's" for 24hrs., and specimens washed in (70%), ethyl-alcohol and the larva preserved with (70%), ethyl-alcohol and then, dehydrated in ascend alcoholic-series 70; 80; 90; 96 and 100%, 1/2hr., for each, then cleared in "xylen" for seconds; and the specimens infiltrated in three-changes of hot wax-paraffin, each lasted 20min., {Xylol dish and three-wax-dishes, wax^{I}; wax^{II} and wax^{III}} in an oven 50 to 52C° for 1/2hr for each respectively. Made the embedding, was in hot wax-paraffin, using plastic-cups standard. The blocks of Paraffin's solidified with soaked in water-cold, and stuck to holder of rotary microtome, the sections transvers cut at thickness 5 microns, and sections ribbons floated with slides coated by egg-albumin smear using dropping in hot-water. Hot-plate at 40°C, used in kept the preparations to wax-ribbons separating; after the water evaporations were complete in drying-oven at least 24hrs, slides soaked in {xylol} for 3 - 5min., and in descending series of ethyl-alcohol 100; 90; 80 and 70% for 2min., for each. Then, sections ribbons slides were soaked in distilled-water and stained in "hamatoxylin" for 30 - 45min., the slides were putted under the tape-water for 2min., then in distilled-water. The slides were soaked in "1% eosin", as a counter stain for 5 - 10sec., and rinsed rapidly in descending series of alcohol 70; 80; 90 and 96%, then, putted in absolutely alcohol for 10min., until the removing of water-residues occurred, then, passed to 2-changes of "xylol" for 10 - 15min., for each. In the end, slides mounted in Canada-balsam and kept by cover-glass and dried at 40°C, for one day. Every section was examined, and then examined sections were photographed by a light-microscope, Sharaby and El-Nujiban 2016.

RESULTS AND DISCUSSION

Biological parameters:

Efficiency of the four insecticides was evaluated on 2nd instar larvae of *S. littoralis*. The results indicated that, Table (2) shows the efficacy of tested insecticides on the larval, pupal and adult durations.All treatments cased high significant effects on various durations comparing with those in the control. The larval duration in the treatments ranged 11.26 days (Owner 5%EC) to 13.59 days (Emafel 4% ME) comparing with 14.33 days in the control. The pupal duration was 10.80 days at the Owner 5%EC while it was 12.38 days at the control. The highest decrease of adult longevity was 6.33 days with Owner 5% EC comparing with 8.95 days in control.

Table 2. Impact of pesticides on some biological aspects of *S. littoralis*.

Treatments	Duration (Day)		
	Larval	Pupal	Adult
Owner 5%EC	11.26d	10.80b	6.33c
Dimilin 48%SC	12.56bc	11.50 ab	7.5b
Emafel 4% ME	13.59a	11.50 ab	8.6a
Strong 30% SC	13.28ab	11.67ab	8.25ab
control	14.33c	12.38a	8.95a
F value	13.50*	2.76*	9.58*
LSD	0.779	1.070	1.059

Table (3) shows efficacy of the four insecticides on percentage of larval mortality, Pupal weight (g), pupation, and adult emergence. The weight of pupae in all treatments was insignificant between treatments and control. However, larval mortality percentage was 80% in owner 5%EC meanwhile, was 10 %. in control**.** Also owner 5%EC had higher effect than the other treatments**.** Owner 5%EC recorded the highest decrease in pupation. The pupation percentages were 20.0, , 28.89 ,43.33 and 56.67% at Owner 5%EC, Emafel 4% ME , Dimilin 48%, and Strong 30% SC respectively. On the other hand, Adult emergence percentages in all treatments were insignificantly reduced when compared to untreated (control) but the differences between treatments were

insignificant. Generally, Owner 5%EC was the most effective against *S. littoralis* than the other treatments.

Table 3. Percentages of larval mortality, pupation, adult emergence and weight of pupae for *S. littoralis* larvae treated with four insecticides

Treatments	Pupal weight(g)	%		
		Larval mortality	Pupation	Adult emergence
Owner 5%EC	0.303	80_{a}	20.0_{a}	53.7
Dimilin 48%SC	0.340	56.66_{a}	43.33_{b}	61.11
Emafel 4% ME	0.300	71.11_{a}	28.89_{b}	57.41
Strong 30% SC	0.340	43.33_{ab}	56.67_{ab}	69.44
control	0.37	10b	90a	77.46
F value	1.26	12.96	12.96	4.83
LSD	n.s	34.14	34.14	n.s

The present results agree with the findings of Abd El-Rahman *et al*(2019). studied the effect of some pesticides on 4th larval instars of *S. littoralis,* to investigate the impact of antifeedant index results show that owner 5%EC was the most powerfull tested compounds followed by dimilin 48%SC. Also, the relative growth rate (RGR), of 4th larval instars of *S. littoralis* fed on the treated leaves by owner 5% was decreased clearly. However, several researchers investigated the effect of some natural products on the duration of different stages of *S littoralis* after treated larvae. Hussein and Eldesouky (2019) found that, the sublethal-concentrations of chlorfluazuron and chlorantraniliprole, were significant of reduce of pupae and larvae weight; longevity of adults; pupation%; emergence of adults and fecundity of females, while increasing occurred in larvae and pupae durations.

Effect of tested insecticides on histological structure of mid gut of *S littoralis*.

The normal mid- gut ultrastructure:

Microscopic examination showed that the histological structures of normal mid-gut of 4th larval instars of S. *littoralis*, consists of 2-muscular-layers and epithelial-layer lining the lumen, and the intestinal-epithelium contain 4-types of cells; [1]digestive, [2]regenerative, [3]endocrine, and [4]goblet cells, it is distinguished by its order Lepidoptera. The epithelium-layer rest on basement-membrane and the membrane is externally surrounded by circular and longitudinal muscle-fibers. This epithelium-layer consists of goblet and columnar-cells with cluster from regenerative-cells small contain relatively on large-nucleus and strong basophilic of cytoplasm, Fig.,1^{A}. Also epithelium-layers are protected from particles food by a detach sheath peritrophic-membrane surrounding lumen.

A. owner 5%EC:

Figure 1^{B}, illustrated that, the several damages occurred the mid-gut epithelium of 2nd larval instar of *S. littoralis*, treated by owner 5%EC insecticide when examination with Microscopic. The mid gut epithelium cells was the most affected when compared with the untreated mid gut. The Regenerative-cells were separated from each other's according to base of epithelium and muscular-layer becomes shrinkages than, the control, big destruction of epithelium cells, big and some vacuoles present, were "not pronounced" and could "not been identified" in many layers at epithelial-cells base, caused by sever epithelium destructions and increasing goblet cells secretions, Fig., 1^{B}.

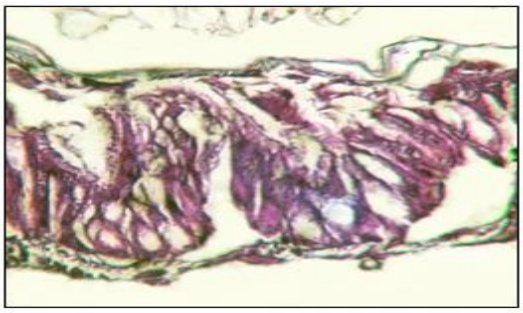

Fig. 1^{A}. Cross section in mid-gut area of larvae shows normal differences tissues in control.

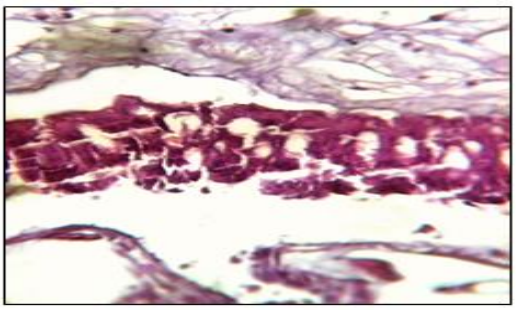

Fig. 1^{B}. Cross section in mid-gut area of larvae shows differences damaged tissues after the treatment by owner 5%EC.

B. Dimilin 48% SC

Examination of mid-gut section of 2nd larval instar of *S. littoralis*, treated by Dimilin48% compound, (Fig., 2^{B}), showed that severe damage effects on the mid-gut layers. Most characteristics effect were mid-gut epithelial cell vacuolization, alterations in cell size and shape, which were quite evident at larvae treated with Dimilin 48%. Also, few epithelial-cells were seen showing vacuolization, increase in slight size, vacuolization was more pronounced and epithelial (Fig., 2^{B}), this is in contrast with the control in (Fig., 2^{A}).

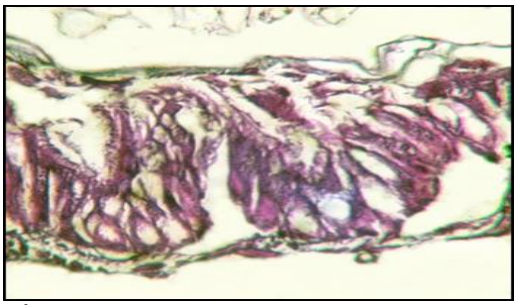

Fig. 2^{A}. Cross section in mid-gut area of larvae shows differences normal tissues in control.

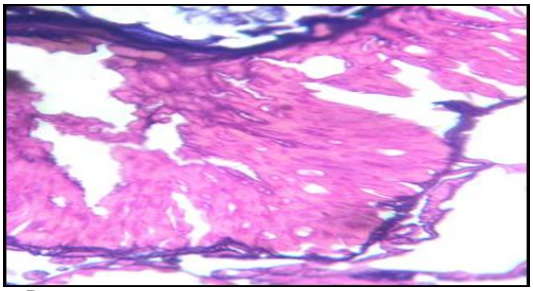

Fig. 2^{B}. Cross section in mid-gut area of larvae shows damaged tissues after treatment by Dimilin 48%SC.

C. Emafel 4% ME

Study of the histological effects of Emafel 4% ME on 4th larval instar of *S. littoralis*, are shown in (Fig., 3^B). The disintegration of the disal end of the mid gut epithelial cells and the muscular lager seems to become thick . Partially lysis in the epithetlial cells, separation of the peritrophic membrane. Apically swelling in gut-lumen, intercellular contacts reducing with neighboring cells and nuclei degenerations and brush borders (Fig., 3^B), when comparison with control (Fig., 3^A).

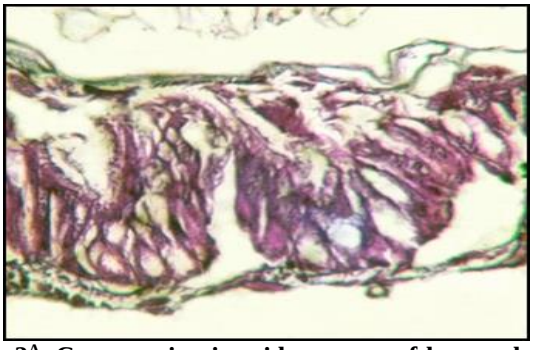

Fig. 3^A. Cross section in mid-gut area of larvae shows different normal tissues in control.

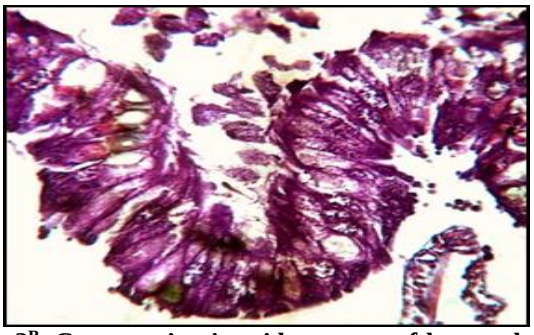

Fig. 3^B. Cross section in mid-gut area of larvae shows damaged tissues after treatment by Emafel 4% ME.

Strong 30% SC

Fig., (4^B), showed the changes histopathological were observed in the mid-gut of 4th larval instar of *S. littoralis*, treated by Strong 30% SC, peritrophic membrane started disintegrating and inure separated so, the peritrophic membrane got more conspicuous. Also apically swelling in gut-lumen, intercellular contacts reducing with neighboring cells and nuclei degenerations and brush borders (Fig., 1^D). Microvilli showed that, completely disorder in some areas, goblet-cells secretion increasing with ruptures of basement-membrane and disruptions in columnar epithelium-cells. When comparison with control that showed that, intercellular contact along the whole lateral plasma membrane is normal, nuclei and adhesive basement-membrane are normal, showed in (Fig., 4^A).

This results were agree with that, mentioned by Youssef (2006) reported that, pyriproxyfen and abamectin caused a histological changes in the mid-gut of treated *Spodoptera littoralis* larvae, in form disruptions in columnar epithelium-cells and stretching's lead to peritrophic membrane tearing. Rawi *et al*., (2011) studied the extracts effect of *A. indica* and *C. colocynthis* at LC_{10} and LC_{25}, on histology of 4th larval instar *S. littoralis*. The extract of *A. indica*, at two doses induced histological damages in larval mid-gut, some epithelial-cells were vacuolated and destructed of nuclear content also, the *C. colocynthis* extract, at dose level $LC_{10,}$ caused degenerations of columnar epithelial-cells and vacuolations. On the other hand, the treatments with LC_{25} of extract formulation of *C. colocynthis*, caused vacuolations, and detachments of columnar epithelium-cells.

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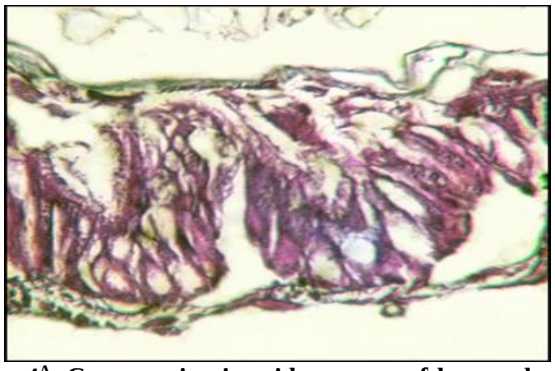

Fig. 4^A. Cross section in mid-gut area of larvae shows normal differences tissues in control.

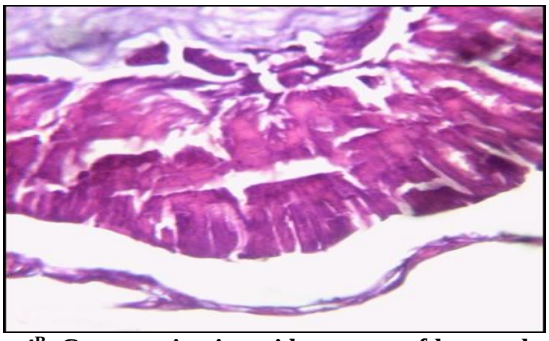

Fig. 4^B. Cross section in mid-gut area of larvae shows damaged tissues after treatment by Strong 30% SC.

Sharaby and El-Nujiban (2016), found that, the histopathological changes were in mid-gut of *A. ipsilon*, larvae, treated with oils "Garlic+ Mint", showed that, the cells differences of mid-gut exhibited a swelling appearance and microvilli, showed completely disorder in some areas, the goblet cells secretion increasing with ruptures in basement-membrane.

Additionally, similar or other histopathological changes had been researched by (Abdel-Salam *et al.*, (2018), showed that, the treatments of the 4th larval instar

of *S. littoralis*, with 2-chitin synthetic inhibitors showed that, many ultrastructure alterations in mid-gut of 6th larval instar as the vacuolations, elongations and disintegrations of epithelial-cells, also, the disappearances of musculoris and regenerative cells, detachment of basement-membrane.

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التأثيرات البيولوجية والهستولوجية لبعض المبيدات الحشرية على دودة ورق القطن

حسام احمد صالح ، سهير فاروق عبدالرحمن ، علي ربيع محمد الجبلي ، شنودة سيد يعقوب و علاء محمد خورشيد

معهد بحوث وقاية النباتات ، مركز البحوث الزراعية ، الدقي ، الجيزة

يلقى هذا البحث الضوء على كفاءة ليوفينيرون (اونر ٥ %) داى فلوبنزرون (ديملين ٤٨%) وايمامكتين بنزوات (ايمافيل ٤%) واندوكساكارب (سترونج ٣٠%) على العمرين الثانى والرابع لدودة ورق القطن تحت الظروف المعملية . وقد اظهرت النتائج ان مبيد ليوفينيرون (اونر ٥ %) كان الاكثر فاعلية ضد يرقات دودة ورق القطن ، وسبب نقص فى فترات العمر اليرقى والعذرى والحشرة الكاملة بمقدار ١١,٢٦، ١٠,٨٠ ، ٦,٣٣ يوما على الترتيب . وامتدت الدراسة الى التأثير المميت للمبيدات وحقق ليوفينيرون اعلى نسبة مئوية للموت ٨٠% ،واقل نسبة تعذير ٢٠% واقل نسبة بزوغ للحشرات الكامل ٥٣,٧ %. واظهرت كل المعاملات بالتركيز المختبر اعلى نسبة اضرابات هستولوجية فى المعى الاوسط شملت تهتك فى العضلات واختلال فى الخلايا الطلائية وانفصال كلا من الغشاء القاعدى والغشاء حول البلعة الغذائية وظهور فجوات . واخيرا اظهرت هذه النتائج اسباب زيادة نسبة الموت لليرقات المعاملة عن المقارنة .