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HISTOPATHOLOGICAL STUDIES ON THE EFFECT OF SOME ALTERNATIVE INSECTICIDES ON *Spodoptera littoralis* (Boisd.)

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AUTHOR'S CONTRIBUTION

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

The insecticidal activity biological and histological effects of a bacterial bioagent spinosad (24% SC), an insect growth regulator methoxyfenozide (24% SC) and extrem (36 % SC), Ready-made mixture (Spinetoram 6% and methoxyfenozide 30%) on the 4th larval instar of the cotton leaf warm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), under laboratory conditions, as denoted by the determined LC_{50} which was 7.28, 0.071 and 0.113 ppm, in spinosad, methoxyfenozide and extrem, respectively. The highest rate of decrease in the pupation percentage of 25% was recorded in case of treated with extreme followed by spinosad and methoxyfenozide (76 and 63 % respectively). Also, the adult emergence rate was remarkably reduced in case of extreme to 40% followed by spinosad 56.6% and methoxyfenozide 69.8%. The male moths recorded lower rate of adult longevity than the female moths. On the other hand both toxicants induced drastic effect on fecundity and fertility of adult moths. Highly histopathological disturbances in the midgut of this pest including destruction of the muscle layers, disorganization in the epithelial cells, separation of the peritrophic membrane as well as detachment of the basement membrane and appearance of vacuolizations. Also caused severe histological aberration of the ovarioles.

Keywords: Spinosad; methoxyfenozide; extreme; *Spodoptera littoralis*; biological study; histology..

1. INTRODUCTION

"Cotton is one of the major sources of fiber. Besides the fibers, cotton plants produce a large amount of seeds" [1]. "These seeds are rich in protein and have been considered as a valuable source of oil and fodder" [2]. "The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), is a destructive polyphagous insect pest of diverse field crops in different regions including; tropical and subtropical" [3]. "*S. littoralis* feeds on approximately 90 species of economic crops in 40 plant families" [4]. "The regular use of chemical insecticides against *S. littoralis* resulted in the development of resistances to most of the traditional insecticides" [5, 6, 7]. "Also, application of synthetic pesticides is financially expensive" [8]. "Therefore, searching for new alternative and safer agents for human health, economic animals and environment, is prerequisite need" [9]. "Also, it is necessary to develop specific compounds for the pest control which are selective for the non-target organisms" [10,11].

"Spinosad, belongs to a new class of polyketide-macrolide insecticides. It is a combination of

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spinosyns A and D, derived by fermentation from the naturally occurring soil actinomycete, a metabolite of *Saccharopolyspora spinosa"* [12], "which is currently registered in several countries. Spinosad acts in two unique ways on nicotinic acetylcholine and Gamma-Aminobutyric acid (GABA) receptors. The extensive global testing spinosad provided an efficient control to key pests in copious crops, including vegetables and cotton" [13, 14, 15]. "Spinosad is particularly active against lepidopteran, dipteran, and thysanopteran pests; it has been reported to be safe to many predatory insects, but in some cases, harmful to parasitoids" [16]. "In addition, it has good environmental performance (quick degradation, low toxicity to humans, and low doses of use) makes spinosad a choice for integrated pest management (IPM) programs in vegetables and ornamentals" [17].

"Insect growth regulator (IGRs) are claimed to be safer for beneficial organisms than conventional products, and they have been successfully used in IPM programs against many tree and small fruit pests" [18]. "Methoxyfenozide belongs to a novel class of IGRs, the molting accelerating compounds or nonsteroidal ecdysteroid agonists, discovered by the company Rohm and Haas (Spring House, PA). The compound mimics the biological function of the natural insect molting hormone 20-hydroxyecdysone, inducing a premature and lethal larval molt by direct binding to the ecdysteroid receptors" [19]. "In addition, methoxyfenozide is highly selective against lepidopterous larvae" [20], and "its effectiveness against many economically important agronomic and forest pests have been reported" [21]. Extrem, Ready-made mixture (spinetoram and methoxyfenozide) are considered promising candidates.

Current work was conducted to evaluation and comparison efficacy of bioagent spinosad (24% SC), an insect growth regulator methoxyfenozide (24% SC) and extrem (36 % SC), Ready-made mixture (Spinetoram 6% and methoxyfenozide 30%) on the 4th larval instar of the Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisd.), on some biology and histology aspects of this insect.

2. MATERIALS AND METHODS

2.1 Technique of *Spodaptera littoralis*

The strain of larvae used in the present study were obtained from laboratory colony that was reared on castor bean leaves in the *S. littoralis* rearing laboratory, Cotton Leafworm Research Department, Plant Protection Research Institute (PPRI), Agricultural Research Center (ARC), which were reared under constant laboratory conditions of 25 + 2°C and 60 + 5% RH as described by El-Defrawi, et al. [22]. Larvae were reared on castor oil leaves (*Ricinus communis* L.), the 4th instar larvae were selected for bioassays and biochemical assessments. Male and female pupae were separated to avoid mating. Emerged moths were supplied with a 10% sugar solution. For a limited number of experiments, assays were conducted separately in another room equipped with a dim bright red backlight, but under the same rearing conditions.

2.2 Tested Compound

The following three commercial chemicals were evaluated for their effect on *S. littoralis* (Boisd.):-

- Bio- pesticides, Tracer® (Spinosad ,24% SC) from Dow Agroscience Co..
- Insect growth regulators, (Non-steroidal ecdysteroid agonists) Runner® (methoxyfenozide, 24% SC) from Dow Agroscience Co..
- Ready-made mixture, extrem®, 36 % SC (spinetoram 6% and methoxyfenozide 30%) from Dow Agroscience Co.

2.3 Bioassay Tests

These tests were carried out on the 4th larval instars of *S. littoralis* fed on castor bean leaves using immersion technique. One hundred larvae divided into four replicates; each 25 larvae were used for each concentration (0.8, 0.4, 0.2, 0.1 and 0.05 ppm for Runner, 50, 25, 12.5, 6.25 and 3.12 ppm for Spinosad and 1, 0.5, 0.25, 0.125 and 0.0625 ppm for Extrem). A control experiment was performed using castor been oil leaves dipped in water. Serial concentrations of each insecticides were prepared. Castor leaves were dipped for 30 sec. in each concentration as per Abo El-Ghar et al. [23]. Control was dipped in distilled water. Then treated and untreated leaves were left to dry for about 1 hr under room conditions. Then treated and untreated leaves were left to dry for about 1 hr under room conditions. The 4th larval instar was starved for about 3 hrs then fed for 48 hrs on the treated leaves and later transferred to fresh untreated leaves for 7 days. Three replicates for all treatments and control were used, with 25 larvae in each replicate. Concentration-mortality percentages were calculated daily and corrected according to Abbott's equation [24]. LC_{50} & LC_{90} values were calculated by using the probit- analysis method of Finney [25].

2.4 Biological Investigation

Newly moulted 4th instar larvae were treated with the LC_{50} concentration of the three tested compond

(spinosad, methoxyfenozide and extrem). Treated larvae were examined daily to determine the post treatment effects on those insects survived the treatments. These biological aspects include; pupation%, adult emergence, adult longevity, number of laid egg per female and percent of hatchability

2.5 Histopathological Changes

The histology of the midgut after 6 days following treatment of 4th instar larvae with the LC_{50} of spinosad; methoxyfenozide and extreme was studied. Similarly, investigation of healthy untreated larvae in the same instar was considered as a control. Larvae were dissected in Ringer's solution. The histopatholgical studies of the midgut were obtained according to the method, isolated and fixed in Bouin's solution and then embedded in paraffin. Many sections of 5 µm thickness were obtained and stained with hematoxylin and eosin (H & E stain) as per the method of Junqueira and Carneiro [26] and Suvarna et al. [27]. The sections were examined by microscope under 400X.

Also, histopathological studies of the ovaries were obtained from the adult female pretreated larvae. The surviving virgin treated and untreated females were dissected in ringer's solution on the first day of emergence. The ovaries were fixed in carnoy's solution, embedded in paraffin wax, and stained with heamatoxylin and eosin.

2.6 Statistical Analysis Procedure

The significance of the main effects was determined by using analysis of variance (ANOVA). The significance of various treatments was evaluated by Duncan's multiple range tests ($p<0.05$). All analysis was preceded using a software package "Costat", a product of cohort software Ine. Berkley, California [28].

3. RESULTS AND DISCUSSION

3.1 Toxicological Studies

The efficiency of three tested compound: spinosad, methoxyfenozide and extrem against the 4th instar larvae of *S. littoralis* is given in Table 1.

LC_{50} values of three tested compounds, treated on fourth instar larvae were determined as 7.28, 0.071 and 0.113 ppm, in spinosad, methoxyfenozide and extrem, respectively. The Slope were 0.359, 0.866 and 2.069 in spinosad, methoxyfenozide and extrem, respectively. Mohamed et al. [29] reported that spinosad showed high toxicity against 4th instar larvae of *S. littoralis*. Marwa [30] studied the effect of adding mint oil to spinosad insecticide on the toxicity of the compound against the cotton leaf worm, *S. littoralis*. The results showed that adding 0.3% mint oil to spinosad solution increased its toxicity.

The data presented in Table 2 demonstrated that, the highest reduction in the pupation percentage (25%) was recorded in the case of larvae treated with extreme. In addition, treatment of larvae with spinosad and methoxyfenozide caused a reduction in pupation percentage to 76 and 63 % respectively compared to 90% in the case of the untreated control. The adult emergence rate was remarkably reduced in the case of extreme to 40% followed by spinosad, 56.6% and methoxyfenozide ,69.8% compared to 92.9% in the case of the untreated control.

The male moths has lower rate of adult longevity than the control. The highest increment recorded 10.0 day in case of methoxyfenozide and extreme for female moths compared to 9.0 day in the untreated control, while it recorded 9 day for spinosad. However, in male treatment of with spinosad, methoxyfenozide and extreme caused a reduction in adult longevity compared with control in all treated which being 9.7, 10.7 and 11.0 day compared to 12.0 day in the untreated control. El-Sheikh [31] who found that, spinosad reduced female longevity when larvae *S. littoralis* treated as 4th instar larvae.

The reproductive potential of mated moths emerging from larvae treated as 4th instar with spinosad, methoxyfenozide and extreme at LC_{50} value showed that their reproductive potential was highly significantly affected [Table 3]. The means of cumulative eggs/female recorded were 1380.3, 1712.3 and 914.7 eggs spinosad, methoxyfenozide and extreme, respectively, while it was 2111.0 eggs in the untreated control. However, the egg hatch percentages were also decreased, the highest reduction in the egg hatch percentages (46.2%) was recorded in case of extreme followed by methoxyfenozide 68.3% and spinosad 72.7% compared to 94.2% in case of the untreated control. Similarly Seham [32] showed a significant reduction in fecundity and fertility in treated larvae of *S. littoralis* with spinosad when compared with control. In the field of study, Ahmed et al. [33] found that the methoxyfenozide LC_{50} caused significant decrease in fecundity and fertility of *Spodoptera littoralis* adults when applied on the newly molted 6th instar larvae.

Table 1. Lethal toxicity values (ppm) of insect growth regulators spinosad, methoxyfenozide and extrem tested against *S. littoralis* 4th instar larvae

Treated compound	LC_{50} (ppm)	Confidential limits for (95%)		Slope±S.E.
		Lower	Upper	
Spinosad	7.28	3.759	14.125	0.359 ± 0.24
Methoxyfenozide	0.071	0.015	0.128	0.866 ±0.26
Extreme	0.113	0.076	0.146	0.069 ±0.34

Table 2. Rate of pupation, percentage adult emergence and adult longevity of 4th instar larvae of *S. littoralis* treated with LC_{50} of spinosad, methoxyfenozide and extreme

Treated compound	Pupation %	Adult emergence %	Adult longevity	
			Female ♀	Male ♂
Spinosad	76	56.6	$9.0^{b} \pm 0.29$	$9.7^{c} \pm 0.2$
Methoxyfenozide	63	69.8	$10.0^{a} \pm 0.18$	$10.7^{b} \pm 0.3$
Extreme	25	40.0	$10.0^{a} \pm 0.24$	$11.0^{b} \pm 0.5$
Control	90	92.9	$9.0^{b} \pm 0.12$	$12.0^{a} \pm 0.4$
F value			$.0107^{*}$	$.0005^{***}$
L.S.D.			0.6918	0.6918

Numbers of the same letters have no significant difference.

Table 3. Fecundity and fertility of *S. littoralis* moths treated as 4th instar larvae with spinosad, methoxyfenozide and extrem at LC_{50} value

Treated compound	No. of eggs/female ±S.E.	No. of egg hatch/female ±S.E.	Egg hatchability %
Spinosad	$1380.3^{c} \pm 45.29$	$1003.3^{b} \pm 17.3$	72.7
Methoxyfenozide	$1712.3^{b} \pm 24.9$	$1170.0^{b} \pm 34.71$	68.3
Extreme	$914.7^{d} \pm 37.06$	$422.3^{c} \pm 51.68$	46.2
Control	$2111.0^{a} \pm 47.06$	$1989.0^{a} \pm 88.24$	94.2
F value	$.0000^{***}$	$.0000^{***}$	
L.S.D.	263.794	303.373	

Numbers of the same letters have no significant difference

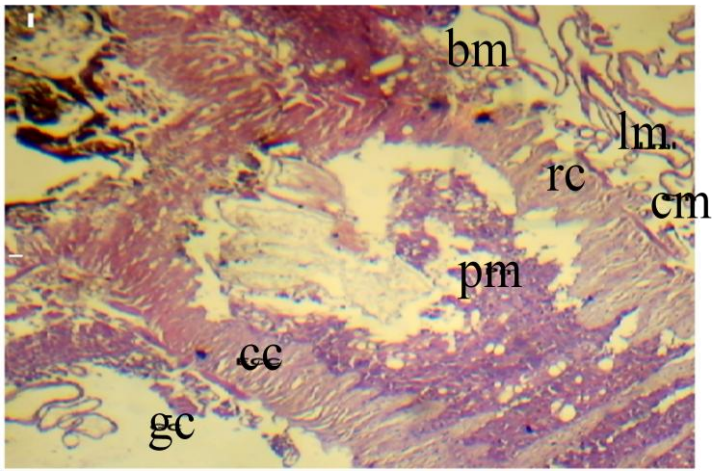

Fig. 1. T. S. of the midgut of *S. littoralis* control larvae that showed normal type tissues.

- *lm: longitudinal muscle layer, - cm: circular muscle layer.*
- *bm: basement membrane, - rg: regenerative cell.*
- *Pm: peritrophic membrane, - cc: columnar cell.*
- *gc: goblet cell*
-

Light microscopic examination shows that the histological structure of the midgut of 6th instars of *S. littoralis* is given in Fig. 1. It is composed of two layers of muscle fibers, the outer longitudinal fibers and inner circular ones (musculosa). Next to it inwards is a basement membrane, which is followed by an columnar cells that forms a lining in the midgut cavity. Within the lumen there is a thin peritrophic memebrane, surrounding the food mass.

Treatment of *S. littoralis* 4th instars with the LC_{50} of spinosad (Fig. 2) caused exfoliation and vacuolization of the midgut epithelium from the underlying circular muscle fibers, leaving a large vacuole or space in surviving larvae after 6 days following treatment. Disruption of both the peritrophic membrane and the striated borders were evident. Some of the degenerated columnar cells fuse with the disrupted peritrophic membrane.

The treatment of *S. littoralis* 4th instars with the LC_{50} of methoxyfenozide (Fig. 3) showed that the muscularis lost their compact appearance. Led to fused, disintegrated, vacuolization and exfoliation of the columnar cells, also, disrupted peritrophic membrane.

On the other hand treatment of 4th instar *S. littoralis* larvae with the LC_{50} of extreme (Fig. 4) resulted in loss of musculosa layer after 6 days following treatment. The peritrophic membrane was considerably deteriorated, and the striated border together with the columnar cells were highly obliterated. The lumen of the midgut epithelium was highly shrunken. Few cytoplasmic fragments were seen pinching off from the tip of the columnar cells underneath the pertrophic membrane and the regenerative cells lost their integrity.

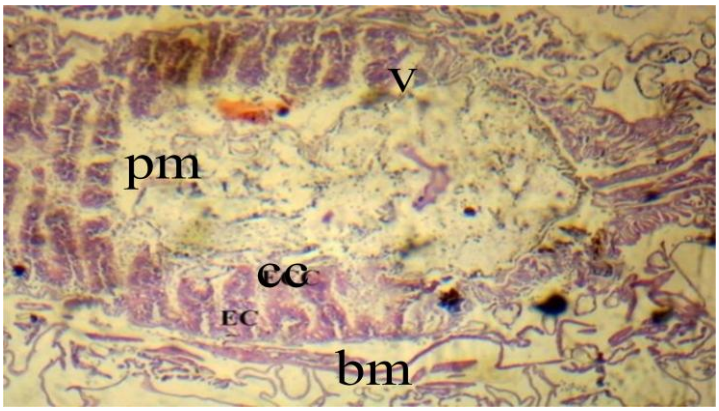

Fig. 2. T. S. of the midgut of 4th instar larvae of *S. littoralis*, 6 days post treatment with LC_{50} of spinosad

- *bm: basement membrane, - rg: regenerative cell.*
- *Pm: peritrophic membrane, - cc: columnar cell, – v: vacuoles*

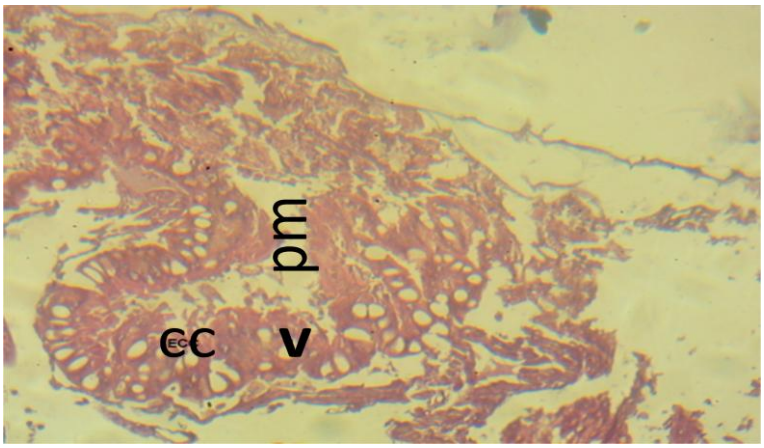

Fig. 3. T. S. in the midgut of 4th instar larvae of *S. littoralis* 6 days post treatment with LC_{50} of methoxyfenozide

- *Pm: peritrophic membrane, - cc: columnar cell, – v: vacuoles*

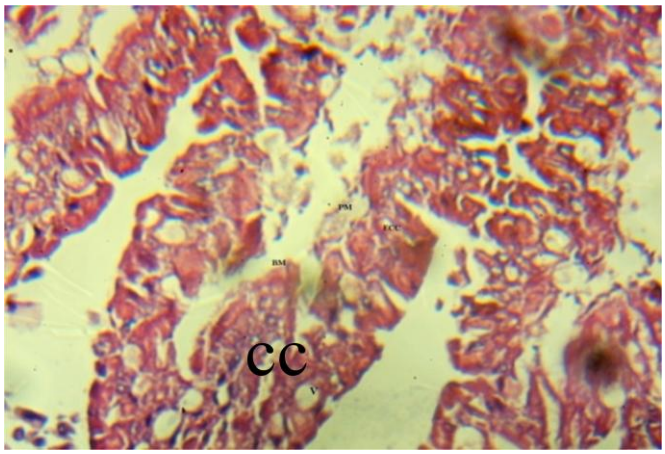

Fig. 4. T. S. in the midgut of *S. littoralis* larvae 6 days post treatment with LC_{50} of extreme
- *cc: columnar cell*

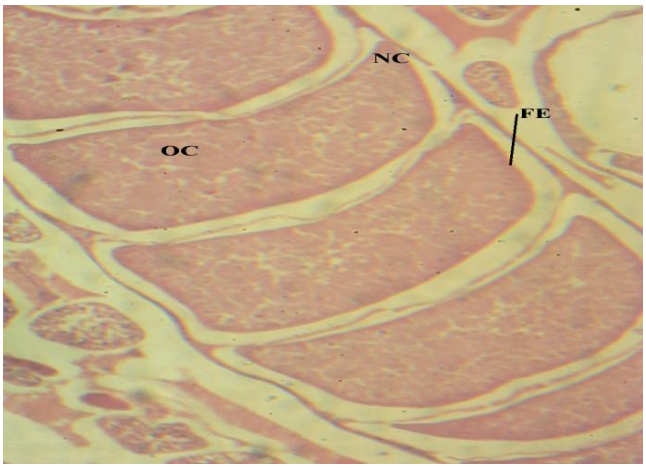

Fig. 5. L.S through normal ovarioles of female *S. littoralis* larvae
NC: nurse cell, FE: follicular epithillum, OC: oocyt

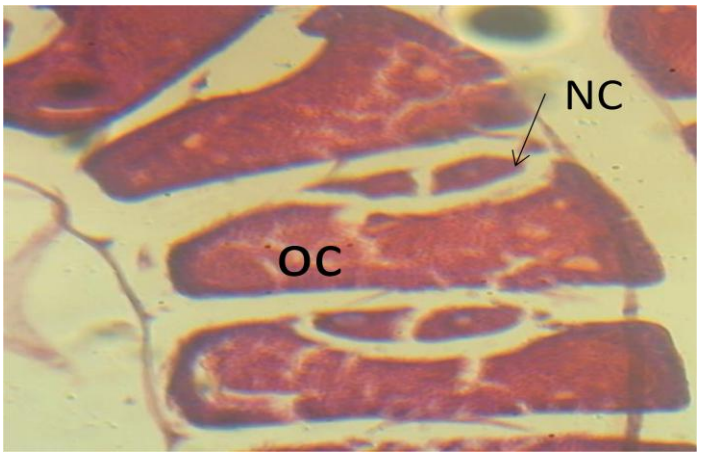

Fig. 6. L.S through ovarioles of female of *S. littoralis* larvae post treatment with LC_{50} of spinosad as 4th larvae instar
NC: nurse cell. OC: oocyt

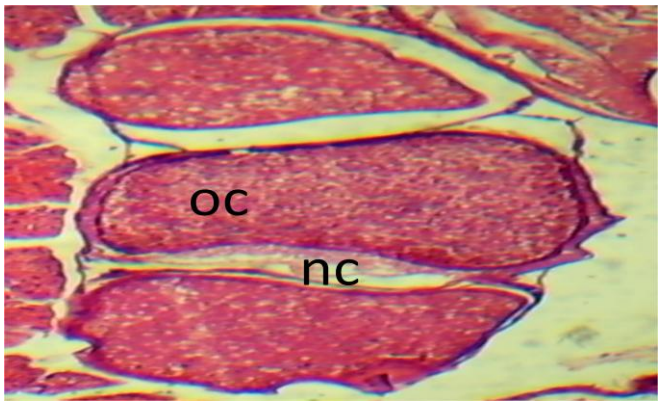

Fig. 7. L.S through ovarioles of female *S. littoralis* larvae post treatment with LC_{50} of methoxyfenozoid at 4th larval instar

NC: nurse cell, OC: oocyt

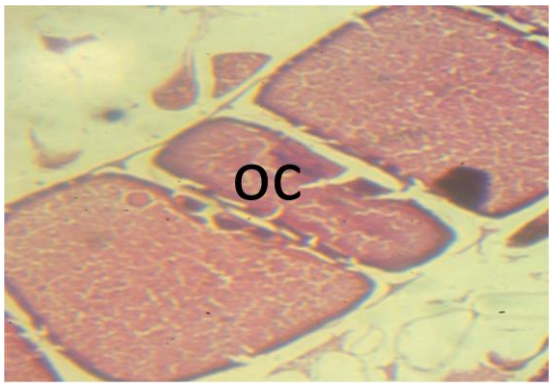

Fig. 8. L.S through ovarioles female of S. littoralis larvae post treatment with LC50 of Extreme at 4th larval instar

OC: oocyt

The results agree with that mentioned by Ibrahim [34] who recorded similar histological changes in *Spodoptera littoralis* larvae treated with spinosad and tebufenozide and reported to cause a histological changes in the mid-gut in the form of disruptions in columnar epithelium-cells and stretching's that lead to peritrophic membrane tearing. Also showed many midgut histological aberrations as reported by Saleh et al. [35] in the case of *S. littoralis* larvae treatment with lufenuron and diflubenzuron, The tested 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.

The normal female of *S. littoralis* have well developed ovaries with "8" polytrophic ovarioles. Each ovariole consists of a chain of developing ova, each chain have many oocyte, evervry oocyte have follicle body and 2 nurse cell and which has nucleus (Fig. 5).

The histological deformities to the ovarioles as the result of treatment with spinosad were recorded in (Fig. 6), absence of follicular epithelium.

Absence of follicular epithelium for methoxyfenozoid treatment the egg follicle had masses of cells so mixed that it was very difficult to differentiate between the nurse cells and oocyte (Fig. 7).

histological damages for the follicular epithelium and nurse cell shrinkage of the oocyst recorded for extreme treatments **(**Fig. 8).

The treatment Lc_{50} of spinosad, methoxyfenozide and extreme at LC_{50} against 4th larval instars of S. littoralis induced several structural changes in moths' ovary as compared to controls. Abdel-Aal and Abdel-wahab [36] reported complete damage for *S. littoralis* female ovariolar cells following feeding of 4th instars larvae for 48 hours on castor oil leaves treated with lufenuron and spinosad. Similar observations were also reported by Saleh et al. [37] in insects treated

with LC_{50} of two insect growth regulators, Diflubenzuron (Dimilin®) and chromafenozide (Virtu®), on the cotton leaf worm, *S. littoralis*, ovaries against the 2nd and 4th larval instars and reported to induce several structural changes in moths' ovary as compared to controls.

4. CONCLUSION

Our results concluded that Ready-made mixture extrem (Spinetoram 6% and methoxyfenozide 30%) have more effect on the cotton leaf worm, *Spodoptera littoralis* were methoxyfenozide have senyrgistic effect to spinosad. Therefore, it is recommended to use this compound within the integrated pest management (IPM) program against *S. littoralis.*

COMPETING INTERESTS

Author has declared that no competing interests exist.

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