Pyridalyl Effectiveness on Some Biological and Physiological Parameters of Cotton Leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae)

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Abstract: The present investigation aim to throw light on the efficiency of the median lethal concentration (LC_{50} value) for the novel insecticide pyridalyl on 2nd, 4th, and 6th larval instars of cotton leafworm *Spodoptera littoralis* (Boisd.) under laboratory conditions .The results showed that the pyridalyl is more effective on 4th instar larvae due to the larval mortality percent estimated by 78.0%. Also fertility % was 0.0 in comparison to control and the number of eggs/female was the smallest one in comparison with other, estimated by 365.7 eggs. Marked biochemical changes however, being recognized in pest as marked SDS-polyacrylamide gel electrophoresis representing molecular weights in protein showed there are three ,two and one bands were found to be specific to treated 4th, 2nd, and 6th larval instars, respectively. Also the activity of both α & ß esterases enzymes analysis showed differences in esterase pattern in the treated 4th instars than control. The tested LC_{50} value of pyridalyl showed highly histopathological disturbance in the epithelium of mid gut. The histochemical observation showed a conspicuous depletion in total protein content in both 4th and 6th treated larval instars.
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1. Introduction

The cotton leafworm *Spodoptera littoralis* in particular which considered as one of the major and important economic pests not only in Egypt but also in many parts of the world which attacking 112 plant species belong to 40 families (**Hosny *et al.*, 1986**)

In Egypt, *S. littoralis* developed resistance to organophosphorus, synthetic pyrethroid and other insecticides have been used, with appearance of resistance and cross resistance in many cases (**Issa *et al.*, 1984; Abo – El Ghar *et al.*, 1986).** It requires that highly selective insecticides should be used for pest control. Moreover, much safer insecticides need to be developed in order to decrease the burden on the environment by using less chemical in comparison to conventional insecticides. Pyridalyl flowable (active ingredients: pyridalyl was discovered and has been under worldwide development by Sumitomo Chemical .Co, Ltd. This novel insecticide exhibit high insecticidal activity against Lepidoptera and Thysanoptera (**Sakamoto *et al.*, 2003)** also it has effect on Diptera insect (**Satio *et al.*, 2002**).

Many existing insecticide- resistance strains of lepidopterous pests can be adequately controlled by pyridalyl as well as susceptible strains. Since pyridalyl develop quite unique insecticidal symptoms, it is considered that has a different mode of action from any other existing insecticides. It does not act upon the nervous system as do organo-phosphoric pesticides and synthetic pyrethroide ; does not inhibit the respiratory system (**Sakamoto *et al.*,2005**). It posses a certain type of toxicity for insect cells. it inhibited the cell growth **(Satio *et al.*, 2006**) pyridalyl flowable has been verifies as an environmentally friendly insecticides that is suitable for use in IPM system it possesses a high level of safety for human, animals and fish ,and it has minimal impact upon natural pest predator as well as pollinating insects **(Sakamoto *et al.*, 2005) .**

2. Material and Methods

Tested insects:

A laboratory strain of *S. littoralis* was obtained from Plant Protection Research Institute, Agriculture Research Centre, Dokki, Giza, Egypt. Insects were reared on castor oil bean leaves at 27±2°C and 65±5 % R.H. The bioassay tests were carried out using newly moulted 2nd, 4th and 6th instars. The formed pupae were collected and placed in clean jar with moist saw dust placed at the base to provide the pupation site. Adult were provided with 10% sugar solution.

Toxicity test:

Serial concentrations of the insecticide pyridalyl (50 % EC) were prepared by dissolving in

distilled water. In the bioassays, 2nd, 4th, and 6th instar larvae of *S. littoralis* were treated by feeding. Three replicates (each of 20 larvae/ concentration) were used. Control larvae were treated with distilled water only .Pyridalyl doses of 1.95, 3.9, 7.5, 15.6, 31.25 and 62.5 ppm were used for 2nd instar , for the 4th , instar doses of 7.8 ,15.6 , 31.25 ,62.5 ,125 and 250 ppm were used and doses of 3.9 , 7.8 ,15.6 ,31.25 and 62.5 ppm for 6th instars. All leaf discs were dipped into solution containing different concentrations of pyridalyl for 5 seconds and then they were air dried for one hour .Control discs were dipped into distilled water. Mortality percentages were measured after 24 hrs .LC_{50} of pyridalyl were calculated by using probit analysis (**Finney, 1971**), through software computer program .Statistical significant differences individual means were determined by one way analysis of variance (ANOVA).

Biological activity:

Effects of pyridalyl on some biological aspects of *S. littoralis* were determined using a leaf dip bioassay. The newly ecdysis 2nd, 4th and 6th instars larvae were treated by feeding on castor leaves treated with LC_{50} concentration for each instar. Daily inspections were carried out until adult emerge occurred and the number of individuals that managed to develop was recorded. Data regarding larval duration, pupation %, pupal duration, pupal weight and adult emergence % were recorded. Adult obtained from tested larvae sexed and transferred to cages. The effect of pyridalyl on the number of eggs deposited by each female (fecundity), percent egg hatch (fertility) and longevity of each sex were determined. Cages were examined daily to record the number of eggs laid and hatchability percentage from eggs of each treatment were estimated. The data were analyzed using analysis of variance (ANOVA) with Duncan's new multiple range test (**Duncan's 1955**) to separate treatment means at the $P \leq 0.01$ levels.

Biochemical methods

Protein extraction methods

Protein extraction was performed using 2nd, 4th, and 6th larval instars tissues treated with LC_{50} value of pyridalyl. SDS-polyacrylamide gel electrophoresis (SDSPAGE) was performed for total storage proteins according to the method early described by **Laemmli (1970).**

Electrophoratic separation of esterase

Esterase patterns of the larval tissues of untreated and treated samples of *S.littoralis* cotton leaf worm were separated by using poly-acerylamide gel electrophoresis into groups based on their relative mobility using ᾱ-naphthal acetate as substrate, according to (**Sell *et al.*, 1974).**

Histopathology studies

The effect of LC_{50} of pyridalyl on the histological structure of the mid-gut of 4th instar *S .littoralis* was determined by fixing treatment and control larvae overnight in Bouin's solution. Specimens were then dehydrated in a series of ethanol, cleared in xylene and embedded in parablast. Embedded tissues were sectioned on a rotary microtome at 5 µm for the histopathological study, these sections were stained with hematoxyline and eosin Stained sections were finally mounted on glass slides with DePeX mounted medium. Microscopic examination and photography were done using a Leitz microscope.

Histochemistry:

Treated and untreated larvae were fixed in 10% normal saline. After then washed carefully in water consequently, were dehydrated in ascending grades of ethyl alcohol, cleared in xylol and mounted in molten paraplast 58-62 ̊C processed for the formation of paraffin blocks. Serial histological sections 5 microns were cut. Stained with Harris hematoxilin, eosin and investigated under microscope.

3. Results and Discussion:

The results in Table (1) summarized the efficacy of pyridalyl at different concentrations against the 2nd, 4th, and 6thlarval instars of *S. littoralis* .The data indicate that the percentage mortality of larvae had positive correlation with pyridalyl concentrations. The probit analysis indicated that the LC_{50} value of the 2nd, 4th and 6th larval instars after 24hrs of treatment were 6.77, 8.926 and 16.0 ppm, respectively. These results indicate that pyridalyl bioassay under laboratory was effective against *S. littoralis*. These results agree with **Satio *et al.*, 2002,** who reported that pyridalyl possesses excellent insecticidal activity against numerous lepidopterous pests. In particular, it demonstrates a high level of insecticidal activity against important vegetable crop pests, such as *Helicoverpa armigera, S. exigua* and the tobacco cutworm *S. litura*. It is expected to be a pests control compound for use in insect resistance management (IRM). The present data consistent with results reported by **Nair *et al.*, (2008),**who indicate that pyridalyl provided excellent control of the two bollworm species of cotton and **Satio *et al.*, (2005)** who reported that pyridalyl caused 100% mortality in the 4th instars larvae of *S. litura* at concentration of 500 mg /L.

Data in Table (2) showed the effect of LC_{50}

value of pyridalyl on larval, pupal duration, mortality and adult emergence resulted from treated different larval instars. It is clear that the tested compound significantly prolonged the duration of the treated 2nd larval instars than that of untreated. The larval duration was 12.98 days and it was 11.72 days for control. On the other hand, the duration of treated 6th larval instar decreased than the control .This period was 3.24 and 5.0 days for treated and untreated larvae, respectively. In case of 4th larval instar, there is no significant difference in duration between treated and untreated larvae. Also data indicate that the treatment of different larval instars with LC_{50} of pyridalyl increased the larval mortality of *S. littoralis* in comparison with control. Mortality rate of 2nd, 4th and 6th larval instars was 46.0, 78.0 and 66.0%, repectively.46.0, 78.0 and 66.0% resulted from treated 2nd, 4th and 6th larval instars, respectively. It is obvious that treatments with this compound resulted in decrease in pupation percent (55.0%) for treated 2nd larval instars as compared with 92.0 % in check experiments, it was 22.0% for treated 4th larval instars compared with 100% in untreated and 34 for treated 6th larval instars as compared with 88.0 % in untreated (Table 2).

It is clear that there is no significant difference between pupal duration resulted from treated and untreated of different larval instars. These results agree with **Abdel Rahman *et al.* (2007).** They found no significant differences in period of larvae, pre-pupae and pupae when they used different concentration of the growth inhibitor Lfenuron EC.

The result in Table (2) also indicated that the treatment of 6th larval instar succeeded to suppress the pupal weight to 0.234g. . It was 0.225g. as compared with 0.341g. for control .The female pupal weight was 0.243 g. as compared with 0.3611 g. in untreated.

Data in Table (2) also indicated that the percentage of pupal mortality was averaged 7.4 , 0.0 and 23.5 % .Resulted from treatment of 2nd, 4th and 6th larval instars, respectively .The percentage of adult emergence were 92.6 ,92.0 and76.9 resulted from 2nd 4th and 6th larval instars, respectively.

Data in Table (3) illustrated that the treated 6th larval instar with LC_{50} of pyridalyl shifted the sex ratio as it increased the male and decreased the female ratio than that of control this percent was 69% males compared to 45% for control .On contrast, in case of treated 2nd and 4th, larval instars ,the sex ratio increased the females and decreased the males ratio than the check , this percent was 54 males developed from treated 2nd larval instar compared to 55 males in control, while the percentage male developed from treated 4th larval instar was 45 compared to 50 males for control

The pyridalyl showed significant reduction in adult males developed from treated 6th larval instars. The decrease was 8.2 days, while the control value was 9.8 days (Table 2) The data also showed that the longevity of males are reduced significantly when treated the 6th larval instars that of males 8.2 days compared with 9.8 days for control. On the other hand, this compound did not affect the longevity of males and females adults resulted from treated 2nd and 4th larval instars. The data in the same table showed that no significant reduction of the number of eggs laid by each female developed from treated 2nd, 4th and 6th larval instars compared with the control.

Table (3) described the hatchability of deposited eggs by female developed from treated 2nd, 4th and 6th larval instars by pyridalyl; the percentage of hatchability was high. The hatchability percentages were 62.0, 00.0 and 88.0% for eggs deposited females emerged from treated 2nd, 4th and 6th larval instars, respectively as compared with 92.0 ,91.0 and 93.0% in control, respectively . These data indicate that the test compound caused failure of the insects embryonic development, showing the lowest egg hatchability percentage compared with control. These results agree with **Sammour *et al.* (2008)** they found a reduction in fecundity and egg hatchability of cotton leaf worm after treated larval instars with chorfluazuron and leufenuron and failure of egg hatchability may be due to the penetration of insecticide into the eggs and prevent hatchability by interfering with embryonic cuticle synthesis so the new hatch probably cannot use its muscles to free itself from egg wall.

Enzymatic activities (ᾱ-& β - esterase)

As shown from electrophoretic separation banding pattern presented in Fig. (1) and (Table 4), no wide range of variation either in density or intensities was found in α- esterase between treated and untreated larvae. There were only two bands (bands 6&7) which appeared in untreated 4th larval instar, and absent in treated 4th larval instar, so they were considered as normal bands for healthy larvae.

Electrophoresis patterns of β-esterase are presented in (Fig. 2) and (Table 5). The results show that no variation either in density or intensities in β-esterase between treated and untreated 2nd and 6th larval instar. There was only some variations appeared in 4th larval instar, bands number 2 and 4 appeared only in untreated larvae, so they were considered as normal bands for healthy larvae. Furthermore, there was one band (band 3) appeared in treated larvae and not found in untreated larvae,

so this band was specific for larvae treated with pyridalyl.

Table (1): Susceptibility of different larval instars of *S. littoralis* to different concentrations of pyridalyl after 24hrs of treatments.

Larval instar	Concentration	Mortality %	LC_{50}	Upper –lower limits	Slope
2nd	1.95	5	16.00	20.1-12.9	2.08±9.64
	3.9	15			
	7.5	73.3			
	15.63	81.7			
	31.25	88.3			
	62.5	100			
	0	0			
4th	7.8	56.6	8.93	137.7-63.03	1.622±1.92
	15.63	70			
	31.25	85			
	62.5	90			
	125.00	93			
	250.00	98			
	0	0			
6th	3.9	26	6.77	40.8-23.2	2.601±5.25
	7.8	24			
	15.63	56			
	31.25	64			
	62.5	92			
	125.00	100			
	0	0			

Table (2): The biological aspects for *S. littoralis* when treated as 2nd , 4th and 6th instar larvae by pyridalyl Lc_{50}.

Biological aspects	2nd instar		4th instar		6th instar	
	Treatment	Control	Treatment	Control	Treatment	Control
Laval duration (days ± SE)	12.98 ± 0.49	11.72 ±0.10 *	11.01 ±0.83	9.56 ±0.24 ns	3.24 ±0.25	5.0 ±0.0 **
Larval mortality %	46.0	8.0	78.0	0.0	66.0	12.0
Pupation %	55.0	92.0	22.0	100.0	34.0	88.0

Horizontally (* Significant ns non significant ** Highly significant for each instar.

Table (3): The biological aspects for *S. littoralis* adult when treated as 2nd , 4th and 6th instar larvae by pyridalyl Lc_{50}.

Biological aspects	2nd instar		4th instar		6th instar	
	Treatment	Control	Treatment	Control	Treatment	Control
Sex ratio (♂:♀)%	54 :46	55 : 45	45 : 55	50 : 50	69 : 31	45 : 55
Male longevity (days± SE)	9.5 ±0.61	10.4 ±0.54 ns	7.3 ±0.54	9.0 ±0.57ns	8.16 ±0.61	9.77 ±0.44 *
Female longevity (days ± SE)	10.8 ±0.55	11.0 ±0.49 ns	11.7 ±0.54	9.8 ±0.44 ns	10.6 ±0.43	10.47 ±0.36 ns
Fecundity (No. eggs/ ♀)	3369.0 ± 61.7	2269.8 ± 26.5 ns	365.7 ±9.3	2416.0 ± 43.0**	2653.5 ± 118.5	2541.9 ±61.2 ns

Fertility %	62.0	92.0	0.0	91.0	88.0	93.0

Horizontally (* Significant ns non significant ** Highly significant for each instar).

Table (4): Gel documentation analysis data of electrophoresis of patterns of treated and untreated of *S. littoralis* larvae with (SLMNPV) isolates (1=present, 0=absent).

RF	2nd instar larvae		4th instar larvae		6th instar larvae	
	Control	Treated	Control	Treated	Control	Treated
Band1	1	1	1	1	1	1
Band2	1	1	1	1	0	0
Band3	1	1	1	1	0	0
Band4	1	1	1	1	0	0
Band5	1	1	1	1	0	0
Band6	0	0	1	0	1	1
Band7	0	0	1	0	0	0

Table (5): Gel documentation analysis data of electrophoresis of β -Estrase patterns of treated and untreated of *S. littoralis* larvae with (SLMNPV) isolates (1= present, 0=absent).

RF	2nd instar larvae		4th instar larvae		6th instar larvae	
	Control	Treated	Control	Treated	Control	Treated
Band1	1	1	1	1	1	1
Band2	1	1	1	0	0	0
Band3	0	0	0	1	1	1
Band4	1	1	1	0	0	0
Band5	1	1	1	1	1	1

Estearases are proteins that are defined by their ability to catalyze the hydrolysis of ester bonds within lipophilic compounds **(El Bermaway, 2004)**. The electrophoretical analysis showed differences in the activities of the alpha and beta esterase enzymes in the treated 4th instar than control .In addition poly -acerylamide gel electrophorasis has been widely used to help in explanation of different biological process that occur like living organism **(Sharaawi *et al.*,2002).**

Differences in the esterase patterns reflect differential gene expression in the production of proteins, which are important not only for maintenance of the stage itself, but also for the synthesis of proteins that trigger processes of metamorphosis. On the other words, the appearance of extra bands due to the treatment with an insecticide indicates that resulting proteins are probably responsible for the detoxification of the insecticide. These bands are present in untreated individuals in a hidden form or are probably activated by the addition of insecticides **(Mohamed and Hafez, 2000).**

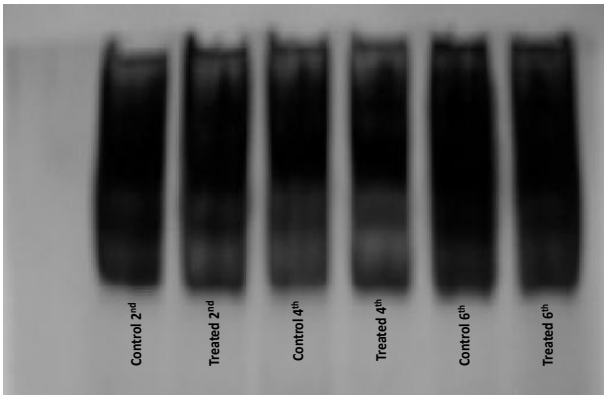

Fig (1): A photograph of poly-acerylamide gel profiles of α-Esterase of treated and untreated *S. littoralis* larvae with Pyridalyl

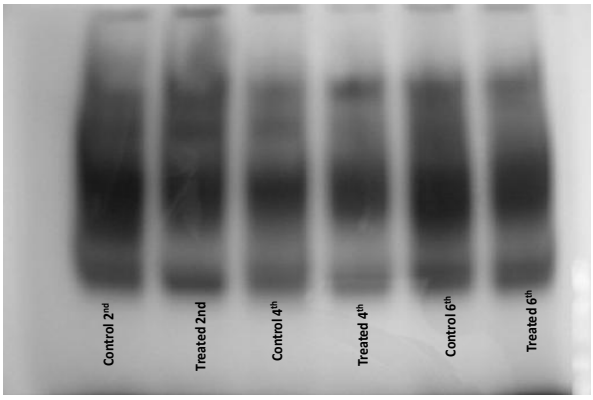

Fig (2): A photograph of acerylamide gel profiles of β-Esterase of treated and untreated *S. littoralis* larvae with Pyridalyl

Rizkallah *et al*., (1982) studied the activities of ā-& β - esterase of synthetic pyrethroids resistant and susceptible strains of *S. littoralis*. They reported that increase of esterase activity was closely associated with the development of resistance.

Protein extraction

The data indicate that a total of 18 protein bands were obtained representing molecular weights (MW) ranging between 0.075 to 0.653 KDa. The highest numbers of protein bands were detected in control and treated 6th instar larvae (9 bands) followed by 8 bands in treated 2nd and 4th instars while the lowest number was 5 bands which was recorded in control of 2nd instars. (Fig. 3 and Table 6). The data presented in the same table show that, two bands with molecular weight of 0.342and 0.585 kDa were found to be specific to the treated 2nd instars larvae. While the three bands with molecular weight of 0.342, 0.585 and 0.414 kDa were found to be specific for the treated 4th instar larvae. Followed by one band with molecular weight of 0.311 kDa can be considered as specific protein marker for control 6th instars larvae and only one band with molecular weight of 0.308 kDa was found in treated 6th larval instar. Also **Zhiwel, 2004** showed that protein level in *Spodoptera littura* significantly influenced. When the protein were separated by 2-DE (two dimentional polyacrylamide gel electrophorases), they observed 10 proteins were significantly affected by azadrichtin treatment when compared to an untreated control. **Moriya *et al.* (2001)** observed rapid and significant inhibition of protein synthesis when they examined the effect of cytotoxicity(LC_{50} mM) pyridalyl on the cellular protein synthesis in Sfg cells derived from *S. frugiperda.* In addition **Powell *et al*.,(2011)** reported that cytochrome P450 action leads to an active pyridalyl metabolite, which results in production of reactive oxygen species (ROS), that leads to damage to cellular macromolecules (e.g., proteins) and enhanced proteasome activity leads to increased protein degradation and necrotic cell death.

Table (6): Survey of SDS-protein markers in the control and treated *S. littoralis* larvae broad- range marker was used to detect MW of extracted protein (1) present and(0)absence

Band No	MW (KD)	Marker	2nd instar larvae		4th instar larvae		6th instar larvae	
			Control	Treated	Control	Treated	Control	Treated
1	0.075	1	1	1	1	1	1	1
2	0.167	1	-	-	-	-	-	-
3	0.122	0	1	1	1	1	1	1
4	0.183	0	1	1	1	1	1	1
5	0.219	1	0	0	0	0	0	0
6	0.256	0	1	1	1	1	1	1
7	0.308	0	0	0	0	0	0	1
8	0.311	0	0	0	0	0	1	0
9	0.342	0	0	1	0	1	0	0
10	0.347	1	0	0	0	0	0	0
11	0.358	0	0	0	0	0	1	1
12	0.394	0	0	0	0	0	1	1
13	0.414	0	0	0	0	1	0	0
14	0.422	0	0	1	1	0	0	0
15	0.447	1	0	0	0	0	0	0
16	0.469	0	1	1	1	1	1	1
17	0.585	0	0	1	0	1	1	1
18	0.653	1	0	0	0	0	0	0

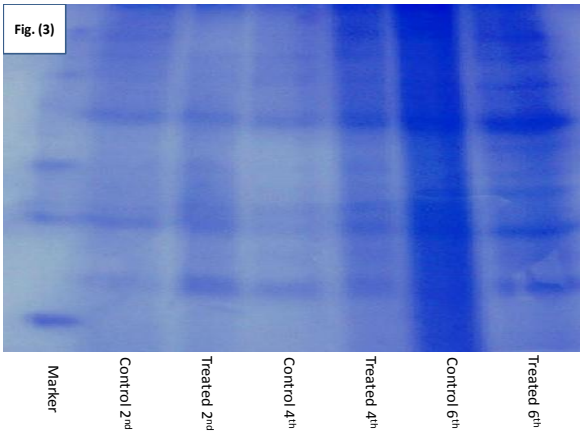

Fig. (3): The SDS-PAGE of total protein extracted from the control and treated S. *littoralis* larva

Histopathological studies:

The mid gut epithelium consists of tall columnar cells and is interspersed apically with the goblet cells and basally with regenerative cells. The columnar cells contain large nuclei in the middle of apical region. The columnar cells possesses a fine brush border facing towards lumen and large number of vesicles discharging into extra peritrophic region of the lumen .The goblet cells are flask shaped with oval nuclei.

The regenerative cells are small spherical or elongated and are basally located. They contain large spherical nuclei at the centre. The peritrophic membrane is well evident in the lumen of the mid gut (Fig. 4) The histological structure of the mid gut of treated larvae show many histological changes than that of control larvae (Fig. 5). Elsewhere, the epithelial cells were completely ruptured after treatment and separated from the basement membrane. The peritrophic membrane was not closely lying to the epithelial cells and the space in between the epithelium and peritrophic membrane was filled with few cytoplasmic vesicles . **Rawi *et al.*, (2011)** reported the histological changes occurred in the larval mid gut of *S. littoralis* treated with *Azadirachta indica* and *Citrulus colocynthis* extracts were vacuolated and necrosis of the epithelial cells and their boundaries and vacuoles occur as a result of cell elongation. Also **Younes *et al.*, (1999)** observed the degeneration of the epithelial cells and decay of its boundaries when *S. littoralis* larvae with extracts of both *Clerodendro inerme* and *Conyza dioscoridis* caused slight sever disintegrations of the epithelium, fading of the boundaries of epithelial cells, and detachment of epithelium. The peritrophic membrane was not closely lying to the epithelial cells and the space in between the epithelium and peritrophic membrane was filled with few cytoplasmic vesicles . The dermal exposure of pyridalyl to *S .litura* larvae caused rapid hydropic degeneration of the epidermal cells, it has common direct and cytotoxic effect on these cells (**Satio *et al.*, 2006).**

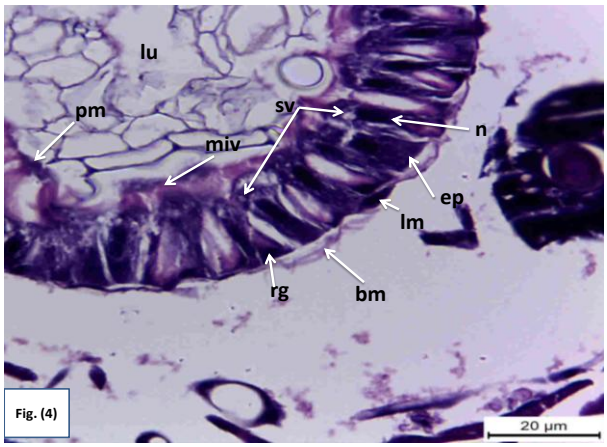

Figure (4): Section showing the anterior midgut epithelium of control fourth instar larvae of *S. littoralis*

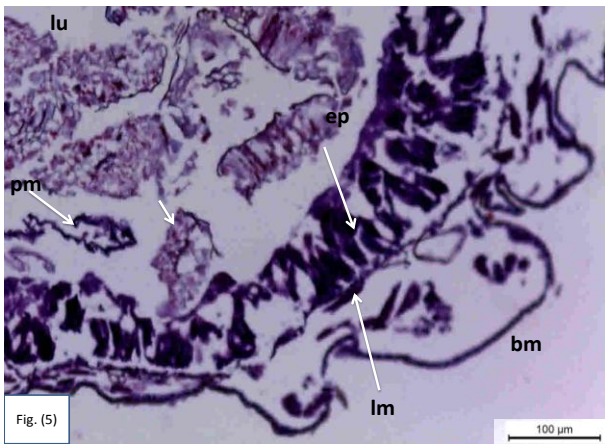

Figure (5): Section showing the anterior midgut epithelium of 48 h post-treatment fourth instar larvae of *S. littoralis* Lumen (**lu**), peritrophic membrane (**pm**), epithelial cell, regenerative cells (rg), secretory vesicles (**sv**) longitudinal muscle (**lm**) and basement membrane (**bm**)

Histochemistry

The total proteins appeared as faintly stained bluish material diffused in the ground cytoplasm of the intestinal cells of the control 4th larval instar (Fig 6 No. 1) .The nucleus was hardly distinguished from the cytoplasm in the most intestinal cells. However, few ones appeared light and containing prominent nucleoli. While in the treated 4th larval instar (Fig. 6 No.2) the total proteins appeared as more faintly

stained bluish homogenous material diffused in the ground cytoplasm of the intestinal cells as compared with the control group .The nucleus was hardly distinguished from the cytoplasm in the most intestinal cells and the cellular protein contents appeared as a syntheium (compact cellular mass). Also we examined the control 6th larval instar (Fig. 6 No.3) and observed the total proteins appeared as moderately stained bluish granules of variable sizes randomly distributed in the ground cytoplasm. The nucleus appeared large containing nuclear chromatin bodies at the nuclear margins and acquired intense stain ability. While the treated 6th larval instar (Fig. 6 No.4) appear a conspicuous depletion in the total protein content was observed in the present group, where the cytoplasmic areas appeared vacuolated containing few cytoplasmic granules observed at the cell margins. The nucleuses are pyknotic reveling strong protein reaction.

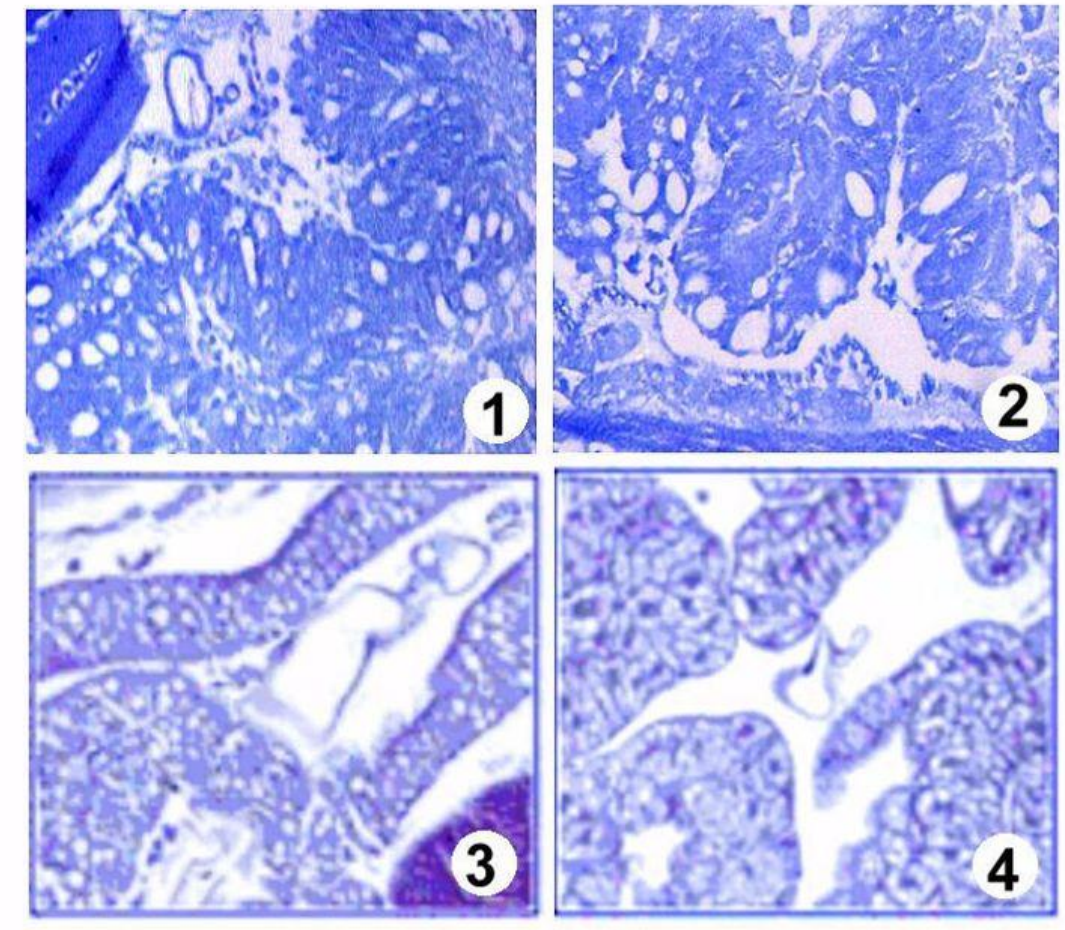

Fig. 6 (No.1,2,3,4) The histochemical effect of pyridalyl on the mid gut of 4th larval instar (1) control (2) treated and 6th larval instar (3) control (4) treated

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