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Effects of specific insecticides on biological functions and histological changes of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith)

Mohamed R G Abo Elela, Salem M S, Antonous M Mekhael, Ali R El-Gabaly
Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt

Abstract
The aim of this study investigated the effectiveness of Lufenuron (Cymex 5% EC), diflubenzuron (Dimilin 48% SC), Emamectin benzoate (Pasha 1.9% EC) and Indoxacarb (Strong 30% SC) on the second and fourth larval instar of the fall armyworm. Cymex 5% EC was the most effective insecticide against *S. frugiperda* larvae, resulting in the shortest larval, pupal, and adult durations of 12.27, 11.81, and 7.34 days compared to control 15.34, 13.39, and 9.96 days respectively. Also, the current study examined lufenuron's insecticidal effect and caused the following results, the percentages of adult emergence (64.8%), pupation (25.1%), and larval mortality (91%). All treatments' tested dose levels revealed significant histopathological disturbances in the insect midgut, including muscle layer destruction, epithelial cell breakdown, peritrophic membrane separation, basement membrane detachment, and the emergence of vacuolations. In addition, the tested insecticides, especially Lufenuron, act as potent physiological disruptors, interfering with the insect hormonal balance, digestive efficiency, impaired developmental rates and histopathological damage. These findings may help to explain why treated larvae died at higher rates than control.

Keywords: *Spodoptera frugiperda*, Biological aspects, histological aspects, insecticides

Introduction

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is one of the most significant invasive polyphagous pests that live in more than 353 plant species, such as maize, sorghum, sugarcane, turfgrass, cotton, and vegetable crops (Gamil, 2020) [10]. According to Kassi *et al*. (2020), *S. frugiperda* has rapidly expanded over 44 West African countries since 2016. According to the Food and Agriculture Organisation (FAO) 2019 [8] report, *S. frugiperda* was first discovered in Egypt on maize fields in Komombo, Aswan Governorate, in 2019. Then, in 2021, it moved northward over the Upper Egypt governorate on sorghum and maize crops (Mohamed *et al*., 2022) [16]. It caused significant harm to Africa's maize and sorghum crops (Hailu *et al*., 2021) [12], leading to a loss of up to 17.7 million tonnes of production annually (Kassie *et al*., 2020) [15].

The two main approaches to controlling insects are chemical and biological. Insecticide use has a number of problems, including lowering the number of natural enemies, polluting the environment, and creating insect-resistant pesticides (Salahuddin *et al*., 2004 and Abo Elghar *et al*., 2005) [2, 19]. As part of IPM programs, new control techniques are required to reduce dependency on traditional insecticides (Hamama *et al*., 2015) [13].

Insecticides, such as synthetic insect growth regulators (IGRs), perform the biochemical control of some insect pests, particularly when it comes to critical agent action on insect development and growth. Disruption of the insect growth regulator "IGRs" can lead to hormone regulation, insect metamorphosis, and finally insect mortality (Gholami *et al*. 2013) [11]. Emamectin benzoate (1.9% EC) (Methylamin: Avermactin) is a member of the vermactin series of compounds, which block the neuromuscular junction's post-synapsic potential, causing paralysis and ultimately death (Fritz *et al*., 1979) [9]. As a novel class of oxidiazine insecticide, indoxacarb effectively inhibits the passage of sodium ions, causing paralysis and stopping feeding in Lepidoptera pests while having very no effect on non-target insects (Dinter and Wiles, 2000) [5]. According to Abdel-Aziz (2012) [1], these compounds interfere with the typical growth and development of insects and may have a further impact on the insects' capacity for reproduction in addition to other physiological impacts. The purpose of the current study was to compare and assess the effectiveness of Cymex 5% EC, Dimilin 48% SC, Pasha 1.9% EC, and Strong 30% SC on certain histology and biology in *S. frugiperda* larvae.

Material and Methods

Rearing insects

Specimens of *S. frugiperda* were continuously reared for multiple generations at the Plant Protection Research Institute. Following the methodology described by El-Sawaf (1971) [6], the colony was reared on fresh castor bean leaves (*Ricinus communis L.*). Environmental conditions were strictly regulated within an incubator at 27±2˚C and 65±2% relative humidity.

Bioassay

The selected second larval instar of *S. frugiperda* was starved for approximately four hours prior to consuming castor bean leaves treated with the following compounds. Each concentration was applied by submerging castor leaves for a two-minute period. Following complete desiccation, these leaves were positioned inside 250 ml glass jars to facilitate the testing process.The larvae that survived were permitted to consume untreated castor bean leaves until they reached pupation and emerged. Three repetitions (20 larvae each) were employed for each concentration, while three replicates that contained larvae fed on untreated leaves served as a control. Every experiment was maintained at 27±2°C and 65±2% relative humidity.

Tested insecticides

Table 1: Lists the tested insecticides used on *S. frugiperda* larvae in their second instar.

Trade name	Common name	Recommended application rate	Obtained from
Cymex 5% EC	Lufenuron (Insect growth regulator)	160 cm3 /fed.	Shoura Chemicals-Egypt
Dimilin 48% SC	Diflubenzuron (Insect growth regulator)	125 cm3 /fed.	Eristia life co.
Pasha 1.9% EC	Emamectin benzoate (Bio-insecticide produced by the soil microorganism, Streptomyces avermitilis). They block (GABA) the transmittance of electrical activity in nerves and muscle cells	250 ml/feddan	ElHelb Pesticides and Chemicals- Egypt
Strong 30% SC	Indoxacarb (voltage- gated sodium ion channels blockers)	12.5 cm3/100L	Al-Qawafel Technical Ind. Agr.Co.

Biological Aspects

To evaluate the long-term biological impact of the tested pesticides on *S. frugiperda*, surviving larvae from both the experimental and control groups were transferred to individual glass jars and provided with fresh, untreated castor leaves. These specimens were maintained under controlled laboratory conditions (27 ± 2ºC and 60 ± 2% R.H.) and monitored daily to record the larval developmental period. Following the individual weighing of newly formed pupae, they were transferred to glass vials (4 × 10 cm) and covered with muslin fastened by rubber bands. These vials were kept under the same environmental conditions until adult eclosion. Key biological parameters documented included pupal duration, pupation success rate, pupal mass (mg), and the emergence rate of adults.

Additionally, Adults were moved into a fresh glass vial and given a 10% honey solution. To document the adult longevity, the vials were additionally covered with muslin using rubber bands and checked every day.

Statistical Analysis

The variance analysis "ANOVA" and F test according to Fisher (1954) [7], were used to statistically evaluate the collected data. The significance between treatments was assessed using the COSTAT computing program and the F value test.

Mid-gut histological preparation for light microscopy

The four insecticides' histopathological effects on *S. frugiperda* larvae in their fourth instar were tested by feeding treated leaves with 1/16 of a concentration field of Cymex 5% EC, Dimilin 48% SC, Pasha 1.9% EC, and Strong 30% SC for 48 hours. Following a 24-hour treatment period, approximately ten larvae from each treatment and control group were individually dissected in petri dishes containing Bouin's alcoholic solution, following a 24-hour exposure period.

Following a rinse in 70% ethyl alcohol, the specimens were preserved in the same medium. A gradual dehydration process followed, involving 30-minute exposures to an upward alcohol series (70%, 80%, 90%, 96%, and 100%). Lastly, the larvae were briefly cleared with xylene.

The specimens were then infiltrated in three changes of hot wax-paraffin, each lasting 20 minutes, in a Xylol dish and three wax dishes, waxI, wax II, and wax III} in an oven set at 50 to 52Cº for half an hour each1 used normal plastic cups to make the embedding, which was in hot wax paraffin. To solidify the paraffin blocks, they were chilled in cold water, ensuring they were sufficiently hardened for secure mounting onto the rotary microtome holder. Transverse sections were obtained at a 5 µm thickness using a rotary microtome. The resulting wax ribbons were subsequently floated in a warm water bath to facilitate expansion before being mounted onto slides pre-treated with an egg-albumin adhesive. Following a minimum 24-hour incubation in a drying oven to ensure complete moisture evaporation, the preparations were deparaffinized in xylene for 3–5 minutes. Rehydration was then achieved through a descending ethanol gradient (100, 90, 80, and 70%) for 2 minutes at each concentration. Throughout the process, a hot plate maintained at 40°C was utilized to ensure the proper flattening and separation of the sections. Following a 30 to 45-minute hematoxylin staining period, the slides were immersed in distilled water and then again in tape water. Subsequent to counterstaining with 1% eosin for 5–10 seconds, the slides underwent rapid dehydration through an ascending ethanol series (70%, 80%, 90%, and 96%). The specimens were then immersed in absolute alcohol for 10 minutes to ensure complete dehydration, followed by two changes of xylene (10–15 minutes each) for clarification. Finally, the sections were mounted in Canada balsam, protected with coverslips, and cured in a 40°C incubator for 24 hours. Following preparation, the segments were examined and documented using a light microscope (Sharaby and El-Nujiban, 2016) [18].

Results and Discussion

Biological parameters

The effectiveness of the four pesticides was assessed on *S. frugiperda* larvae in their second instar. The findings showed that the effectiveness of the tested pesticides on the durations of larvae, pupae, and adults is displayed in Table (2). All treatments demonstrated highly significant effects throughout a range of time periods when compared to the control group. In comparison to 15.34 days in the control, the larval duration in the treatments varied from 12.27 days (cymex 5% EC) to 14.60 days (pasha 1.9% EC). At the cymex 5% EC, the pupal duration was 11.81 days, but at the control, it was 13.39 days. Cymex 5% EC caused the highest reduction in adult longevity, lasting 7.34 days as opposed to 9.96 days in the control group.

Table 2: Insecticide effects on some *S. frugiperda* biological traits.

	Cymex 5% EC	Dimilin 48%Sc	Pasha 1.9% EC	Strong 30% SC	Control	F value	LSD
Larval duration (Day)	12.27^{d}	13.57^{bc}	14.60^{a}	14.29^{ab}	15.34^{c}	13.50*	0.779
Pupal duration (Day)	11.81^{b}	12.51^{ab}	12.51^{ab}	12.68^{ab}	13.39^{a}	2.76*	1.070
Adult duration (Day)	7.34^{c}	8.6^{b}	9.7^{a}	9.26^{ab}	9.96^{a}	9.58*	1.059

The effectiveness of the four pesticides on the percentage of larval mortality, pupal weight (g), pupation, and adult emergence is displayed in Table (3). There was no significant difference in pupae weight between treatments and the control in any of the treatments. However, the larval mortality rate was 21% in the control group and 91% in the cymex 5% EC group. The effects of cymex 5% EC were also greater than those of the other treatments. The highest reduction in pupation percentage was observed with cymex 5% EC. At cymex 5% EC, pasha 1.9% EC, dimilin 48%SC, and strong 30% SC, the pupation percentages were 25.1, 33.90, 48.34, and 61.68%, respectively. However, the differences between treatments were insignificant, and the adult emergence percentages in all treatments were marginally lower than those in the untreated (control) group. In comparison to the other treatments, cymex 5% EC was generally the most effective against *S. frugiperda*.

Table 3: Weight of pupae, adult emergence, pupation percentages, and larval mortality percentages for *S. frugiperda* larvae treated with four insecticides

	Cymex 5% EC	Dimilin 48%SC	Pasha 1.9% EC	Strong 30% SC	Control	F value	LSD
Pupal weight(g)	0.404	0.441	0.401	0.441	0.471	1.26	n.s
Larval mortality %	91[a]	67.67[a]	82.12[a]	54.34[ab]	21[b]	12.96	34.14
Pupation %	25.1[a]	48.34[b]	33.90[b]	61.68[ab]	95[a]	12.96	34.14
Adult emergence %	64.8	72.12	68.42	80.45	88.47	4.83	n.s

The current findings confirm with those of Abd El-Rahman *et al*. (2019) [3] claimed that cymex 5% EC was the most potent tested ingredient, followed by dimilin 48%SC, according to a study that examined the effects of various pesticides on four *S. littoralis* larval instars. Additionally, there was a noticeable decrease in the relative growth rate (RGR) of *S. littoralis* fourth larval instars fed on the cymex -treated leaves by 5%. Nonetheless, a number of researchers looked into how several natural chemicals affected the length of time that various stages of *S. frugiperda* lasted following treated larvae. Hussein and Eldesouky (2019) [14] discovered that sublethal concentrations of chlorfluazuron and chlorantraniliprole significantly decreased the weight of pupae and larvae, as well as the longevity of adults, pupation percentage, adult emergence, and female fecundity, while increasing the duration of larvae and pupae.

Impact of the studied pesticides on the midgut histological structure of *S. frugiperda*.

1. The typical ultrastructure of the control midgut

According to microscopic analysis, the normal mid-gut histological structures of *S. frugiperda* larval instars four include two muscular layers and an epithelial layer lining the lumen. The intestinal epithelium contains four different cell types: goblet, endocrine, regenerative, and digestive cells. This structure is unique to the lepidoptera order. Circular and longitudinal muscle fibres encircle the basement membrane, which supports the epithelial layer. The goblet and columnar cells that make up this epithelial layer are clustered from regenerative cells, which are tiny cells with a comparatively big nucleus and strong cytoplasmic basophiles (Fig. 1). Additionally, the peritrophic membrane that surrounds the lumen protects the epithelial layers from food particles.

2. The typical ultrastructure of treated midgut with Cymex 5% EC

When examined under a microscope, Figure 2 showed that the mid-gut epithelium of *S. frugiperda* fourth larval instar, which had been treated with Cymex 5% EC pesticide, had sustained several damages. When compared to the controlled midgut, the cells of the midgut epithelium were most impacted. As a result of severe epithelium destructions and an increase in goblet cell secretions, regenerative cells were separated from one another based on the base of the epithelium, and the muscular layer shrank more than the control. Large epithelium cell destructions, along with some vacuoles, were "not found" and could "not be identified" in many layers at the base of the epithelial cells (Fig. 2).

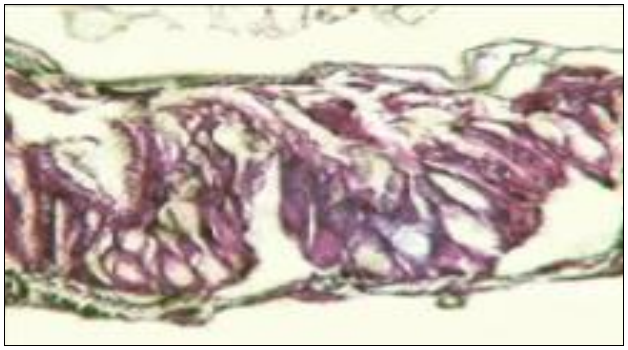

Fig 1: A cross section of the larvae's midgut reveals no variations in the control tissues.

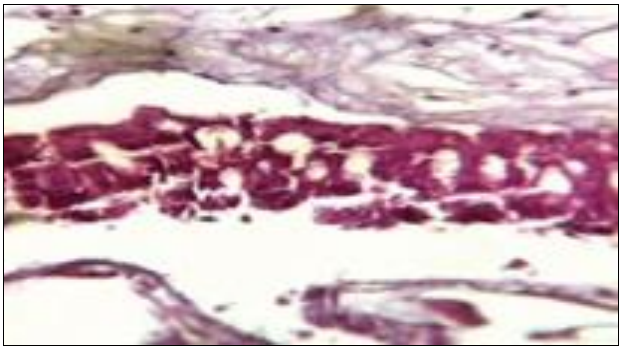

Fig 2: A cross section of the larvae midgut reveals different damaged tissues following Cymex 5% EC treatment.

3. The typical ultrastructure of treated midgut with Dimilin 48% SC

An analysis of the midgut segment of a fourth larval instar of *S. frugiperda* treated with the chemical Dimilin48% SE (Fig. 3) revealed significant harm to the midgut layers. Mid-gut epithelial cell vacuolization and changes in cell size and shape were the most noticeable effects in larvae treated with Dimilin 48% SC. Additionally, compared to the control in (Fig. 1,) a small number of epithelial cells exhibited vacuolization, increased slightly in size, and vacuolization was more prominent and epithelial (Fig., 3).

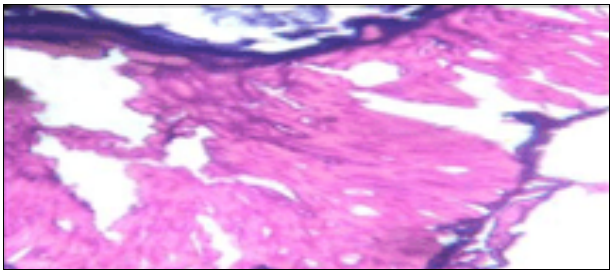

Fig 3: A cross section of the larvae midgut reveals different damaged tissues following Dimilin 48% SC treatment.

4. The typical ultrastructure of treated midgut with Pasha 1.9% EC

The histopathological effects of Pasha 1.9% EC on *S. frugiperda* larvae in their fourth instar are examined in (Fig., 4). The muscular lager appears to thicken when the distal end of the midgut epithelium cells disintegrates. The peritrophic membrane partial lysis is detachment of the epithelial cells. In contrast to the control (Fig. 1), there is apically swelling in the gut-lumen, a decrease in intercellular connections with nearby cells, nuclei degenerations, and brush boundaries (Fig. 4).

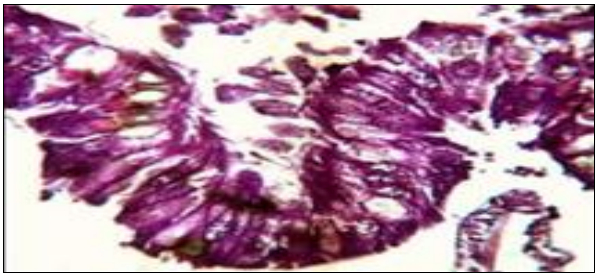

Fig 4: A cross section of the larvae midgut reveals different damaged tissues following Pasha 1.9% EC treatment.

5. The typical ultrastructure of treated midgut with Strong 30% SC

Histopathological alterations in the midgut of the fourth larval instar of *S. frugiperda* were seen in Fig. 5. After being treated with Strong 30% SC, the peritrophic membrane began to disintegrate and the inure divided, making it more noticeable. Additionally, there is brush boundaries, apically enlarged gut-lumen, decreased intercellular connections with nearby cells, and nuclei degenerations. Microvilli revealed total disorder in some places, goblet cell secretion rising in conjunction with basement membrane ruptures and columnar epithelial cell disruptions. Comparison with the control revealed that the adhesive basement membrane, nuclei, and lateral plasma membrane were normal compared to control (Fig. 1).

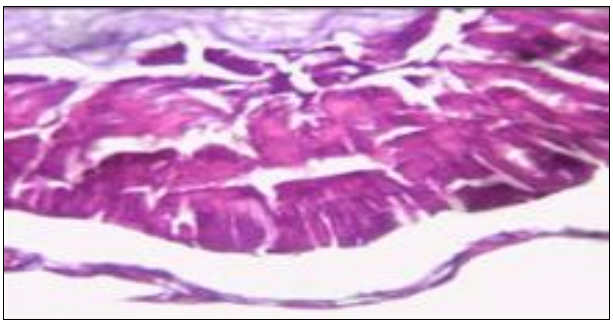

Fig 5: A cross section of the larvae midgut reveals different damaged tissues following Strong 30% SC.

Histological examinations by Youssef (2006) [21] revealed that both abamectin and pyriproxyfen induce structural deformities in the larval midgut of S. littoralis. These treatments resulted in the disorganization of the columnar epithelium and induced enough stretching to cause ruptures in the peritrophic membrane. These results were consistent with that study. Rawi *et al*. (2011) [17] investigated how the extracts of *A. indica* and *C. colocynthis* affected the histopathology of *S. littoralis* larvae in their fourth larval instar at LC10 and LC25. At two dosages, the *A. indica* extract caused histological damage in the larval midgut, causing some epithelial cells to vacuolate and lose their nuclear content. At dose level LC10, the *C. colocynthis* extract caused columnar epithelial cells to degenerate and vacuolate. Conversely, the application of LC25 extract formulation of *C. colocynthis* resulted in vacuolations and columnar epithelial cell detachments.

Histopathological alterations in the midgut of *A. ipsilon* larvae treated with oils "Garlic+Mint" were discovered by Sharaby and El-Nujiban (2016) [18]. These changes included a swelling appearance of the cells in the midgut, microvilli that were completely disordered in some areas, and an increase in goblet cell secretion along with basement membrane ruptures. Furthermore, research on similar or other histopathological changes was conducted by Abdel-Salam *et al*. (2018) [4]. They found that when 2-chitin synthetic inhibitors were used to treat the fourth larval instar of *S. littoralis,* the mid-gut of the sixth larval instar showed numerous ultrastructure alterations, including vacuolations, elongations, and disintegrations of epithelial cells, as well as the disappearance of musculoris and regenerative cells and the detachment of the basement membrane.

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