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Assessing Toxicity and Histopathological Effects of a Novel Group Acetyl-CoA Carboxylase Insecticides (Tetramic and Tetronic Acids-Based) Against *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae)

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ABSTRACT

In a laboratory study, this research focused on the effects of the insecticides spirodiclofen, spiromesifen, and spirotetramat, which are Acetyl-CoA carboxylase (ACC) inhibitors, on the mortality rates of *Pectinophora gossypiella* neonates. A comparative assessment of toxicity through contact and ingestion was conducted at 6, 12, 24, 48, and 72 hours. Results indicated that spirotetramat exhibited higher toxicity as the median lethal concentration (LC_{50}) values significantly decreased over time (21.619 to 6.872 µg/ml). While spirodiclofen demonstrated moderate effectiveness, otherwise spiromesifen was considerably weaker, especially during the initial hours. Biological evaluation revealed prolonged larval developmental time and reduced weight in the treated larvae, with spirodiclofen causing the most significant effect. The highest mortality rate was observed for the spiromesifen treatment (81.7%), closely followed by spirodiclofen and spirotetramat (85.7% and 84.7%, respectively). There was a notable decrease in the pupation percentage and a significant increase in the pupal malformation percentage across all treatments compared to the control group. Histopathological examination revealed significant alterations in integument and gut structures, including degeneration of cuticle layers and destruction of epithelial tissues. These results highlight the potential utility of spirodiclofen, spiromesifen, and spirotetramat in integrated pest management for controlling *P. gossypiella*.

INTRODUCTION

Cotton fibers, derived from the *Gossypium* genus, have significant commercial value as one of the most esteemed natural fibers. Their popularity stems from their softness, strength, durability, breathability, and absorbency (Varghese and Mittal, 2017).

In Egypt, the pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechidae) is one of the most destructive pests threatening the cotton crop. This insect damages various parts of the cotton plant. Its harmful effects include flower deformation and

rosetting, shedding bolls, reduced fiber length, and deterioration of lint quality due to contamination from rotting bolls (Singh *et al*., 1988).

Globally, pesticides are essential pest management tools widely used to control harmful insects and reduce crop losses (Casida and Durkin, 2017). However, the frequent and intensive abuse of such noxious substances adversely impacts plant and natural enemies, causing increased pest problems in several field crops (Armes *et al.,* 1997; Ramana *et al.*, 1988). Consequently, this results in economic losses to crops and poses health risks to livestock and humans (Doucette, 2023).

The need arose to identify safer and less hazardous alternative insecticides for incorporation into integrated pest management (IPM) strategies against the domestic pest *P. gossypiella.*

Tetramic acid derivatives Spirodiclofen, Spiromesifen, and Spirotetramat are receiving attention because of unique modes of action targeting key metabolic pathways in insects (Baker *et al*, 2020). Tetronic and Tetramic acid derivatives emerge as a promising alternative to traditional nerve and muscle- targeting insecticides. Also, they have demonstrated great agricultural value as fungicides (Li *et al*., 2000), herbicides (Graupner *et al*., 2003; Chen and Qiang, 2017), and nematocidal (Lee *et al*., 2017).

Using ACC inhibitors to control lepidopteran pests such as *P. gossypiella* is a promising and effective method. These compounds adversely affect target organisms by inhibiting lipid biosynthesis and causing various physiological and developmental abnormalities, which enhances their efficacy in pest management (Zhang *et al*., 2019).

Prior studies have shown that ACC results indicate that the addition of spiromesifen in the preimaginal diet affects growth, development and reproduction. reduced fecundity and fertility could be explained by the alteration of lipid metabolism during the vitellogenesis process (Hamida *et al*., 2021). Their effects as lipid biosynthesis inhibitors decreased the fertility and fecundity of *Earias insulana* with a significant impact on its physiological activity (El-Bassouiny *et al.*, 2022). In addition, Marium Fatima *et al.* (2013) concluded that Spirotetramat affects the haemocytes in the haemolymph of American Bollworm, *Helicoverpa armigera*. Sleem *et al.* (2019) indicated the effect of Spirotetramat LC_{50} on protein concentrations and enzyme activities in the body homogenate of *S. littoralis* larval instar. Kheireddine *et al.* (2023) reported the effect of spirodiclofen on biochemical and enzymatic parameters of the snail *Helix aspersa* Muller. Generally, these new keto-enol insecticides, including spirodiclofen, spiromesifen, and most prominently Spirotetramat as a commercial product, can be utilized for many crops (Nauen *et al.,* 2008).

A pilot study aimed to assess the efficacy of Tetramic and Tetronic acid-based insecticides, particularly their median lethal concentration (LC_{50}) levels, on *P. gossypiella* concerning larval mortality, developmental duration, and histopathological changes. Results suggested their recommended use in integrated bollworm management as new insecticides to promote sustainable agriculture.

MATERIALS AND METHODS

Biological Materials:

Neonates of *P. gossypiella* larvae were acquired from a laboratory strain reared with no insecticidal contamination in the Bollworms Research Department, Plant Protection Research Institute, Agriculture Research Center, Egypt. The larvae were reared for several generations on an artificial diet as described by Rashad and Ammar (1985).

Chemical formulations:

The study utilized commercial formulations of the active ingredients, Spirodiclofen (Envidor® 24% SC), Spiromesifen (Oberon® 24% SC), and Spirotetramat (Movento® 10%

SC), all manufactured by Bayer Crop Science, Germany, and purchased from Egyptian distributors.

Laboratory Evaluation of *P. gossypiella:*

The impact of three acetyl-CoA carboxylase (ACC) inhibitor insecticides (spirodiclofen, spiromesifen, and spirotetramat) on neonates of cotton pink bollworm *Pectinophora gossypiella* from a laboratory strain was examined through bioassays under laboratory conditions.

Toxicity Tests:

The toxicity of Spirodiclofen, Spiromesifen, and Spirotetramat compounds against *P. gossypiella* neonate larvae was assessed using various concentrations prepared in distilled water as follows: Spirodiclofen at 7.5, 15, 30, 60, 120, and 240 µg/ml; Spiromesifen at 7.5,15,30,60 and 120 µg/ml; and Spirotetramat at 3.125, 6.25, 12.5, 25, 50,100, and 200 µg/ml. Each concentration was sprayed over three grams (3 g) of artificial diet distributed in Petri dish using clean hand sprayer and allowed to dry under laboratory conditions. Three replicates for each concentration were established, each containing 30 transferred neonates. The larvae were allowed to contact and feed on the treated diet under controlled conditions of 26 ± 1 °C and 75 ± 5% RH. A control group was maintained with an untreated diet. Mortality percentages were recorded after 6, 12-, 24-, 48-, and 72-hours post-treatment to evaluate the acute toxicity of the compounds.

Biological Assay:

The effects of the tested compounds on some biological aspects of exposed larvae were estimated. After toxicity evaluation, a biological assessment was performed using the calculated LC_{50} after 24 hours for each compound. One millilitre of the LC_{50} for each compound was sprayed on 3 grams of artificial diet. Ninety *P. gossypiella* larvae were separated into three replicates of 30 larvae each and fed with the treated diet. Another group was maintained on an untreated diet. All the groups were kept at constant conditions of (26 ± 1°C and 75 ± 5% RH) for 24 hours.

Alive larvae from both treated and untreated groups were transferred individually to glass tubes (2x7.5cm) each containing two grams of artificial diet. Each tube was tightly closed with a piece of medical cotton stopper and maintained under the same conditions in an incubator, with daily inspections until pupation. Various biological aspects were evaluated, including cumulative mortality, malformations, durations of immature stages, pupation percentage, and adult emergence percentage.

Histopathological Assay:

Histological studies were conducted on the full-grown (4th instar) larvae treated as neonate with the LC_{50} of the most effective compound, compared to untreated larvae. Three samples were collected from both treated and untreated larvae to evaluate the effects on the cuticle, mid-gut, and hind-gut due to treatment. The tested larval samples were homogenized and preserved in 10 % formal saline for 24 hours. After fixation, the specimens were washed with tap water and dehydrated using 10% alcohol (methyl, ethyl, and absolute ethyl). The specimens were cleared in xylene and embedded in paraffin for 24 hours at 56 °C in a hot air oven. Paraffin beeswax tissue blocks were prepared and sectioned to a thickness of 4µm using a sled microtome. The obtained tissue sections were collected on glass slides, deparaffinised, and stained with hematoxylin and eosin for examination under a light microscope (Banchroft *et al.*, 1996).

Statistical Analysis:

The LC_{50}, chi-square (χ 2), fiducial limits, and slope values were estimated according to probit analysis (Finney, 1971) using Ldp line software, as described by Bakr (2000). Results were expressed as mean ± SE and statistically analyzed, using SPSS Statistical Program Software.

RESULTS

Comparative Toxicity Studies:

The relative toxicity of the three tested insecticides (spirodiclofen, spiromesifen, and spirotetramat) against the *Pectinophora gossypiella* neonates under laboratory conditions was reported at different exposure times (6, 12, 24, 48, and 72 hours) (Table 1).

Table 1: Comparative toxicity of three Acetyl-CoA carboxylase inhibitor insecticides to the susceptible strain of *Pectinophora gossypiella* neonates under controlled conditions (26 ± 1 °C and 75 % RH.) .

Treatment	Time (hours)	LC_{50} (µg ml^{-1}) (Fiducial Limits)	Slope ± SE	χ^2 (df)	h	g	R^2
spirotetramat	6	21.619 (18.302– 25.673)	1.553± 0.122	1.383 (4)	0.346	0.0237	0.997
	12	16.683 (14.195 -19.577)	1.640 ± 0.124	2.908 (4)	0.727	0.0221	0.992
	24	12.850 (10.594 -15.402)	1.402 ± 0.118	2.654 (4)	0.6635	0.0273	0.9921
	48	9.223 (7.448 -11.134)	1.403 ± 0.121	5.988 (4)	1.497	0.0286	0.983
	72	6.872 (5.159 -8.722)	1.228 ± 0.197	4.777 (2)	2.389	0.0986	0.945
spiromesifen	6	48.879 (39.882 – 61.340)	1.562 ± 0.155	1.313 (2)	0.656	0.0378	0.995
	12	44.884 (37.068 -55.585)	1.617 ± 0.131	0.667 (3)	0.222	0.0.252	0.998
	24	17.169 (13.473 -21.762)	1.161 ± 0.113	2.903 (3)	0.968	0.0365	0.989
	48	14.266 (11.038 -18.133)	1.137 ± 0.114	3.655 (3)	1.218	0.0384	0.985
	72	10.954 (8.265 -14.339)	1.119 ± 0.154	3.653 (2)	1.827	0.0730	0.971
spirodiclofen	6	26.866 (23.655 – 30.781)	2.535 ± 0.235	3.607(2)	1.804	0.0331	0.987
	12	26.666 (23.402 -30.757)	2.393 ± 0.196	4.866 (3)	1.622	0.0258	0.989
	24	20.554 (18.241 -23.259)	2.621 ± 0.199	6.483 (3)	2.161	0.0221	0.986
	48	14.058 (12.582 -15.690)	2.986 ± 0.216	3.088 (3)	1.029	0.0201	0.994
	72	11.812 (10.671 -13.084)	3.567 ± 0.291	2.091 (2)	1.045	0.0255	0.995

Obtained results indicated that the spirotetramat exhibited the highest toxicity against *Pectinophora gossypiella* neonate larvae, with LC_{50} values of 21.619, 16.683, 12.850, 9.223, and 6.872 µg/ml after exposure periods of 6, 12, 24, 48, and 72 hours, respectively. spirodiclofen showed moderate effectiveness against *P. gossypiella* neonates with LC_{50} values of 26.866, 26.666, 20.554, 14.058, and 11.812 µg/ml, respectively) for the same exposure periods. Spiromesifen was the least toxic insecticide against *P. gossypiella* neonates, particularly at 6 and 12 hours, with LC_{50} values of 48.879, 44.884 µg/ml, respectively; however, it ranked second in effectiveness after 72 hours with LC_{50} values of 10.954 µg/ml.

Effect of Acetyl-CoA Carboxylase Inhibitor Insecticides on Some Biological Aspects:

Exposed *P. gossypiella* larvae to the LC_{50} calculated doses of spirotetramat (12.850 µg/ml), (17.169 µg/ml) and spirodiclofen (20.554 µg/ml), affected some biological aspects as follows:

Immature Duration and Weight:

The results obtained in Table 2, declare that larval development time was increased to 26.00 days in the treatmeant of spirodiclofen followed by spirotetramat and spiromesifen

treatments (23.33 and 20.67) days, in respect compared with untreated check (15.90 days). In the same boat, the pupal duration was also extended to 19.33, 18.33, and 19.33 days for spirotetramat, spiromesifen, and spirodiclofen, respectively, compared with 9.60 days in the control. The larval and pupal weight was deeply affected by the treatment where ranged from 0.02-0.03gm for larval and 0.01-0.02gm for pupal in comparison to the untreated check, compared (0.07 and 0.06 gm.) respectively, A Subsequent elongation duration was drastic affect by the treatments means recorded 45.33, 42.67, and 39.00 days for spirodiclofen, spirotetramat, and spiromesifen, respectively, compared with the untreated check (25.50) days.

Table 2: Life table aspects of neonate larvae of pink bollworm treated by Acetyl-CoA carboxylase inhibitor insecticides.

Treatments	Larval duration days (Mean ± SE)	Larval Wight (Mean ± SE)	Pupal duration days (Mean ± SE)	Pupal Wight (Mean ± SE)	Time mature Larval & Pupa days (Mean ± SE)
Spirotetramat	23.33±3.76 ^{ab}	0.03±0.01 ^{b}	19.33±0.88 ^{a}	0.02±0.00 ^{b}	42.67±3.53 ^{a}
Spiromesifen	20.67±1.20 ^{ab}	0.02±0.00 ^{b}	18.33±1.20 ^{a}	0.01±0.00 ^{b}	39.00±2.08 ^{a}
Spirodiclofen	26.00±3.79 ^{a}	0.02±0.00 ^{b}	19.33±0.88 ^{a}	0.02±0.00 ^{b}	45.33±2.91 ^{a}
Untreated Check	15.90±0.99 ^{b}	0.07±0.02 ^{a}	9.60±0.44 ^{b}	0.06±0.01 ^{a}	25.50±1.42 ^{b}
$LSD_{0.05}$	9.05	0.03	2.91	0.28	8.51
***P* value**	0.143	0.015	0.000	0.004	0.003

Column values followed by different letters are different significant (*P*=0.05, L.S.D test)

Immature Mortality and Malformation %:

Larval mortality and malformation rates were significantly affected, with spirodiclofen giving the highest percentages (85.70, 5.00 %), followed by spirotetramat (84.20, 3.50%), then spiromesifen (81.70, 3.34%), respectively, compared with untreated check (6.06, 0.00 %). Regarding the pupal stage was deeply affected by spirotetramat, spirodiclofen, and spiromesifen significantly reduced the pupation rate to 18.30, 15.80, and 14.20%, compared to the untreated check (93.90 %). however, Pupa Malformation percentages were significantly increased by spirodiclofen (35.30 %), followed by spirotetramat (18.70%), and spiromesifen (8.32%), contrary to zero % in the untreated check (Table 3).

Table 3: Biological aspects of neonate larvae of *P. gossypiella* treated by three Acetyl-CoA carboxylase inhibitor insecticides.

Treatments	Larval mortality (%)	Larval Malformed (%)	Pupation (%)	Pupa Malformed (%)	Emergency (%)
Spiroteramat	84.20±0.96^{a}	3.50±0.17^{a}	18.30±1.27 ^{b}	18.70±4.04^{b}	84.80±1.09^{b}
Spiromesifen	81.70±2.21^{a}	3.34±0.48^{a}	14.20±1.27 ^{b}	8.32±1.33^{c}	74.40±0.56^{c}
Spirodilofen	85.70±2.40^{a}	5.00±0.83^{a}	15.80±0.96 ^{b}	35.30±1.21^{a}	72.10±2.35^{c}
Untreated Check	6.06±1.75^{b}	0.00±0.00^{b}	93.90±1.75 ^{a}	0.00±0.00^{d}	94.60±1.08 ^{a}
L.S.D $_{0.05}$	4.47	1.59	4.39	7.22	4.67
***P* value**	0.0001	0.0001	0.0001	0.0001	0.0001

Column values followed by different letters are different significant (*P*=0.05, L.S.D test)

Figure (1) showed that malformation in the immature stages (larval and pupae) of *P. gossypiella* after exposure to the compound under study. Where affected on the larvae displayed distinct abnormalities such as reduced sizes with altered coloration, particularly in

spirotetramat treatment, which lost their pink hue to become white. Moreover, spiromesifen and spirodiclofen-treated larvae appear to have compressed bodies with very compacted cuticle and atrophy with dark spots in some areas along the body. While in the pupae malformed were small and highly compressed.

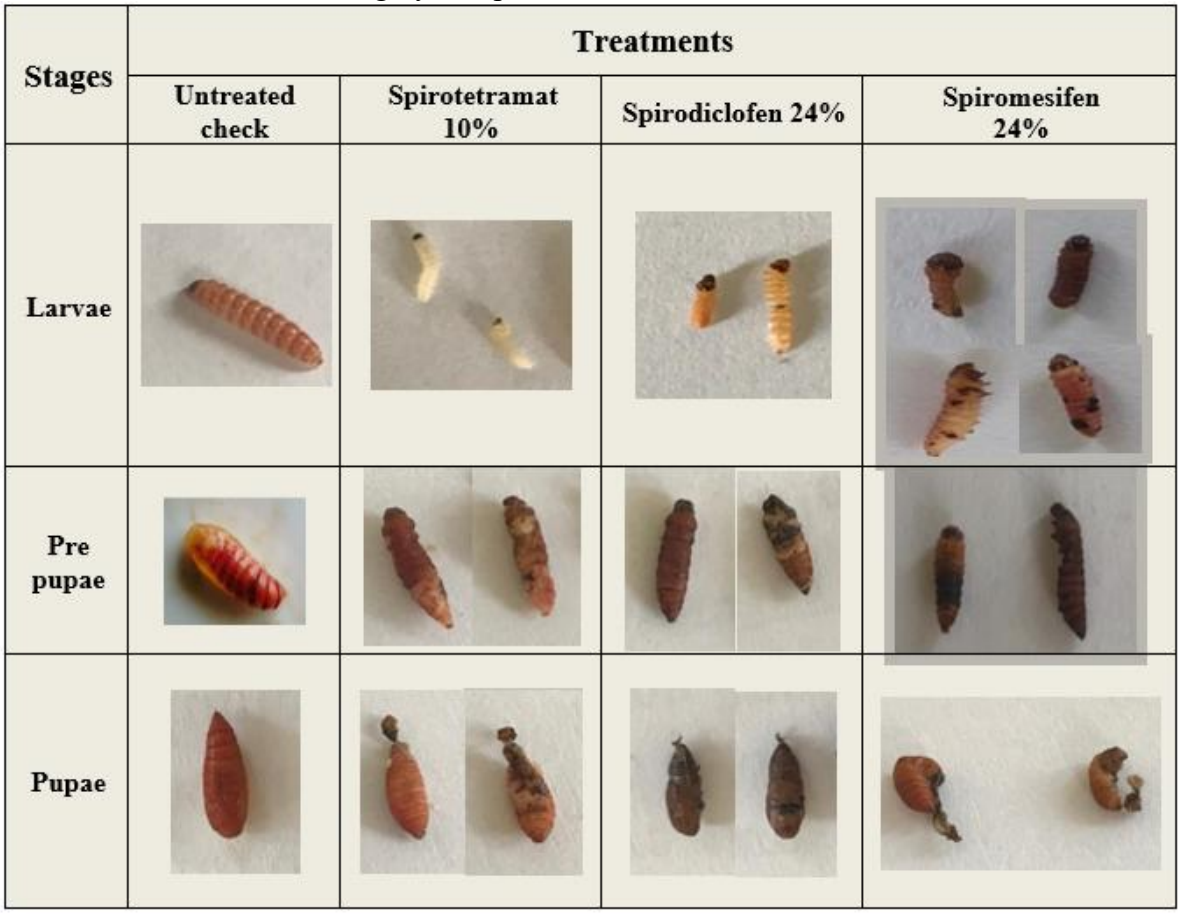

Fig. 1: Malformation of *P. gossypiella* immature stages after Acetyl-CoA carboxylase inhibitor insecticide treatments compared with the untreated check.

Adult Emergence %:

Treating *P. gossypiella* larvae with spirotetramat, spiromesifen, and spirodiclofen negatively affected the percentage of adult emergence (Table 3). The corresponding values of adult emergence significantly decreased to 84.80, 74.40, and 72.10%, respectively, with the highest efficacy of spirodiclofen compared to the untreated check (94.6%).

Histopathological studies:

Integument:

Findings revealed that spiromesifen insecticide demonstrated the highest efficacy against *P. gossypiella* in terms of mortality and malformation percentages. Consequently, it was selected for histological examination. Various histopathological changes in specific tissues were observed after treating *P. gossypiella* newly hatched larvae with the LC_{50} of the tested compound. Severely folded and corrugated layers of cuticle with disintegration of epicuticle and exocuticle, and disappearance of body spikes of treated cuticle compared to the control, were detected. Moreover, signs of lysis and irregular size of epidermal cells were observed in some cuticle areas of the treated cuticle (Figs. 2&3).

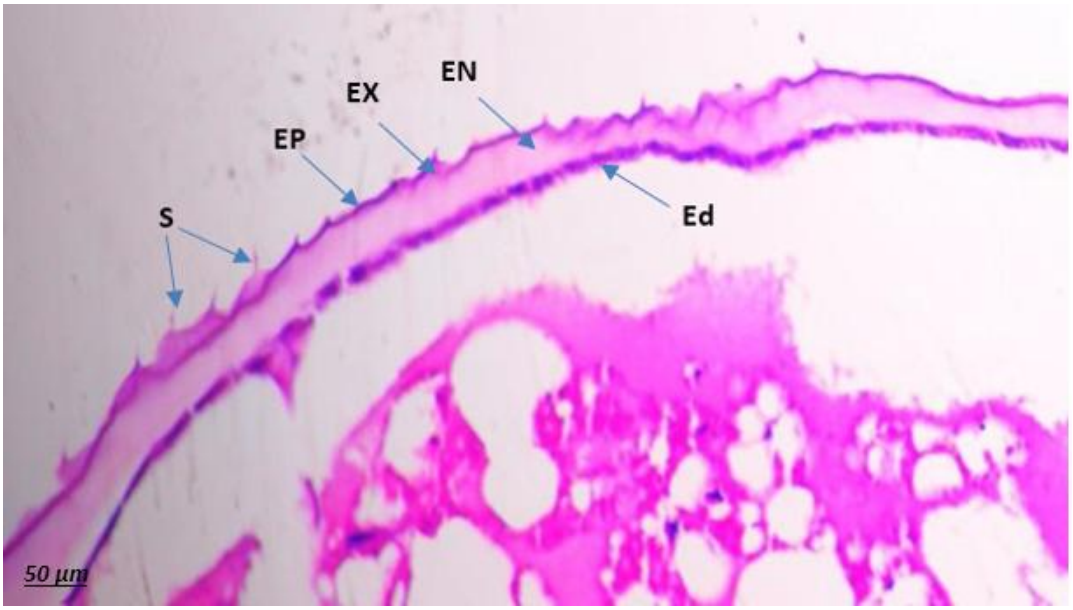

Fig. 2: Photomicrographs of transversal section of *P. gossypiella* showing normal cuticle structure consisting of: Epicuticle (EP) with spiny spikes (S), Exocuticle (EX), and Endocuticle (EN) all resting on the Epidermis layer (Ed).

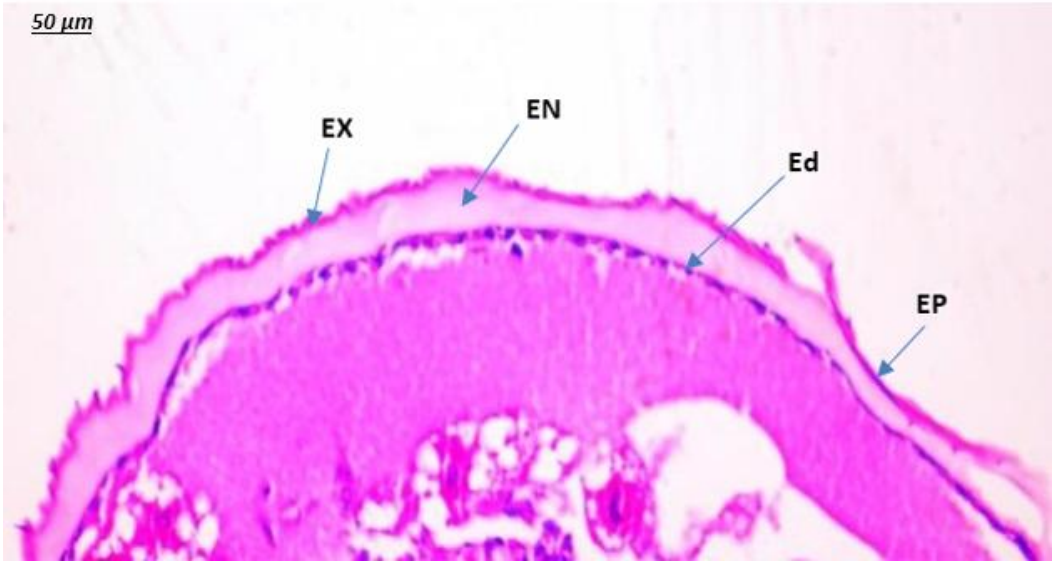

Fig. 3: Photomicrographs of transverse section of *P. gossypiella*-treated larvae showing severely folded cuticle, disintegration of the epicuticle (EP) and exocuticle (EX), and the disappearance of body spikes. Some areas have signs of lysis and irregular sizes of epidermal cells (Ed).

Mid and Hindgut:

Treating newly hatched larvae of *P. gossypiella* with the LC_{50} of the tested compound causes signs of pathological changes at the level of the insect gut compared to the gut normal structure of the untreated larva displayed in Figures (4 & 6). Following treatment, the general appearance of the cytoplasm was reticular and coagulated. Exfoliation and fusion of the midgut epithelium, with disruption of the peritrophic membrane, were evident. The epithelial layer lost its integrity and identity as compact, closely arranged, granulated columnar epithelial cells. The musculosa lost its compact appearance. Vacuolization, exfoliation, and loss of circular muscle were induced for both mid and hindgut (Figs. 5 & 7), respectively.

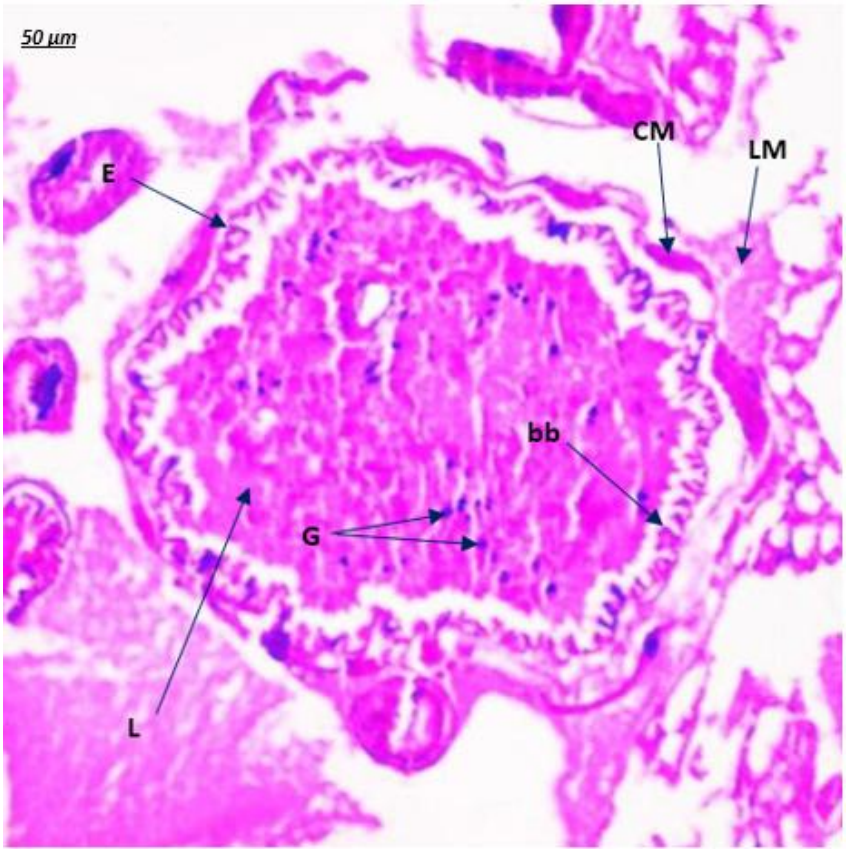

Fig. 4: Photomicrographs of transversal sections of *P. gossypiella* larval midgut reveal simple columnar epithelium (E) with a distinct brush border (bb) resting on an intact basement membrane, surrounding the lumen (L) filled with food particles (ingesta) (G). The epithelium is enclosed by a muscular layer of circular (CM) and longitudinal (LM) muscle bundles.

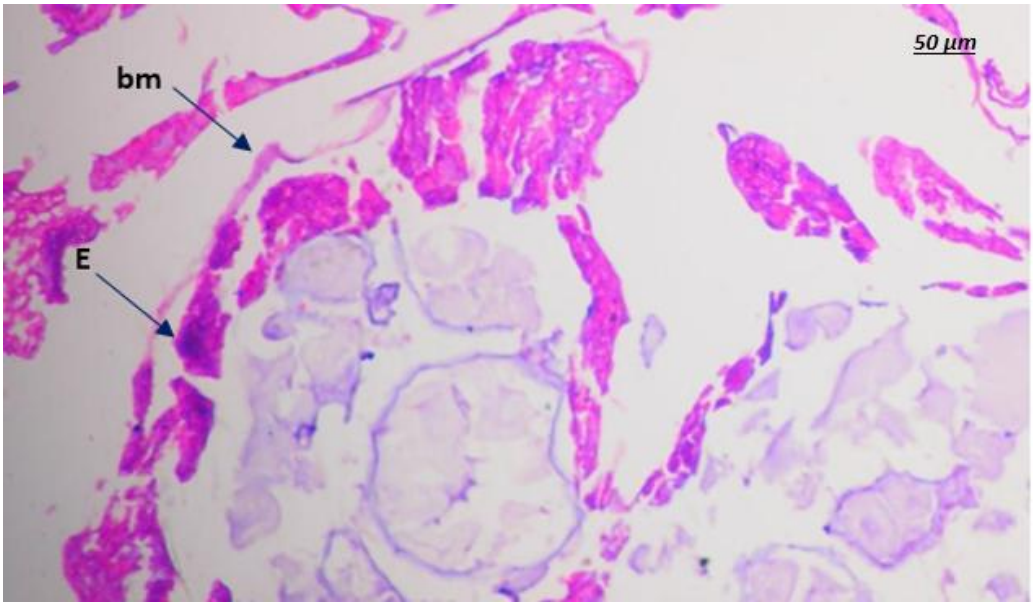

Fig. 5: Photomicrographs of transversal section of *P. gossypiella* treated larva showing signs of pathological changes of midgut, loss of normality and coagulated necrosis in epithelial cells (E), large vacuolization, loss of demarcations in between epithelial cells, detached basement membrane (bm) and muscle layer with departure of brush border.

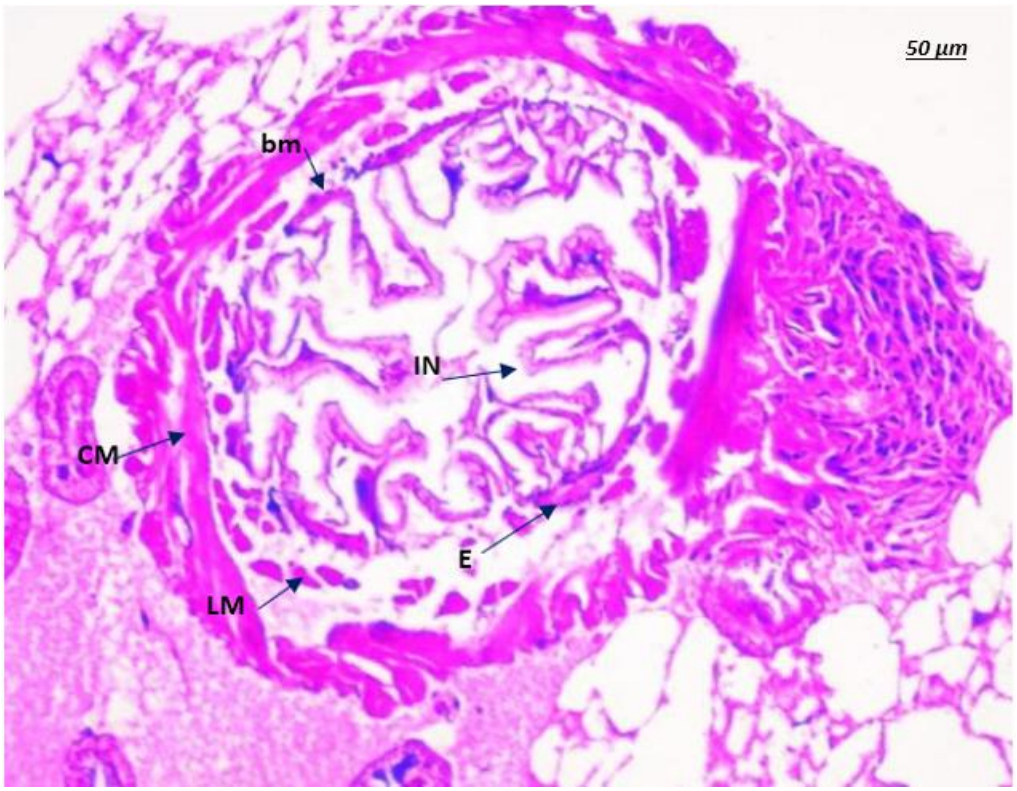

Fig. 6: Photomicrographs of transversal section of the histological structure of *P. gossypiella* larval hindgut showing continuous epithelium (E) covered by a thin chitinous cuticular intima (IN) resting on intact basement membrane (BM), surrounded by longitudinal (LM) and circular (CM) muscular layers.

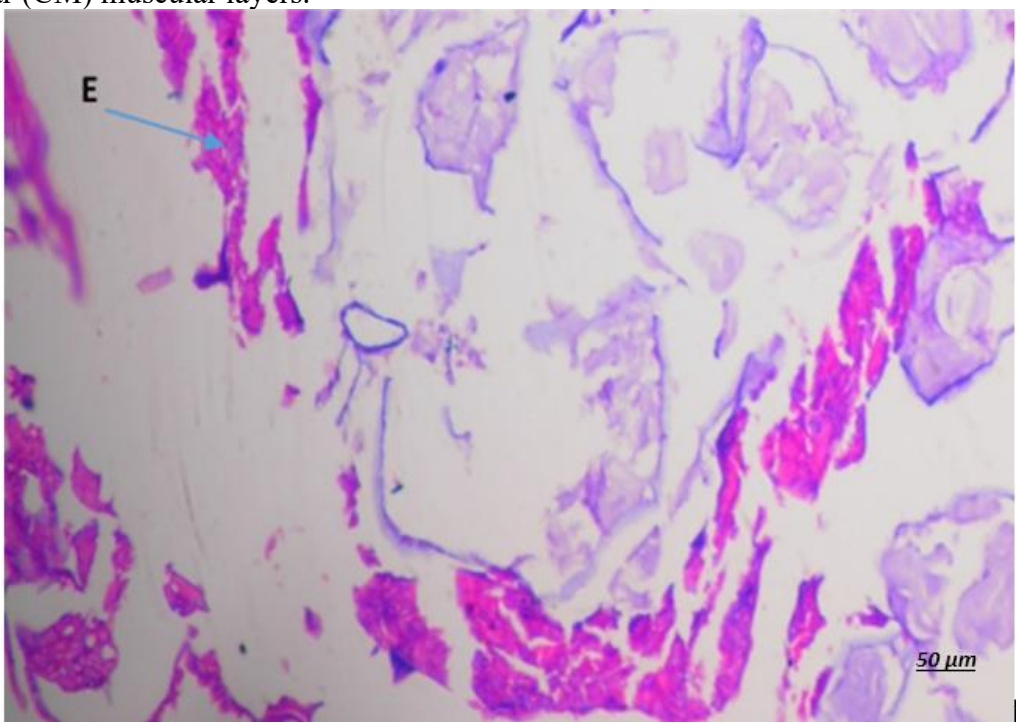

Fig. 7: Photomicrographs of transversal section of the hindgut histological structure of *P. gossypiella* treated larvae, showing damage to the architecture, loss of continuity, and corrugations of the basement membrane, and severe degenerative and necrotic changes within the epithelial cells(E) and dislocation of epithelial cells away from their muscle layer**.**

DISCUSSION

Our findings demonstrated for the first time the increasingly toxic effect of the three Acetyl-CoA carboxylase inhibitor insecticides spirodiclofen (Envidor®), spiromesifen (Oberon®), and spirotetramat (Movento®) to neonatal larvae of *P. gossypiella.* The LC_{50} values of the tested insecticides gradually decreased over three days and effort slowly, with a respectable residual activity. The data agree with El-Bassouiny *et al*. (2022), who found that the 24 h-LC_{50} of spirodiclofen, spiromesifen, and spirotetramat were toxic and gradually decreased over three days against neonatal larvae of the spiny bollworm *Earias insulana*. Sleem *et al*., (2019) indicate that Spirotetramat showed the steepest toxicity line (slope = 3.781), also it is affected on the activity of protein concentrations and enzymes activity in whole body homogenate of survivor second larval instar of *S. littoralis* after application with LC_{50}. Also, Gong *et al.,* (2016) and Nauen *et al.* (2008) indicated that spirotetramat (Movento®) exerts a delayed yet highly effective action against immature cotton aphids. Consistent with its known mode of action, spirotetramat induces mortality in immature stages of aphids and whiteflies within 2 to 10 days post-application and demonstrates good residual activity. Comparable results were improved by Kay and Herron (2010), who sluggishly used Movento® to kill *Frankliniella occidentalis* larvae, and the mortality was sustained for 9, 10, and 13 days. According to Brück *et al*. (2009), the spirotetramat mode of action is flexible and dependent on the target insect's life cycle and outside factors.

Also, results of the acute toxicity test proposed that exposure to three Acetyl-CoA carboxylase inhibitor insecticides, spirodiclofen, spiromesifen, and spirotetramat, hurt the larval and pupal duration of *P. gossypiella*, as well as the increase in mortality and malformation percentages, which reduced the current adult emergence. These results, consistent with the study of El-Bassouiny *et al*. (2022), who proved that the larval and pupal duration of spiny bollworm *E. insulana* had been affected by the tested Tetramic acid insecticides, which were increased, in addition to the presence of malformation. Kheireddine *et al.* (2023) proved the effect of spirodiclofen on biochemical and enzymatic parameters of the snail *Helix aspersa* Muller. Moreover, Al-Harbi *et al*. (2024) confirmed that when *Eobania vermiculata was* treated by spirotetramat, the total cholesterol increased. Also, it affected the activity of ALT and AST. Lümmen *et al.* (2014) showed that spirotetramat-enol inhibits *Myzus persicae* and *Spodoptera frugiperda*, as well as the spider mite *Tetranychus urticae,* by interacting with the carboxyltransferase domain. The binding modes of SPT-enol and the herbicidal ACC inhibitors are likely different. Systematic mutagenesis of residues in the CT domain should provide further insight into the binding site and the mechanistic details of the keto-enol inhibitors.

The present study is considered the first done to show the effect of Acetyl-CoA carboxylase inhibitor insecticides on the histopathological studies of Lepidoptera pests, *P. gossypiella*. Our result proved that exposure to movento (spirotetramat) record the most effective against *P. gossypiella* where it induced different histological alterations at the level of *P. gossypiella* larval integument, mid, and hindgut. The study is consistent with Marium Fatima *et al.* (2013), who concluded that the spirotetramat effect on the total hemocyte count and observed the abnormalities in the hemolymph of American Bollworm, *Helicoverpa armigera.*

Additionally, Salazar-López *et al.* (2016) expressed that Spirotetramat, as a Tetramic acid-based insecticide, is compelling against the immature stages of parasitic sucking insects where ecdysis can't be completed, the insect cannot shed its exoskeleton, hence prohibiting its development. It inhibits normal development and propagation in *Daphnia magna* (Ying *et al.*, 2018) and is thought to act as a genotoxic agent in *D. melanogaster* ovaries (González-

Marín *et al.*, 2021). Other than that, it causes histopathological changes within the ovaries with an injury within the mitochondria of zebrafish, *Danio rerio* (Zhang *et al*., 2020).

Although the use of pesticides is increasing daily, there is an immediate need for awareness and restriction procedures that must be followed to minimize the ill effects of pesticides. The toxicity information of Tetramic and Tetronic acid-derived pesticides suggests their rational use by following prescribed procedures (Zyoud *et al.,* 2010). The toxicity at different levels like neurotoxicity, reproductive toxicity, carcinogenicity, hepatotoxicity and cell toxicity in general caused by pesticides by acting as acetyl CoA carboxylase enzyme inhibitors (Brück *et al.*, 2009)(Bretschneider *et al*., 2003) is of major concern because at any stages of development they can directly or indirectly inhibit lipid biosynthesis. Essentially, the Tetramic acid-based insecticides, Spirotetramat, Spirodiclofen, and Spiromesifen block lipid biosynthesis by inhibiting acetyl-CoA carboxylase, and decreasing the amounts of carbohydrates and glycogen (Nauen *et al.*, 2003), (Kissoum and Soltani, 2016), (Bouabida *et al*., 2017), (Sleem *et al*., 2019), (El-Bassouiny *et al.*, 2022).

Conclusion:

In summary, the present work is the first to demonstrate the efficiency of Acetyl-CoA carboxylase inhibitor insecticides (spirodiclofen, spiromesifen, and Spirotetramat) against *P. gossypiella.* All observations of this insecticide group are effective on biological aspects, Treatments elongate both larval and pupal times, and so does the total life span of *P. gossypiella.* In addition, noticeable percentages of mortality and malformation of treated and resulting stages were reported as a result of treatments. Also, histological damage was observed at the level of insect tissues produces conspicuous destruction in *P. gossypiella* larval integument, midgut, and hindgut. So , Up to the research concluded effects of Novel Group Acetyl-CoA carboxylase insecticides (Tetramic and Tetronic Acids-Based) against *Pectinophora gossypiella*, we can recommend its employment in the integrated management program of this pest.

Declarations

Ethical Approval: The research does not include human or animal subjects

Competing Interests: The authors declare that they have no competing interests.

Authors' Contributions: Rania M El-Shennawy: Methodology, Histology - Original Draft. Mervat AA Kandel: Supervision, Provision of Treated Insect Rearing, Review; H. M. El-Bassouiny: Validation, Statistical Analysis, Data Curation, Resources, Writing - Review and Editing and Shady Selim: Toxicity tests Analysis, Provision of Chemicals

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Availability of Data and Materials: Data and materials used to support the study's findings are included from the correspondence author upon reasonable request.

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ARABIC SUMMARY

تقييم السمية والتأثيرات النسيجية المرضية لمبيدات الحشرات الكربوكسيلية من مجموعة أسيتيل CoA الجديدة(القائمة علي الأحماض الرباعية والنترونية) ضد دودة اللوز القرنفلية(ليبيدوبترا : جيليكيدي)

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1- **قسم بحوث ديدان اللوز-معهد بحوث وقاية النباتات-مركز البحوث الزراعية-الدقى-جيزة-مصر**
2- **قسم كمياء وتكنولوجيا المبيدات-كلية الزراعة الصحروية-جامعة مطروح-مصر**

في دراسة معملية، ركّز هذا البحث على تأثيرات المبيدات الحشرية سبيروديكلوفين (spirodiclofen) وسبيروميسيفين (spiromesifen) وسبيروتيترامات (spirotetramat) وهي مثبطات لأنزيم أسيتيل- كوكربوكسيليز (ACC)على معدلات الموت لليرقات حديثة الفقس لدودة اللوز القرنفلية (*Pectinophora gossypiella*) . تم إجراء تقييم و مقارنة للسُمية عبر طريقتي التلامس والابتلاع عند فترات 6، 12، 24، 48، و72 ساعة. أشارت النتائج إلى أن سبيروتيترامات أظهر سُمية أعلى حيث انخفضت قيم التركيز القاتل المتوسط (LC50) انخفاضًا ملحوظًا مع مرور الزمن (من 21.619 إلى 6.872 ميكروجرام/مل). بينما أظهر سبيروديكلوفين فعالية متوسطة، **في حين** كان سبيروميسيفين أضعف بشكل ملحوظ، خاصة خلال الساعات الأولى. كشفت التقييمات البيولوجية عن إطالة زمن تطور اليرقات وانخفاض الوزن في اليرقات المعاملة **مقارنة باليرقات الغير معاملة** ، حيث تسبب سبيروديكلوفين في التأثير الأكثر وضوحًا. لوحظ أعلى معدل **لموت اليرقات** في معاملة السبيروميسيفين (85.7%)، يليه عن قرب سبيروديكلوفين وسبيروتيترامات (84.2% و81.7% على التوالي). كان هناك انخفاض ملحوظ في نسبة **العذاري** وزيادة كبيرة في نسبة التشوه في جميع المعاملات مقارنة بالمجموعة الغير معاملة (كنترول). كشف الفحص الهستوباثولوجي عن تغيرات كبيرة في أنسجة الجلد والقناة الهضمية، شملت تنكس also known as cyclic طبقات الكيوتيكل وتدمير الأنسجة الطلائية. تُسلّط هذه النتائج الضوء على الفائدة المحتملة **لإستخدام** سبيروديكلوفين وسبيروميسيفين وسبيروتيترامات في برنامج المكافحة المتكاملة للآفات للسيطرة على دودة اللوز القرنفلية.(*P. gossypiella*).