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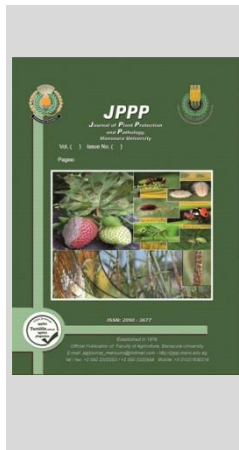
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Field Application of some Pyrethroid Insecticides against Cutworm, *Agrotis ipsilon* and Their Effect on Toxicity and Histological Changes

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ABSTRACT

The field application of five pyrethroid insecticides was carried out in the potato field in Giza Governorate during the seasons 2020 and 2021. Toxicity effect and histological structure of the 4th larval instar of *A. ipsilon* under laboratory conditions were also evaluated. The results illustrated that, regarding the average number of cutworm larvae; there was a significant decrease in the insect number when it was compared before application with the number after application in all treatments. The insecticides showed the highest reduction percentages were lambda cyhalothrin (93.62%) and bifenthrin (93.84%) at 2020 and 2021 seasons, respectively, while the least reduced insecticide was deltamethrin with value 84.87 and 83.62% at the same seasons. As toxicity effect, bifenthrin was the highest toxic compared to the rest of the other tested insecticides with LC₅₀ value of 1.86 ppm while gamma-cyhalothrin recorded the least toxicity with LC₅₀ value 5.18 ppm. Also, the tested insecticides were affected to varying degrees on the midgut, where treatments caused complete detachment of both the basement membrane and the peripheral membrane, as well as the destruction of epithelial cells with lysis of the cells lining the midgut and inability to distinguish cell nuclei to different degrees. All of these changes cause the death of the larvae due to the loss of the midgut's function, which is absorption or digestion.

Keywords: *Agrotis ipsilon*, Pyrethroid insecticides, lambda cyhalothrin, bifenthrin, histological structure

INTRODUCTION

The cutworm, *Agrotis ipsilon* is a species of moth in the family Noctuidae, commonly known as the black cutworm or the greasy cutworm. It is a widespread agricultural pest that feeds on a variety of crops, including corn, cotton, vegetables, and grasses. The larvae of *A. ipsilon* are known to cut down seedlings at or just below the soil surface, and can cause significant damage to crops if left unchecked (Shakur *et al.*, 2007). Control measures for this pest include crop rotation, use of insecticides, and cultivation practices that promote healthy crop growth.

There are several pesticides that can be used to control *A. ipsilon*, but the choice of pesticide and application method will depend on various factors, including the crop being grown and the severity of infestation add reference. Some commonly used insecticides for controlling *A. ipsilon* include pyrethroids, organophosphates, and carbamates (Eldaly, 2022). These insecticides can be applied as foliar sprays, soil drenches, or seed treatments. However, as with any pesticide application, it is important to carefully follow the label instructions and take appropriate safety measures to protect human health and the environment. Additionally, overuse or misuse of pesticides can lead to the development of resistance in insect populations, and can also harm non-target organisms such as beneficial insects and wildlife (Haq *et al.*, 2004; Abd El-Mageed and Shalaby, 2011). Therefore, it is important to use pesticides only as a last resort, and to integrate other pest management strategies such as crop rotation, use of resistant cultivars, and biological control (El-Shershaby, 2010a; Moustafa *et al.*, 2021; Sharaby and El-Nojiban, 2015; Joshi *et al.*, 2021). Pyrethroids are currently

among the main insecticides used against *A. ipsilon* on cotton, maize and various crops against harmful larval stages (Usmani and Knowles, 2001; Tripathi *et al.*, 2003) and causing damage in histological structure in the midgut these changes and caused loss of the midgut function; so they could be used in the integrated pest management control against *A. ipsilon* (Abd-El-Aziz *et al.*, 2019).

This research aims to evaluate the pyrethroid insecticides; deltamethrin, cypermethrin, lambda-cyhalothrin, bifenthrin and gamma-cyhalothrin against *A. ipsilon* in the field, then study the toxic effect and change in the histological structure of *A. ipsilon* larvae.

MATERIALS AND METHODS

Insecticides:

The experiment was carried out to investigate the effect of five compounds belonging to the pyrethroid group against *Agrotis ipsilon*, which are:

- 1) Deltamethrin
Trade Name: Egythrin 2.5% EC
Rate of Application: 400 cc/ Feddan
- 2) Cypermethrin
Trade Name: Cyotin 25% EC
Rate of Application: 250 cc/ Feddan
- 3) Lambda-cyhalothrin
Trade Name: Jinkthrin 10% WP
Rate of Application: 50ml / 100L water
- 4) Bifenthrin
Trade Name: Telecom 25%EC
Rate of Application: 200 cc/ Feddan
- 5) Gamma-cyhalothrin
Trade Name: Vantex 6% CS

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Rate of Application: 100 cc/ Feddan

Baits Preparation:

The poisonous bait was prepared by mixing each insecticide at the recommended rate with the bait that was previously prepared according to Balevski *et al.* (1974) and Abd-El-Aziz *et al.* (2019) by mixing about 25 kg wheat bran and about 10 liters of water, then adding 1 kg of molasses (as an attractant) and then leave it for about 24 hours until fermentation.

Field Trials:

An experiment was conducted to evaluate the efficacy of five insecticides against cutworm, *Agrotis ipsilon*, on potato crop at Kafr Turkey village, El Ayyat, Giza Governorate during two successive seasons 2020 and 2021. The area of the experiment is about one feddan cultivated with potatoes and divided into 24 plots, each plot = 175 m², representing a replicate, and each treatment represented by 4 plots, as well as the untreated. The experiment designed as randomized complete block design. The treatment took place at sunset and when the number of cutworm larvae reached 10 larvae / plant. The experimental field was treated with pre-treated baits with five insecticides (poisonous baits). The number of the larvae / plant (stalks or leaves infected by *A. ipsilon* larvae) was recorded randomly for each replicate immediately before application, then counted after 3 days application, according to the Ministry of Agriculture protocol.

Rearing of Experimental Insect:

Cutworm, *A. ipsilon* obtained from fields of Giza Government then transferred to laboratory and reared on castor bean leaves for several generations under according to (Abdin, 1979) and (Abdou and Abdel-Hakim, 2017).

Toxicity test:

Under laboratory conditions, the toxicity effect was tested for five pyrethroid insecticides against the 4th larval instar of *A. ipsilon*. Using distilled water, a series of concentrations of each insecticide were prepared. One

hundred of 4th larval instars were divided into four replicates. To avoid cannibalism five Petri dish were used as replicates/concentration and only five 4th larval instar in each petri dish. Treated castor bean leaves were introduced for larvae after dipping them in the five different concentrations of each compound. Larvae were transferred to a clean Petri dish 24 hours after treatment and fed on untreated castor leaves, other groups fed on untreated castor bean leaves as control. The mortality was recorded then the LC₅₀ values calculated after 4 days of treatment.

Histological Technique:

Histological technique conducted at the Animal Health Institute of the Agricultural Research Center, according to (Rodríguez-Santiago, 2002) where the larvae were fixed in Buenos solution and a 7 µm length of the midgut was cut with a microtome and dyed with hematoxylineosin, then the slices examined with optical microscope and photographs were taken.

Statistical analysis:

The reduction percentage of *A. ipsilon* were calculated according to Henderson and Tilton (1955). Data was analyzed statistically by one-way analysis of variance (ANOVA) and (Duncan, 1955) Duncan's Multiple Range Test (P= 0.05) using Costat system for windows (Costat, 2006).

RESULTS AND DISCUSSION

Results

During the seasons 2020 and 2021, five pyrethroid insecticides were applied in the potato field in Giza Governorate. The results appear in Table (1) that with regard to the average number of cutworm larvae, there has been a significant decline in the population when comparing before treatment with the number after treatment in all treatments, and thus a significant increase in the percentage of reduction in all treatments compared to the untreated in both seasons.

Table 1. The percentage of reduction and mean numbers of *A. ipsilon* larvae before and after treatment with some pyrethroid insecticides in potato field in Giza Governorate during 2020 and 2021 seasons.

pyrethroid insecticides in potato field in Giza Governorate during 2020 and 2021 seasons.							
Insecticides	Season 2020			Season 2021			Average percentage reduction for the two seasons
	Mean number		Reduction %	Mean number		Reduction %	
	Before	After 3days		Before	After 3days		
Deltamethrin	33.75 ± 4.23 c	4 ± 0.82 bc	84.87	23.5 ± 1.55 b	4 ± 1.00 bc	82.38	83.62
Cypermethrin	43.00 ± 0.71 ab	5 ± 0.91 b	85.16	28.0 ± 3.49 ab	4 ± 1.47 b	85.21	85.18
Lambda-cyhalothrin	40.00 ± 2.04 abc	2 ± 0.58 c	93.62	31.0 ± 1.73 ab	3.75 ± 1.80 c	87.48	90.55
Bifenthrin	40.50 ± 1.71 abc	2 ± 0.75 c	92.91	24.7 ± 1.25 ab	1.25 ± 0.25 c	94.77	93.84
Gamma-cyhalothrin	36.25 ± 1.38 bc	3 ± 0.71 bc	89.44	31.2 ± 2.02 a	3.75 ± 0.63 bc	87.58	88.51
Untreated	45.00 ± 1.08 a	35 ± 1.11 a	-	29.2 ± 3.01 ab	28.25 ± 3.75 a	-	-

Means in the same column followed by the same letter have no significant differences

As the 2020 season, the reduction percentage was recorded as follows: 84.87, 85.16, 93.62, 92.91 and 89.44% for deltamethrin (Egythrin), cypermethrin (Cyotin), lambda-cyhalothrin (Jinkthrin), bifenthrin (Telecam) and gamma-cyhalothrin (Vantex), respectively and mean number of *A. ipsilon* larvae 3 days after treatment recorded a highly significant decrease for all treatments compared to untreated where was 4 ± 0.82, 5 ± 0.91, 2 ± 0.58, 2 ± 0.75 and 3 ± 0.71 larvae for deltamethrin, cypermethrin, lambda-cyhalothrin, bifenthrin and gamma-cyhalothrin, respectively while was 35 ± 1.11 larvae for untreated. The most reduced insecticide was lambda-cyhalothrin (Jinkthrin) with value 93.62% followed by bifenthrin (Telecam) with value 92.91% while the least

reduced insecticide was deltamethrin (Egythrin) with value 84.87%.

As for the 2021 season, the results are similar to the previous season, as there was a slight decrease in the reduction percentage compared to the previous season, 2020, with the exception of bifenthrin, where the reduction percentage was recorded at 82.38, 85.21, 87.48, 94.77, and 87.58% for deltamethrin, cypermethrin, lambda-cyhalothrin, bifenthrin and gamma-cyhalothrin, respectively. The most effective insecticides were bifenthrin (93.84%) and lambda-cyhalothrin (90.55%), while the least effective insecticide was deltamethrin.

The results agreement with (Amitava *et al.*, 2011; Iqbal *et al.*, 1997) treatment of some insecticides against cutworm and found that, significant differences between insecticides compare with untreated, and insignificant differences among them. (Awad *et al.*, 2014) studied six pyrethroids against the cotton bollworm and they found that, there were no significant differences between six pyrethroids and reduction % after the first spray were recorded 89, 86.1, 86.4, 84.2 and 86 % and after the 2nd spray up to 76.6, 80.3, 77.5, 76.6 and 78.4 % and after the 3rd spray up to 82.3, 83.2, 82.5, 81.5 and 81.1% for Icton®, Lamda-Z®, Dora®, Buldock® and Fenerate-S®, respectively.

Toxicity test:

Data in Table (2) and Fig. (1), showed the LC₂₅ and LC₅₀ values for the tested insecticides against the fourth larval instar of *Agrotis ipsilon*. Results explained that, bifenthrin was the highest toxic compared to the rest of the other tested insecticides (LC₅₀ = 1.86 ppm) followed by lambda-cyhalothrin (LC₅₀ = 2.12 ppm), deltamethrin (LC₅₀ = 3.30

ppm) and cypermethrin (LC₅₀ = 4.37 ppm). While gamma-cyhalothrin recorded the least toxicity with LC₅₀ value of 5.18 ppm. (Usmani and Knowles, 2001) reported that, cypermethrin was more effect against *Helicoverpa zea* than permethrin while the two compounds were similar in their toxic effect against *Spodoptera frugiperda* and *A. ipsilon*.

Table 2. Efficacy of tested insecticides against *A. ipsilon* treated as 4th instar larvae.

Insecticide	LC ₅₀ (ppm) Confidence limits	Slope ±SE	LC ₂₅ (ppm) Confidence limits
Deltamethrin	3.30 (2.60-4.67)	1.54 ±0.22	1.20 0.93-1.48
Cypermethrin	4.37 (3.21-7.32)	1.36 ±0.22	1.40 1.07-1.76
Lambda-cyhalothrin	2.12 1.75-2.70	1.57 ±0.20	0.79 0.58-0.99
Bifenthrin	1.86 1.50-2.41	1.36 ±0.19	0.59 0.38-0.79
Gamma-cyhalothrin	5.18 3.74-9.02	1.45 ±0.23	1.77 1.40-2.25

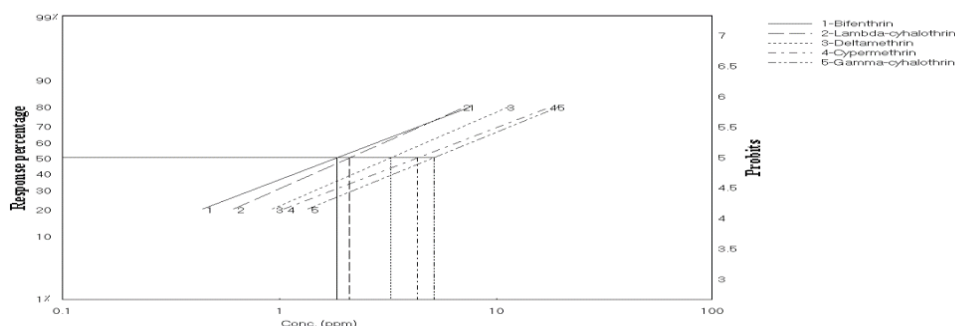


Fig. 1. Efficacy of the tested insecticides against the fourth larval instar of *Agrotis ipsilon*.

Histological Studies:

A microscopic examination revealed that histological structure of the normal (untreated) midgut of larvae *A. ipsilon* is lined with a single layer of epithelial cells. These cells are columnar and have microvilli on their apical surfaces. The epithelial cells also contain scattered secretory cells that produce enzymes and other substances necessary for digestion. Beneath the epithelial layer is the basement membrane, which provides mechanical support to the midgut epithelium. The midgut of *Agrotis ipsilon* larvae also contains circular and longitudinal muscles. These muscles are responsible for peristaltic contractions that move ingested food through the midgut. In summary, the midgut of *Agrotis ipsilon* larvae is a complex structure that is specially adapted for digestion and absorption of nutrients.

The tested insecticides were affected to varying degrees on the midgut as showed in Fig. (2 a, b) where treatment with deltamethrin caused complete detachment of both the basement membrane and the peripheral membrane, as well as the damage of epithelial cells with lysis of the cells lining the midgut. The columnar and longitudinal cells were discrete and partially separated from peripheral membrane (Fig. 2 a,b). Indistinguishability of cell nuclei to different degrees was observed.

Cypermethrin caused obvious histological changes where found damage in epithelial cells, the appearance of big vacuoles between the epithelium and separation at the base of the epithelium and the muscular layer and damage in the muscular layer with broken in many places (Fig. 2 c,d).

Lambda-cyhalothrin caused changes which were sever destruction of the epithelium, lack of visibility of regenerating cells and undifferentiating nuclear contents. Due to lysis in the peritrophic membrane found mixing between the components of the lumen and the lysis cells (Fig. 3 a,b). At Bifenthrin the changes were similar the previous treated, where the epithelium was destroyed and found large vacuoles, cell lysis, separation of the basement, distortion peritrophic membranes and dissolved of nuclei. Likewise, treated larvae with gamma-cyhalothrin led to destruction of epithelial and separation of the epithelial cells from basement membrane with sever breakdown of peritrophic membrane and necrosis cells lining midgut and the collapse of the lumen of midgut cell.

Our results agreed with the results of (El-Shershaby, 2010b; Abdou and Abdel-Hakim, 2017) found histopathological damage, dissolved of nuclei and destroyed of epithelial cells and its separation from the basement membrane of *A. ipsilon* midgut and led to the loss of the midgut function as absorption or digestion. (Sharaby and El-Nujiban, 2016) reported that, the most of the histopathological damage were in the hind gut, mid gut, fat body, trachea and integument after treatment larvae *A. ipsilon* by oil combination (Garlic and Mint oils) and noticed different cells of the midgut a swelling, disorders in many areas with rive of basement membrane and appearance vacuoles in the cell cytoplasm. (Abd-El-Aziz *et al.*, 2019) they studied the effects on the midgut structure was affected with the treated of Anjio and Affact power and found that damage of the peritrophic

membrane and separation of the epithelial cells from the basement membrane and led to mixing of the lumen with the

haemolymph and caused the death of the larvae due to loss of midgut function.

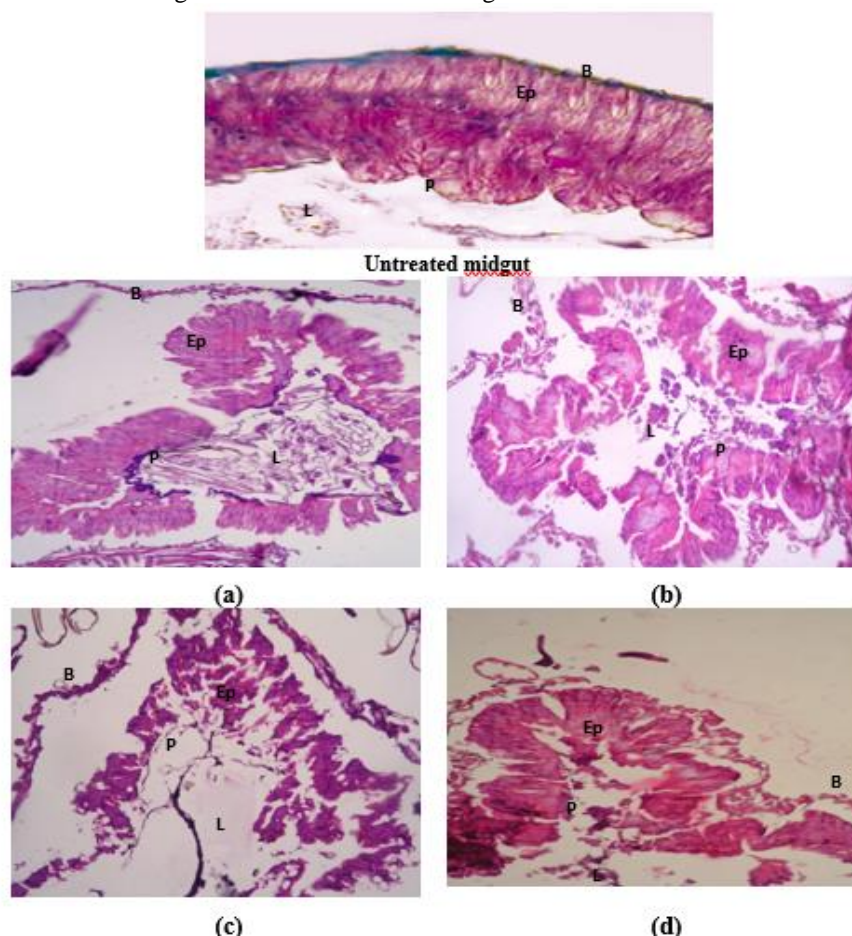


Fig.2. Cross sections in midgut of *A. ipsilon* after 3 days of treatment for 4th instars treated with LC_{50} of Deltamethrin (a,b) and Cypermethrin (c,d) where B: basment membrane , ep : epithelial cell, p: peritrophic membrane and L: lumen.

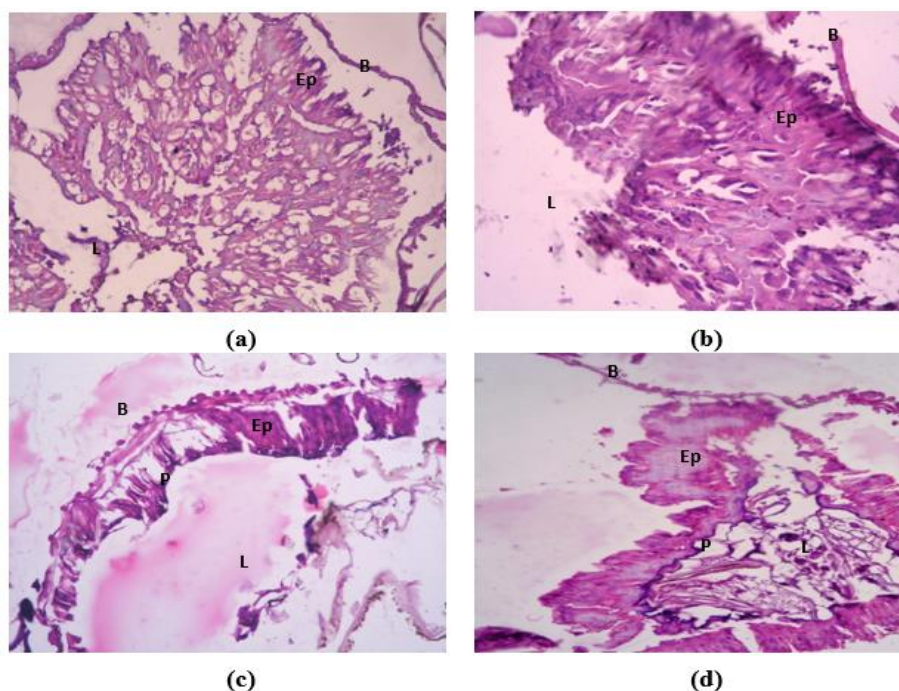


Fig. 3. Cross sections in midgut of *A. ipsilon* after 3 days of treatment for 4th instars treated with LC_{50} of Lambda-cyhalothrin (a,b), Bifenthrin (c,d) and Gamma-cyhalothrin (e,f) where B: basment membrane , ep : epithelial cell, p: peritrophic membrane and L: lumen.

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التطبيق الحقل لبعض المبيدات البيروثرويدية ضد الدودة القارضة وتأثيرهم علي السمية والتغيرات النسيجية

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المخلص

تم التطبيق الحقل لخمس مبيدات حشرية بيروثرويدية في حقل البطاطس بمحافظة الجيزة خلال الموسمين ٢٠٢٠ و ٢٠٢١. كما تم تقييم التأثير السمي والتركيب النسيجي للطور البرقي الرابع لحشرة الدودة القارضة تحت الظروف المعملية. أظهرت النتائج أنه بالنسبة لمتوسط عدد يرقات الدودة القارضة فقد حدث انخفاض معنوي في التعداد عند مقارنة قبل المعاملة مع التعداد بعد المعاملة في جميع المعاملات. وكانت أعلى نسبة خفض هي للمبيدات لامدا سيهاوثرين (٩٣,٦٢%) وبيلفنثرين (٩٣,٨٤%) في موسمي ٢٠٢٠ و ٢٠٢١ على التوالي، في حين كان أقل المبيدات الحشرية في نسبة خفض هو دلتامثرين بقيمة ٨٧,٨٤% و ٨٣,٦٢% في كلا الموسمين. ومن حيث التأثير السمي، كان مبيد بييفنثرين هو الأعلى سمية مقارنة بقيمة المبيدات الحشرية الأخرى التي تم اختبارها حيث بلغت قيمة التركيز النصف المميت ١,٨٦ جزء في المليون بينما سجل جاما سيهاوثرين أقل سمية بلغت قيمة التركيز النصف المميت ٥,١٨ جزء في المليون. كما أثرت المبيدات الحشرية المختبرة بدرجات متفاوتة على المعى المتوسط حيث تسببت المعاملة بالمبيدات في انفصال كامل لكل من الغشاء القاعدي والغشاء المحيطي ولا يمكن تمييز نواة الخلية بدرجات مختلفة. كل هذه التغيرات تسبب في موت اليرقات بسبب فقدان المعى المتوسط وظيفته وهي الامتصاص أو الهضم.