



Field Evaluation of Some Insecticides for Controlling Black cutworm, *Agrotis ypsilon* and Their Effect on Some Histological Aspects.

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ABSTRACT

Field experiments were carried out to evaluate the efficacy of certain insecticides, Lambada cyhalothrin (5% EC) as Affact power, Lambada cyhalothrin (10.6%) + Thiamethoxan (14.1%) as Anjio (24.7% SC) and Chlorantraniliprole formulated as Coragen (20% SC) belonging to different groups against *Agrotis ypsilon* larvae in potato crop in Egyptian governorate; El Qalyubia during two successive seasons, February 2018 and 2019 under field conditions. All the tested plots showed a highly significant reduction in *Agrotis ypsilon* population after 3 days post-treatment with all tested insecticides as poison baits. The reduction was higher in the second season 2019 than the first season 2018 with Coragen and Affact power. The most effective insecticides by the mean of reduction % in the two seasons were Coragen and Anjio by 92.91 and 92.26%, respectively while Affact power by 88.68%. Semi-field technique was used to study the histological changes of two sub-lethal rates 50 and 25% of these compounds on the 4th and 5th instars midgut structure post three days of treatments. The midgut structure was affected with all the treated rates of insecticides with various degrees that concluded in separation or disappearing and lysis of basement membrane which led to separate the epithelial cells from it with undistinguished shapes. The peritrophic membrane was separated or destructed and lysis led to mixing of the lumen content with lysis cells. All or some of these changes led to the loss of the midgut function, nutrition, and digestion; so they could be used in the integrated pest management control against *Agrotis ypsilon*.

INTRODUCTION

The black cutworm, *Agrotis ypsilon* (Hufnagel) (Lepidoptera :Noctuidae) is the most severe and destructive pest in Egypt and other countries in the world (Abdou and Abdel-Hakim, 2017),it attacked corn and many vegetable crops such as potatoes and tomatoes crops. It is a nocturnal pest, the newly hatched larvae attack young seedling and feed on the epidermis of the leaves as larvae become older feed by cutting plant stems below or just above the soil (Shakur *et al.*, 2007). Potato plants are very sensitive to *A. ypsilon* larvae at an early stage especially in the first 4–6 pairs of leaves developed so if we succeeded to protect it by preventing the damage which can happen by this pest in this cultivated period, we could ensure highly significant yield and quality content than the other fields which uncontrolled.

Over the years, the intensive use of conventional pesticides has led to several problems as environmental pollutions, destruction of natural enemies, beneficial insect populations, and insect resistance to different insecticides (Haq *et al.*, 2004). In Egypt, as a result of unregulated and continued application of insecticides, insects began to develop high levels of resistance to insecticides and to overcome this problem and control the black cutworm damage involve using different groups of insecticides in rotation program which may be useful to delay the resistance problem Abd El-Mageed and Shalaby, (2011) the application of insecticides in sequential use induced higher reduction in larval numbers as compared to the lower reduction resulting from several applications of the same insecticide alone. Therefore, we need to develop alternative uses of pesticides to provide crop protection by using different insecticide groups as Lambada cyhalothrin (Affact power) belonging to group 3 synthetic pyrethroid. Lambada cyhalothrin (10.6%) +Thiamethoxan (14.1) (Anjio 24.7%SC) that a mixture of synthetic Pyrethroid and Neonicotinoid which belonging to groups 3 and 4 of insecticides. Coragen (Chlorantraniliprole, Rynaxypr) is the first anthranilic diamide as a new class of insecticide belonging to groups 28 (Lahm *et al.*, 2007) that prevents the build-up of pest populations particularly for controlling of many lepidopterous pests on cotton, rice, potatoes, cabbage, sugarcane and other vegetables and fruits which have developed resistance to other chemical classes of insecticides. It has high biological activity, very low mammalian toxicity, and selectivity to non-target arthropods More and Patole (2015). Acts at relatively low application rates promote eco-friendly insect pests management and excellent control of pest population resistant to

other insecticides. It activates the insect ryanodine receptors (RyRs), which causes uncontrolled release and depletion of internal Ca and prevents further muscle contraction. The insect's death occurs by the rapid cessation of feeding, regurgitation, lethargy, and muscle paralysis (Cordova *et al.*, 2007; Temple *et al.*, 2009).

The present work is aimed to evaluate the effectiveness of some insecticides against the black cutworm in potato fields in El Qalyubia Governorate with rates 200, 100, and 60 ml/ 25kg wheat bran /fed for Affact power, Anjio, and coragen, respectively. Also, their effects were studied on midgut larvae using a semi-field technique for the histological changes post-treatment with rate 100, 50 ml/25kg wheat bran for Affact power, and 50 and 25 ml / 25kg wheat bran for Anjio& Affact power and 30 and 15 ml / 25kg wheat bran for coragen, respectively.

MATERIALS AND METHODS

Three commercial products represented three different groups of insecticides were used in this study. The tested insecticides were; Lambada cyhalothrin (5%) EC (a synthetic pyrethroid insecticide), formulated as Affact power obtained by Aid for insecticides and chemicals. Lambada cyhalothrin (10.6%) + Thiamethoxan (14.1) (24.7%SC) formulated as Anjio (Neonicotinoid) obtained by Syngenta, Co., and Chlorantraniliprole formulated as Coragen (20% SC) 60 ml / fed, as a novel insecticide from class anthranilic diamide and applied by DuPon.

Baits Preparation:

The baits prepared according to Balevski, *et al.*, (1974) with some modification by mixing 25 kg of wheat bran with a suitable amount of water, (8-10 litter) add 1 kg molasses (black honey, as attractive substance) and mix well then left it overnight in dark and worm place until fermentation. Then, mix it with insecticides rates to prepare poison bait. The three insecticides were

applied at the recommended rates by means rate 200 ml for Affact power, 100 ml for Anjio, and 60 ml/ feddan for Coragen with each application.

Field Experiments:

Field experiments were conducted in El Qalyubia Government at EL kharaqaniya village, the experimental area was about half feddan (2100 m²) cultivated with the potatoes crop. Experiments were established in a randomized complete block design with four replicates. The area was divided into plots about 175 m² for each treatment, which divided into four parts, first part for control (baits only), the other three parts were applied with Lambada cyhalothrin (Affact power 5%) Lambada cyhalothrin (10.6%) + Thiamethoxan (14.1) (Anjio 24.7% SC) and Chlorantraniliprole coragen(20%), respectively. Each area divided into 4 replicates. Experiments were carried out when the larval population reach to 10 larvae / 25 plants and the time of application should be before sunset to make sure feeding larvae and to avoid the deleterious effect of UV on the insecticides. The application date of baits was in two successive seasons during February 2018 and 2019. The time of application was either in the early morning or before sunset by putting a suitable amount of baits under plants, according to evaluation protocols of insecticides efficacy against agriculture. Data was obtained the first count just before application and after 3 days from the application by counting the number of larvae/plant or the number of damaged plants (feeding or cutting leaves) randomly in each replicate. The reduction percentage in the larval population were calculated according to Henderson and Tilton (1955).

Semi Field Experiments:

Larvae of *Agrotis ypsilon* obtained from El Qalyubia Government transferred to Cotton Leafworm Department, Plant Protection Research Institute, Dokki, Giza, Egypt and reared on castor bean leaves

Ricinus communis under laboratory conditions at 22 ± 2°C and 50 – 60 % RH without contamination with insecticides for several generations according to (Abdin, 1979) with some modification by Abdou and Abdel-Hakim (2017) to obtained suitable number of larvae to the experiments. Semi-field experiments were carried out to study the histological effects of the three tested insecticides on the 4th and 5th larval instars of *A. ypsilon* midgut by feeding them on baits treated with two sub-field rates 50% and 25% of the recommended rates, other groups fed on untreated baits for three days as control and the whole larvae were collected for histological studies.

Histological Technique:

The larval Samples were fixed in alcoholic Bouins solution and histological technique was done at the Animal Health Research Institute. The midgut cuts of 7µm performed with a microtome and dyed with hematoxylin-eosin, according to protocols described (Rodriguez –Santiago, 2002). Slices were observed with an optical microscope with the lens of 40× and photographs were taken (H α Ex 200).

Statistical Analysis:

Henderson and Tilton (1955) equation was used to calculate the reduction percentages in infestation. All data were subjected to one-way analysis of variance (ANOVA), followed by Duncan multiple range test to determine the significant differences among the treatment means at 0.05 probit (Duncan, 1955).

RESULTS AND DISCUSSION

The field application of the three insecticides was carried out during February of 2018 and 2019 on potato crop in El Qalyubia Governorate. The data in Table (1) and Figure (1) indicated that highly significant reduction in larval population in all tested insecticides compare with the untreated control, where in the first season 2018; the reduction % of larvae post 3 days from the application was 91.47, 92.94 and 88.18% for Chlorantraniliprole

(Coragen), Lambada cyhalothrin (10.6%) + Thiamethoxan (14.1%) (Anjio) and Lambada cyhalothrin (Affact power), respectively. The most reduced insecticide was Lambada cyhalothrin + Thiamethoxan (Anjio) followed by Chlorantraniliprole (Coragen) and finally with Lambada cyhalothrin (Affact power). The correspondent reduction % in the second season 2019 slightly increased values than that observed in the first season at the same period with Chlorantraniliprole (Coragen) and Lambada cyhalothrin (Affact power) to record 94.35 and 89.18% while Lambada cyhalothrin + Thiamethoxan (Anjio) recoded 91.58% less than that recorded in the first season. The most effective insecticides by the mean reduction % of both seasons were Chlorantraniliprole (Coragen) and Lambada cyhalothrin + Thiamethoxan (Anjio) by 92.91 and 92.26% then Lambada cyhalothrin (Affact power) by 88.68% that caused a highly significant reduction in the larval populations in this area. That agrees with Barrania, (2013), the exposure to 100, 50% and 25% field rates of Chlorantraniliprole and Thiamethoxam recorded mortality % (97.5, 95.5 and 95.0%) and (77.5, 67.5 and 60.0 %) against the 4th larvae instar *Spodoptera*, respectively in during cotton season 2011, while recorded (95.0, 95.0 and 92.5) and (90.0, 95.0 and 87.5 %), respectively for all field rates in season 2012 after three days of application. Our findings suggest that Chlorantraniliprole (Coragen) was the most effective insecticide against the larvae of *A. ypsilon* at relatively low rates as compared to other insecticides (pyrethroids group 3 and 4). It had a wide range of activity against black cutworms that makes it an excellent control in an integrated pest management system. Lambada cyhalothrin + Thiamethoxan (Anjio) had highly effective in control *A. ypsilon* than Lambada cyhalothrin (Affact power) because it contains two

active ingredients with low rates as an effective mixture of two different groups contain Lambda-cyhalothrin which is nonsystemic insecticide and Thiamethoxam with systemic activity and both of them have contact, stomach action and knockdown rapidly with taken up into the plant and long residual activity. That agrees with (Maienfisch, *et al.*, 2001 and Elbert, *et al.*, 2008) Thiamethoxan belongs to a class of insecticides neonicotinoids which are considered the most effective insecticides for control many insects as a number of Coleoptera and Lepidoptera pests as *A. ypsilon* in many crops like cotton and potato with most rapidly expanding insecticides due to a unique mode of action, residual activity and perfect protection of young plants against attack insects. Balevski, *et al.*, (1974) treatment would be applied using poison baits for controlling *Agrotis segetum* and *peridroma saucia* before the population of larvae reached to the 6th instar and followed by cultivation a shallow of the soil.

This is in accordance with those findings in *A. ypsilon* by konar *et al.*, (2012) evaluate the efficacy of six insecticides against *A. ypsilon* and *mole cricket* in potato field during 2007 and 2008 which had more or less equal effect to reduce the population of both pests. Significant differences between each of the tested insecticides in the control of *A. ypsilon*, and insignificant differences among them. Reduction % in the larval population; 3 days post-application ranged between 59.2 and 90.2 while ranged between 53.9 and 95.7% for triumph and biotech treatments, respectively in the tested governments (Abo Bakr and El Zoghby, 2016).

These are in harmony with Abd El-Aziz, *et al.*, (2017) on other pests Coragen recorded highly significant effect on *S. littoralis* larvae from 97.99 to 84.85 and from 96.59 to 79.93 in both tested governments, respectively than runner, the effective inspection was the 7 days post-treatment with

insecticides. The most effective time was the zero time and the 3rd day while the less effective was the 7 day. Treated larvae were small in size, sluggish, shrinking, and black in color in Coragen treatment due to dehydration of larvae and may be reflected in its mode of action which acts as selective calcium ion channels. Also, Coragen recorded the highest mortality% ranged from 100 to 87.8% with all-time intervals, for both larval

instars in the two successive seasons (Hassan and Abd El Wahab, 2015). Lambda-cyhalothrin recorded low significant ingestion activity than the combined action of chlorpyrifos +cypermethrin against the control of the sugar beet weevil (Bažok, 2016). Thiamethoxam had a moderate effect basis on the reduction percent of jassid populations during season 2012 (Mandal, *et al.*, 2012).

Table 1: The reduction % and the number of *A. ypsilon* larvae post 3 days of application with certain insecticides in potato field in Qalyubia governorate during seasons 2018 and 2019.

Insecticides	Total number of larvae, damage or cutting leaves during seasons 2018 and 2019						Mean of reduction % in two seasons
	Season 2018			Season 2019			
	Before	After 3 days	Reduction%	Before	After 3 days	Reduction%	
Coragen	149	10	91.47 ^a	133	6	94.35 ^a	92.91 ^a
Anjio	180	10	92.94 ^a	119	7	91.58 ^b	92.26 ^a
Affact power	159	15	88.18 ^b	162	14	89.18 ^c	88.68 ^b
Control	122	96		144	115		
F-value	371062.26***			126745.94***			1845.67***
L.S.D	1.03259			1.60773			1.58

Mean with the same letter are not significantly different. *** high significant (Coragen) Chlorantraniliprole, (Anjio) Lambda cyhalothrin + Thiamethoxan, (Affact power) Lambda cyhalothrin.

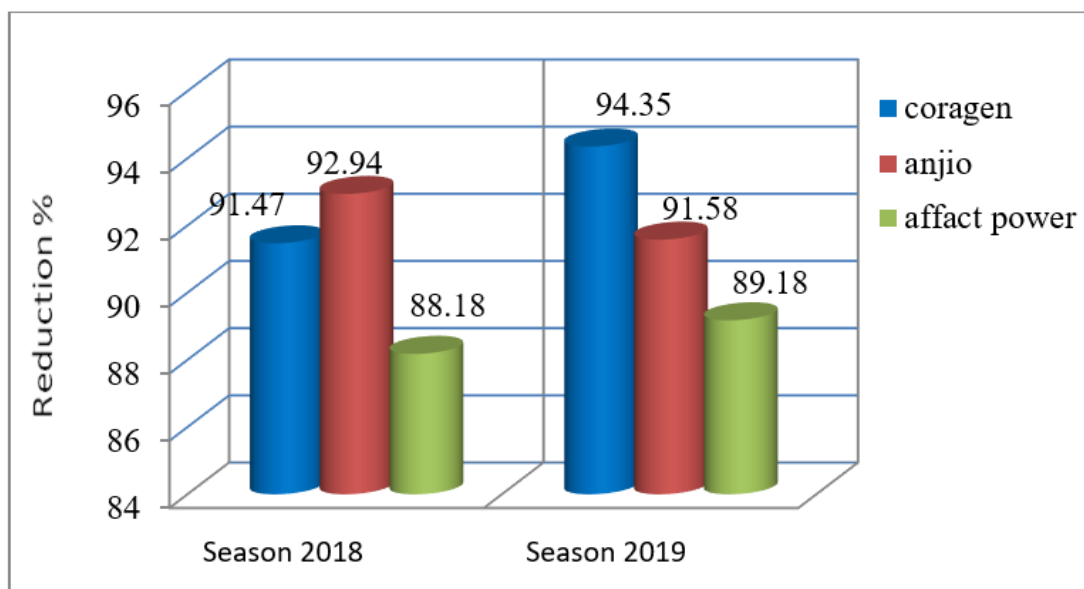


Fig. 1: The reduction % of *A. ypsilon* larvae post 3 days of application with certain insecticides in potato field in Qalyubia governorate during seasons 2018 and 2019.

Histological Studies Using the Semi-Field Technique:

Agrotis ypsilon larvae caused great damage to potato crop by feeding on vegetative leaves especially in the early plantation (Fig.2) the larvae biting the leaves and stems of plantlet, it attacks the plants with different symptoms according to their growth and larval stage founds, that led to reducing the quality and potato yield.

Microscopic examination showed that the histological structure of the normal (control) midgut of *A. ypsilon* (Fig.3), it is the middle larger region of the digestive tract of an insect where the digestion and absorption of food occur. It consists of longitudinal muscle which is the outer layer and the inner layer is the circular muscle. It closed to a thin basement membrane where rested the epithelium cell which is columnar and elongated in shape. In addition to the regenerative cells, which are small in size and present individually or in clusters of few cells in the bases of the columnar cells. The peritrophic membrane is surrounded by the lumen and protects the epithelial cells from mixing with the food contents. So, the cellular modifications are more intense there, the different types of epithelium cells produce enzymes, digestion and others absorb the food (Terra and Ferreira 1994).

The tested insecticides Chlorantraniliprole (Coragen), Lambda cyhalothrin + Thiamethoxan (Anjio) and Lambda cyhalothrin (Affact power) affected on the midgut structure with a different degree as shown in (Figs.3a,b,c&d), the treatment with Chlorantraniliprole (coragen) on *A. ypsilon* midgut caused completely separation of both basement and peritrophic membranes sever breakdown, destruction of epithelial with lysis and necrosis cells lining midgut. The columnar and longitudinal cells were the detached and partial separation of the peritrophic membrane

(Fig. 3d). The lysis cells were unable to repair the damage. Undifferentiating nuclear contents with different degrees depending on tested concentrations and larval age. Coragen more effective on treated 4th instars at a high concentration of 50% than 5th larval instars.

A. ypsilon midgut treated with cyhalothrin + Thiamethoxan (Anjio) observed some histological changes in its structure concluded in (Figs.4 a,b,c&d) undistinguished epithelial cells, the appearance of vacuoles with separation of the basement and peritrophic membranes (a) losing brush border. Muscle fiber separating from each other leaving the degenerating area between them with lysis cells. The most effective instars were the 4th then 5th with a concentration of 50%.

Effect of Lambda cyhalothrin (Affact power) on *A. ypsilon* midgut (Figs.5 a,b,c&d) clear that the basement membrane was completely separated, necrosis of cells with undistinguished cell, scattering nuclear content, disruption of columnar cells and lysis in the peritrophic membrane (Figs.5a&b). Basement and peritrophic membranes were partially separated (Figs.5 c&d), epithelial cells collected in clusters, necrosis of some cells lining midgut. The 5th larval instar was less infected with Lambda than the 4th instars. Lysis in the peritrophic membrane led to mixing the components of the lumen with the lysis cells.

Our studies recorded that the most effective concentration was the highest 50% with all tested insecticides on the 4th instars than 5th instars. That appeared in the histological disturbances in the mid-gut as lysis, separation of the basement, peritrophic membranes, distortion, and cell lysis led to loss the permeability, potassium transport, mixing lumen content with lysis cell. These abnormal structural of midgut may reflect the functional differences between treatment and untreated larval. Where Coragen caused

treated larvae became hard, shrinking, darkening in color, and larvae can't crawl that may due to dehydration as a result of disturbed permeability of plasma membrane and the collapse of the lumen of midgut cell partially paralysis especially in the starved larvae. That agrees with El-Shershaby, (2010), Abdou and Abdel-Hakim (2017) *A. ipsilon* midgut sever breakdown and degeneration of the epithelial cells, peritrophic membrane separated and detachment columnar cells with different degrees according to the used concentrations.

The treatment with the three tested insecticides reduces the efficiency of food utilization due to the disturbance in midgut structure of *A. ipsilon* larvae post-feeding with the sub-lethal rates or /and enzymes secretion that could be affected on larval growth were similar to that observed in the midgut treated with others, that Nasiruddin and Mordue (1993) and Abd-El-Aziz, *et al.*, (2013) hypothesized that azadirachtin had adverse effects on the epithelial cells of the midgut, which due to disrupting enzymes secretion and absorption nutrient by reducing their ability to convert food into biomass led to extend the larval duration and the absence of other nutrients, like amino acids. Larval growth affected by metabolic disturbance or by increasing energetic costs by reducing the ability to utilize dietary nitrogen, which could not necessary to interfere with the digestibility or gut absorption. Other studies related the damage in the midgut structure to the inhibition in other enzymes as Abdou and Abdel-Hakim (2017) and Zhang, *et al.*, (2019) Vip3Aa proteins can bind to *A. ipsilon* midgut brush border membrane vesicles and there are competitive binding between them. Anitha *et.al.*, (1999) histopathological changes are one of the most definitive indicators of fat changes that caused disturbances of fats in different body organs. The destruction of epithelial cells, vacuolization, and

necrosis recognized in midgut cells that led to disturbances in the function of the internal organs and may explain the inhibition of lipid synthesis, amylase is the most sensitive enzyme to the action of a number of molluscicides and this inhibition will, in turn, reduce glucose level in both pests. (Rawi, *et al.*, 2011 and Hashem *et.al.*, 1993). The degeneration in the epithelial lining of the midgut and the peritrophic matrix in *S. littoralis* mid-gut. That may be responsible for the reduction in growth and food utilization, shrinkage of the cells and these cells were unable to replace the damaged cells of the midgut. Contact and ingestion effect of Coragen on *S. littoralis* midgut caused a complete destruction of the epithelial layer and basement membrane separated with severe destruction and necrosis of cells lining midgut, an increase of goblet cells, vacuoles and the muscle fibers were losing their typical pattern. Coragen more effective at high concentration with ingestion than contact all these changes lead to lose its function (Abou El Ghar *et al.*, 2013 and Abdel –Aziz *et al.*, 2020, in press).

The present histopathological disturbance caused by the different investigated insecticides may suggest that any of these insecticides are able to kill this pest in few days by feeding with both the sub-lethal rates as well as recommended rates specially Coragen which has high toxicity effect with low active ingredients. Also, these insecticides have a variety of active ingredients, immediate knockdown effective, and a relation between the progression of toxicity symptoms and the histological changes against the target pest with low rates to reduce costs for controlling. To delay insecticide resistance; we should rotate the use of insecticide with different groups to control the same pests in a field alternative to other insecticides.



Fig.2: Symptoms on potato leaves infected with *Agrotis ypsilon* larvae in field

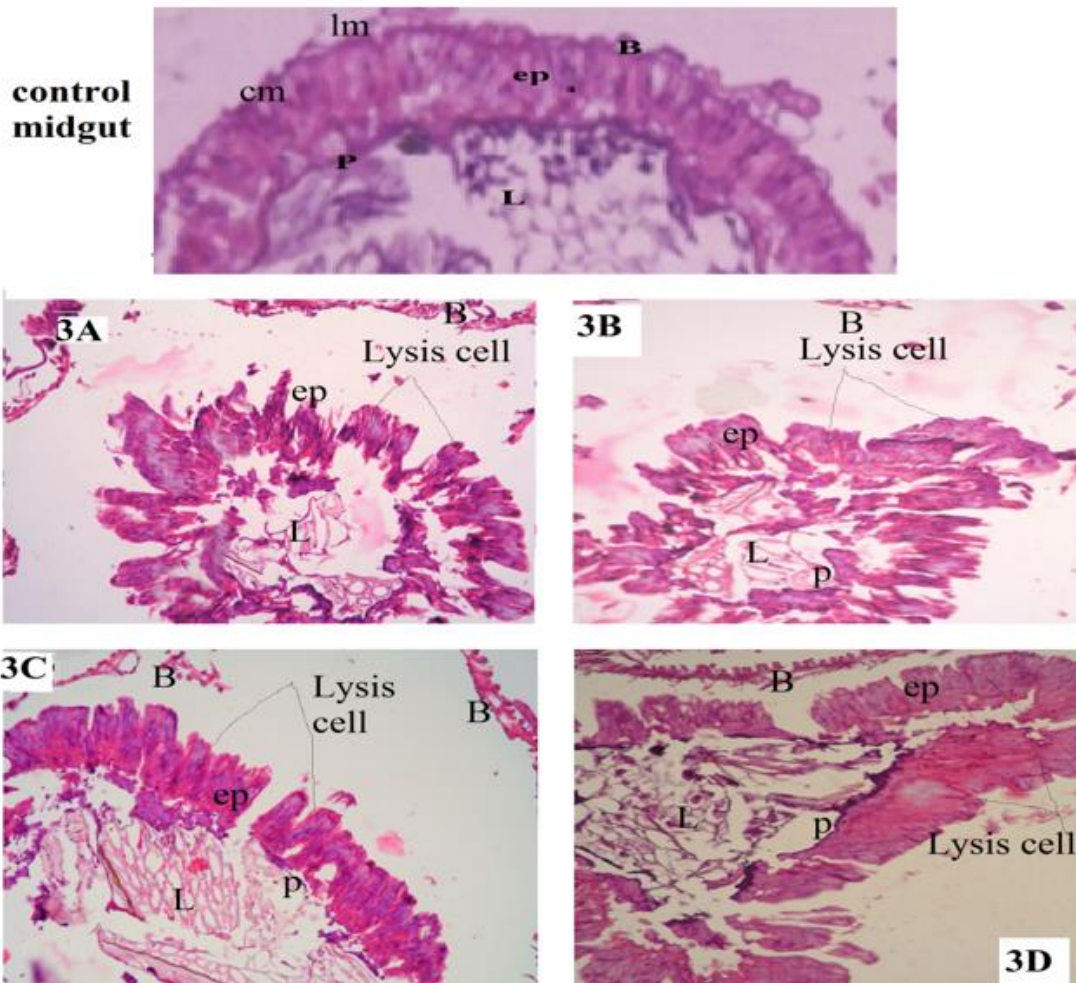


Fig.3 : Cross sections in the midgut of *A. ypsilon* treated with 50 and 25% of recommended rate of Chlorantraniliprole (coragen) showed post 3 days of treatment (a,b) for 4th instars and (c,d) for 5th instars (200xH &E) where lm: longitudinal muscle, cm: circular muscle, **B**: basement membrane, **ep** : epithelial cell, **p**: peritrophic membrane, **L**: lumen.

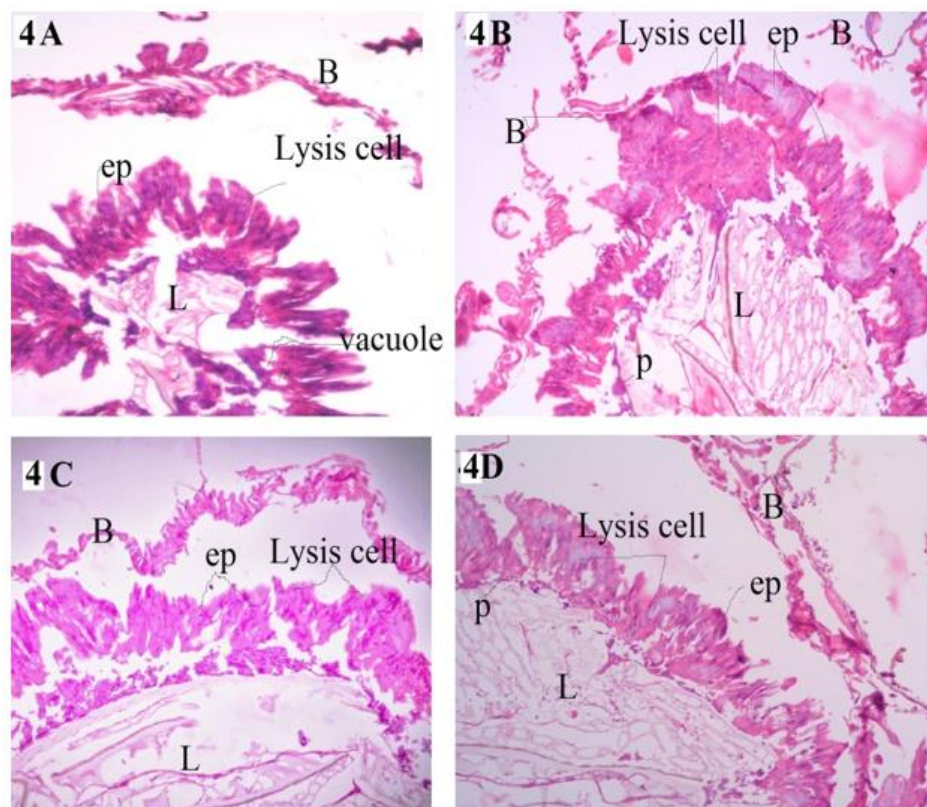


Fig.4 :Cross sections in the midgut of *A. ypsilon* treated with 50 and 25% of recommended rate of Lambda cyhalothrin + Thiamethoxan (Anjio) showed post 3 days of treatment (a,b) for 4th instars and (c,d) for 5th instars (200xH &E) where lm: longitudinal muscle, cm: circular muscle, B: basement membrane, ep : epithelial cell, p: peritrophic membrane, L: lumen

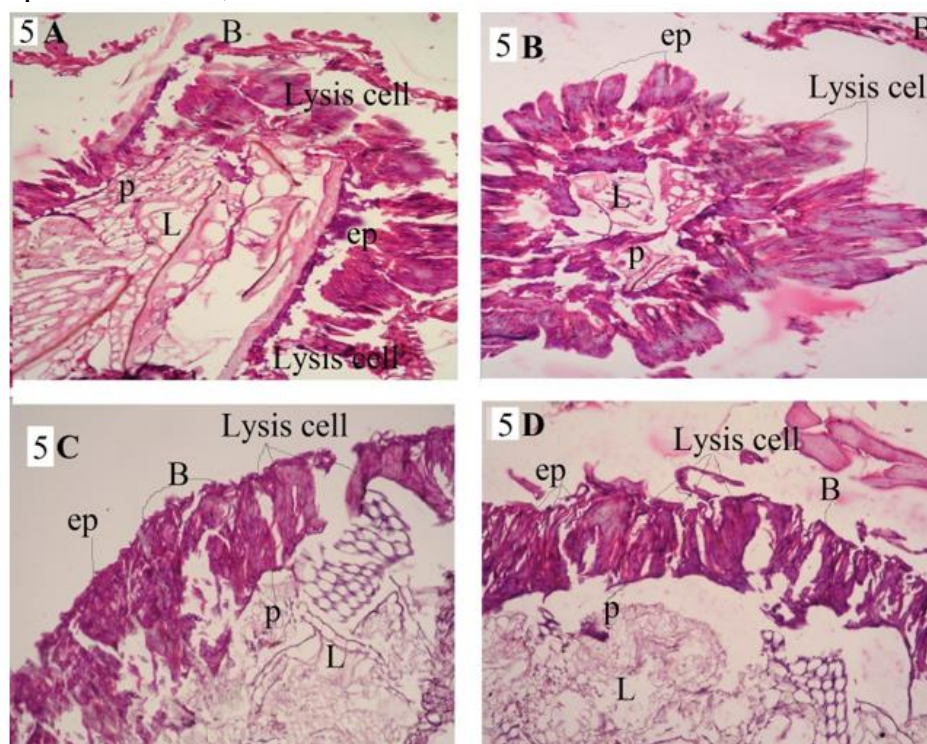


Fig.5 : Cross sections in the midgut of *A. ypsilon* treated with 50 and 25% of recommended rate of Lambda cyhalothrin (Affect power) showed post 3 days of treatment (a,b) for 4th instars and (c,d) for 5th instars (200xH & E) where lm: longitudinal muscle, cm: circular muscle, B: basement membrane, ep : epithelial cell, p: peritrophic membrane, L: lumen.

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ARABIC SUMMARY

تقييم بعض المبيدات في مكافحة الدودة القارضة اجروتس ايسيلون في الحقل وتأثيرهم على بعض النظم الهستولوجية

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تم اجراء تجارب حقلية لتقييم فاعلية بعض المبيدات, لامبادا ثيهالوثرين (افكت باور ٥٪) و لامبادا ثيهالوثرين ١٠,٦٪+ ثياميثوزان ١٤,١٪ (انجيو ٢٤,٧٪) و كلورانترانيليبيرول (كوراجين ٢٠٪) بالمعدلات الحقلية و التي تنتمي الى مجموعات مختلفه ضد الدوده القارضه على محصول البطاطس خلال موسمين متتالين فبراير ٢٠١٨ و ٢٠١٩ بمحافظة القليوبيه المصريه تحت الظروف الحقلية. وقد اظهرت كل القطع المختبره انخفاضاً شديداً المعنويه في تعداد اليرقات بعد معاملتها بثلاث ايام كطعوم سامه بالمبيدات المختبره. حيث كان معدل الخفض في الموسم الثاني ٢٠١٩ اكثر من الموسم الاول ٢٠١٨ بالنسبه الى الكوراجين والافكت باور. وأن الكوراجين والانجيو كانوا أكثرهم تأثيراً في كلا الموسمين استناداً الى متوسط نسبه الخفض ٩٢,٩١٪ و ٩٢,٢٦٪.

كما تم دراسته التغيرات الهستولوجية التي احدثتها الجرعات ٥٠٪ و ٢٥٪ من الجرعه الحقلية لهذه المركبات على تركيب المعى المتوسط ليرقات العمرين الرابع والخامس باستخدام تقنيه الحقلى معملى حيث وجدت انها تأثرت بدرجات مختلفه بكل المعدلات المستخدمه وتتلخص فى وجود انفصال او اختفاء مع تحلل فى الغشاء القاعدى الذى ادى الى انفصال وتهتك فى الخلايا الطلانية وعدم تميزها وكذلك تحلل وتهتك فى الغشاء حول الغذاء الخلايا مما أدى إلى إختلاط الطعام بالخلايا المتحللة. لذلك كل او بعض هذه التغيرات ادت الى فقد المعى المتوسط لوظيفته كالهضم والتغذية. لذلك هذه المبيدات يمكن استخدامها ضمن المكافحه المتكامله للدودة القارضه.