RESIDUES OF RYANODINE RECEPTOR MODULATORS (DIAMIDES) AND CHLORIDE CHANNELS ACTIVATOR (AVERMECTIN) ON BIOLOGICAL AND HISTOPATHOLOGICAL CHANGES OF COTTON LEAF WORM *SPODOPTERA LITTORALIS*.

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ABSTRACT:

Chlorantraniliprole, Flubendiamide (Diamides) and Emamectin benzoate (Avermectin derivative) belong to a recently developed class of insecticides. Diamides disrupt ryanodine receptors while; avermectin' acts as chloride channels activator. So, the objectives of this study were to evaluate median lethal concentration for *Spodoptera littoralis* larvae in laboratory bioassay, to confirm field efficacy and to determine residual efficacy of these recent insecticides. In addition, the histopathological changes of both muscles and mid gut of treated *S. littoralis* 6th larval instars were recorded. Leaves dipping technique were used in bioassay. Based on LC_{50} values against 4th instar larvae Coragen, under common name Chlorantraniliprole, was the most effective insecticide followed by Emox, under common name Emamectin benzoate, whereas Takumi, under common name Flubendiamide, was the last toxic insecticide, the corresponding LC_{50} values were 0.11, 0.29 and 0.33 ppm, respectively. All the treated larvae with LC_{50} values for each three tested compounds were biologically affected. Both pupation and adult emergence rates were decreased as a result of larval treatment. The larval treatment by diamide compounds had the strongest decrease on the pupal weight. Also, it was remarkable that sex ratio directed to the female side with both Coragen and Takumi while it directed to the male side with Emox. Field experiment was carried out at Kaha Research Station, Toukh district, Qalyobia Governorate, Egypt, during the summer seasons 2013 and 2014 of potato plants. Semi-field technique was used to determine the initial and residual activity of tested insecticide against 2nd and 4th instar larvae of *S. littoralis*. At zero time (Initial kill) all tested compounds gave percentage larval mortality ranged from 100 to 98.9% after 120 hrs during the two successive seasons. On the other hand, the residual effect of the tested insecticides which estimated at intervals after field application of 3, 5, 7 and 10 days indicated that, Coragen recorded the highest mortality at all time intervals of the experiment which recorded percentage mortality ranged from 100 to 87.8% for both tested larval instars followed by Takumi and Emox. Histopathological study showed that, LC_{50s} of both Coragen and Takumi caused serious influences on the muscles of 6th larval instar of *S. littoralis*. They cause complete destruction of sarcolemma and sarcoplasm shrinkage of myofibrils, vacuolation and fissures. The attachment of the muscles to the body wall by tonofibrils was severely affected due to disruption of these tonofibrillae. While, Emox had no effect on the attachment of the muscles to the body wall by tonofibrils. The midgut histopathology showed elongation of the epithelial cells and degeneration of some cells with all tested compounds. Also, the cytoplasmic matrix was released in the space between the epithelium and the peritrophic membrane. Coragen had more obvious effects on the histology of the midgut than other compounds.

Key words: Chlorantraniliprole, Flubendiamide, Emamectin benzoate, *Spodoptera littoralis*, bioassays, field efficacy, residual efficacy, histopathological changes.

INTRODUCTION

Diamides belong to a recently developed class of insecticides that ryanodine receptors, intracellular calcium channels play a central role in muscle and nerve function. There are presently two diamide insecticides available for use on tomatoes and potatoes in Egypt: chlorantraniliprole and flubendiamide. Chlorantraniliprole is also referred to as rynaxypyr. Chlorantraniliprole is the common name of Coragen and flubendiamide is the common name of Takumi. Diamide insecticides have been assigned the mode of action classification number 28 by the Insecticide Resistance Action Committee (IRAC). This number appears on the label of any insecticide containing diamides. Anthranilic diamides have very low vertebrate toxicity due to a >500-fold differential selectivity toward insect over mammalian ryanodine receptors (**Cordova *et. al.*, 2006**). Both newly developed diamide compounds, flubendiamide and chlorantraniliprole show specificity for insect RyR's and not affect isoforms of their mammalian counterparts, which show significant differences in their amino acid sequence, thus explaining the excellent toxicological profile of both compounds (**Sattelle *et al.*, 2008**).

Avermectins have emerged as a promising new group of insecticides (**Venkateswari *et al.*, 2008**). Avermectins, a family of 16-membered macrocyclic lactones produced by the fermentation of the soil actinomycete *Streptomyces avermitilis*. The major component of the fermentation, avermectin B1 (abamectin), is a mixture of B1a (≥80%) and B1b (**Lasota and Dybas 1991**). Emamectin benzoate is a semisynthetic derivative of abamectin and has been developed for the purpose of controlling lepidopterous pests on a variety of vegetable crops worldwide (**Jansson *et al.*, 1997 and Babu 1988**). It stimulates the release of the neurotransmitter γ-aminobutyric acid (GABA) (**Jansson *et al.*, 1997**). In general, the chloride ion flux produced by opening ion channels in neuronal cells as a result of GABA signaling results in the loss of cell function and the disruption of nerve impulses (**Jansson *et al.*, 1997**). Consequently, invertebrates are irreversibly paralysed and starved.

Potato plants (*Solanum tuberosum* L.) considered as one of the most important vegetable crop in Egypt. It gained a considerable impotence as an export crop to European markets as one of the national income resources (**El-Sirafy *et al.*, 2008 and El-mougy, 2009**). On the other hand, the cotton leaf worm, *Spodoptera littoralis* (Boisd) is a member of the economically important polyphagous pest that feeds on several economic importance species, such as cotton, corn, tomato, potato, okra, onion, cabbage, cucurbits and so on, (>120 host plants) and causes serious crop losses. In Egypt, control of this lepidopterous insect is mostly performed through the chemical insecticides. As a result of continuous use of pesticides, the field population of these major insect pests has developed resistance. The new chemistry insecticides bearing novel modes of action are now gaining attention (**Wang *et al.*, 2008**). It is a matter of great interest to find the susceptibility of this polyphagous insect pest towards modern insecticides. So, the objective of these studies was to generate median lethal concentration responses for *S. littoralis* larvae under laboratory bioassays, to confirm field efficacy and to determine residual efficacy of these new chemistry insecticides. In addition, any histopathological changes of both muscles and mid gut of treated *S. littoralis* 6th larval instars will be recorded.

MATERIALS AND METHODS

Tested compounds

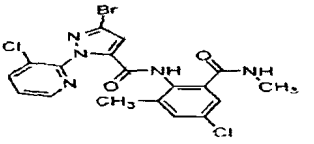
1- Coragen 20% SC (Chlorantraniliprole). It was provided by DuPont Du Nemours Company.

Common name: Chlorantraniliprole

Chemical class: Anthranilic diamide

Molecular formula: $C_{18}H_{14}BrC_{l2}N_5O_2$

Structural formula:



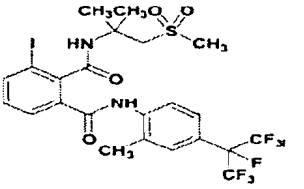
2- Takumi 20%WG

Common name: Flubendiamide

Chemical class: phthalic acid diamide

Molecular formula: $C_{23}H_{22}F_7IN_2O_4S$

Structural formula



3- Emox 2% EC.

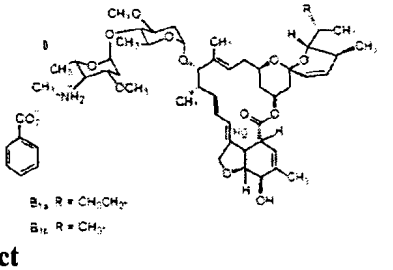
Common name: Emamectin benzoate

Chemical class: Avermectin

chemical formula: $(C_{49}H_{75}NO_{13}C_7H_6O_2) + (C_{48}H_{73}NO_{13}C_7H_6O_2)$.

(4"R)-4"-deoxy-4"- (methylamino) avermectin B1 benzoate (MAB1), which is a mixture of 4"R)-4"-deoxy-4"- (methylamino) avermectin B1a benzoate (MAB1a or emamectin B1a benzoate) and 4"R)-4"-deoxy-4"- (methylamino) avermectin B1b benzoate (MAB1b or emamectin B1b benzoate) The avermectins in emamectin benzoate are specified as a ratio MAB1a:MAB1b = 90:10 (w/w) and differ by a methylene group at the C26 alkyl substituent: –CH2CH3 for MAB1a and –CH3 for MAB1b.

Structural formula



The Experimental Insect

Spodoptera littoralis obtained from the laboratory culture of Plant Protection Research Institute, Agricultural Research Center (Cairo, Egypt). It were reared in the laboratory at 27 ± 2 C° and 65 ± 5% R.H. to get the number needed in this study, as described by **(El-Defrawi *et al.*, 1964).**

Bioassays

The bioassay was performed on fourth instars larvae of *S. littoralis*. Leaf dipping method using discs of Castor leaves, was employed. The washed and dried leaf discs were allowed to dip in the serial concentrations of water in one of Coragen, Takumi and Emox for 10-20 second. Also, castor leaves were dipped in only distilled water and used as control. The leaf discs were allowed to be air dried. The discs were then placed in glass jar and each concentration was repeated four times. 25 larvae per jar were exposed to treated leaf discs and the mortality percentage was taken after 48 hours and corrected according to **Abbott's formula, 1925** to estimate the LC_{50} values, the corrected mortality percentages were subjected to probit analysis according to **Finney, 1971**. The different biological effects such as larval mortality, pupation and adult emergence percentage, pupal weight, and sex ratio were determined at the LC_{50} values of the three insecticides. Statistical analysis of results was according to **(SAS, 1996).**

The semi-field trial

Field experiment was carried out at Kaha Research Station, Toukh district, Qalyobia Governorate, Egypt, during the summer seasons 2013 and 2014. Experimental area was divided into 16 plots, each plot 1/100 feddan (42m²) and planted with potato (*Solanum tuberosum* L.). Randomized complete blocks design was used with four replicates for each treatment. The insecticides, Coragen, Takumi and Emox were applied as foliar spray at the recommended rates 60 ml, 100 g and100 ml per fadden respectively using a knapsack sprayer. The 4th treatment was left untreated to serve as control. plant samples were randomly taken from each treatment after zero time, 3, 5, 7 and 10 days and transferred directly to the laboratory for feeding 2nd and 4th larval instars of *S. littoralis* five experiments were applied to estimate the mortality percentage at different time intervals Exp.(1) at zero time, Exp.(2) at 3rd day, Exp.(3) at 5th day, Exp.(4) at 7th day, Exp.(5) at 10th day from application. hunderd 2nd and 4th instar larvae of *S. littoralis* (newly moulted) were used. Four replicates of twenty five larvae each were used in each treatment, as well as, the control. In each replicate, larvae were confined with treated leaves for 24 hours, after which they were transferred to clean glass jars and provided regularly with fresh leaves. The jars were examined daily to determine the larval mortality. The percentage of mortality was corrected according to the Abbott formula **(Abbott, 1925).**

Histopathological studies:

All tested compounds were applied to the 4th instar larvae using the leaf dipping technique method at LC_{50} values. Ten individuals of the 6th larval instar were taken from each of the treated groups as well as the control one. These individuals were dissected in 0.85% Saline solution, mid gut and muscles were taken and fixed in carnoy's fixative for half an hour, then two changes (for about 15 minutes) in absolute ethyl alcohol (100%)were used for dehydration. The dehydrated organs were cleared in methyl benzoate for about 24 hrs. washed in toluene for 3-5 minutes, then transferred to liquid Paraffin (melting point 58°C) for 2-3 hrs. Serial sections at five microns were made by microtome and mounted on clean slides using Mayer's albumin. Sections were mounted on glass slides and stained with Haematoxylene and Eosin prepared for observation and photomicroscopy **(Humason and Freeman, 1979).**

RESULTS AND DISCUSSION

The LC_{50} values of tested compounds against 4th larval instars of *S. littoralis*.

The result of present investigation indicated that the LC_{50} values of Coragen, Takumi and Emox varied according to insecticide classes as shown in Table (1) and Figure (1).

Table(1): Median lethal concentrations of tested compounds against 4th larval instars of *S. littoralis*.

Tested compounds	LC_{50} after 48 hrs (ppm)	95% fiducially limits		Slope
		Lower	Upper	
Coragen 20% SC (Chlorantraniliprole)	0.11	0.09	0.12	2.87±0.071
Takumi 20% WG (Flubendiamide)	0.33	0.28	0.37	2.42±0.037
Emox 2% EC (Emamectin benzoate)	0.29	0.24	0.34	1.85±0.028

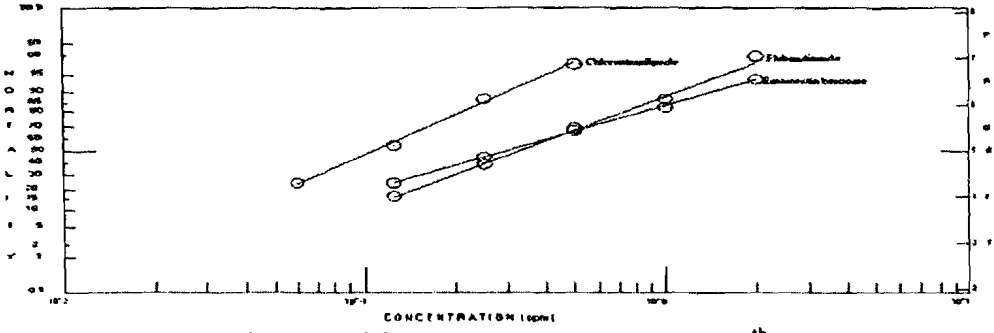


Figure (1): The regression lines of Coragen, Takumi and Emox on 4th instar larvae of *S. littoralis*.

Susceptibility of the 4th larval instars of cotton leaf worm, *Spodoptera littoralis* was carried out at different concentrations of tested compounds. The mortalities were recorded after 48h post treatment. LC_{50s} were ranged from 0.09 to 0.12 ppm of Coragen, 0.28 to 37 ppm of Takumi and 0.24 to 0.34 ppm of Emox i.e. Coragen was the most effective followed by Emox and Takumi. These results are agreement with **(Lahm *et al.*, 2007)** and **(Temple *el al.*, 2009)** who investigated that Rynaxypyr® demonstrated very good activity at relatively low rates against all three major caterpillar pests of cotton evaluated in this study, including tobacco budworm, bollworm, and fall armyworm. Also, **(Hardke *et al.*, 2011)** declare that LC_{50} values of chlorantranilprole and spinetoram were significantly lower than the $LC_{50's}$ of all other tested insecticides against fall armyworm, *Spodoptera frugiperda.* Also, **(Khaliq *el al.*, 2014)** stated that, Chlorfenpyr Coragen and Spinosad were highly effective against *H. armigera* and *S. litura* but Flafenoxuaron, Flubendamide and Leufenuron were found to be least effective in toxicity bioassay.

Effect of the three tested compoundes on some biological aspects of 4th instar larvae of *S. littoralis*.

Effect of LC_{50} values of Coragen, Takumi and Emox on some biological aspects; larval mortality, pupation percentage, pupal weight, adult emergence and sex ratio of 4th instar larvae of *S. littoralis* were demonstrated as shown in Table (2). It was noticed that a significant decrease on percentage of pupation, pupal weight and adult emergence of *S. littoralis* were occurred with all tested compounds. Also, it was remarkable that sex ratio directed to the female side with both Coragen and Takumi while to the male side with Emox. These results are going in line with those findings by **(Abdel-Rahim and Zidan 2012)** who indicated that sex ratio of *S. littoralis* adults directed to the female side with both takumi and methomyl treatment.

The Semi-field trial On 4th larval instar of *S. littoralis* during two successive seasons.

The resultin table (4) declared similarity trend with all tested compounds in reducing the larval population of *S. littoralis* 4th instar with difference in the time needed to induce this reduction as compared with the 2nd instar larvae that mentioned previously. At zero time all tested compounds gave percentage o larval mortality ranged from 100 to 98.9% after 120 hrs during the two successive seasons. On the other hand, the residual effect of the tested insecticides which estimated at intervals after field application of 3, 5, 7 and 10 days indicated that, 2nd larval instars of *S. littoralis* were significantly more susceptible than the 4th larval instars (tables 3 and 4). In general, the percentage of mortality decreased gradually from Exp. (1) to Exp. (5). Coragen recorded the highest mortality at all time intervals of the experiment which recorded percentage mortality ranged from 100 to 87.8% for both tested larval instars followed by Takumi and Emox. The obtained results are agreement with (**Arrod *et al.*, 2011**) who declared that fall armyworm mortality on all insecticide treated tissue at 0 DAT (% Mortality 72 hour after exposure) ranged from 90.6 to 100%. Mortality on chlorantraniliprole (96.9%) and cyantraniliprole-treated tissue (93.8%) significantly differed from that for all other treatments, except methoxyfenozide (89.1%) at 7 days from treatment. At 14 days after treatment, mortality for chlorantraniliprole, (85.9%), cyantraniliprole (75.0%), flubendiamide (26.6%), and methoxyfenozide treated tissue (53.1%) was significantly different as compared with the untreated larvae. In addition, chlorantraniliprole caused significantly higher mortality than all insecticides except cyantraniliprole. At 21 days after treatment, chlorantraniliprole (82.8%) and cyantraniliprole (75.0%) caused significantly higher mortality than the non-treated control (0.0%) and all other insecticide treatments (6.3 to 14.1%) except methoxyfenozide (29.7%).

Table (4): Corrected mortality % for the 4th instar larvae of *S. littoralis* after treated *Solanum tuberosum* field by recommended rate of tested compounds during two successive seasons.

compounds	Recommended rate/ feddan	Summer Season 2013														
		Corrected larval mortality % at different time intervals														
		Exp.(1) Zero time			Exp.(2) 3rd day from application			Exp.(3) 5th day from application			Exp.(4) 7th day from application			Exp.(5) 10th day from application		
		24h	72h	120h	24h	72h	120h	24h	72h	120h	24h	72h	120h	24h	72h	120h
Coragen 20% SC (Chlorantraniliprole)	60 ml	86.0	100	100	84.8	90.9	97.9	64.9	86.5	93.8	66.0	83.0	87.8	52.0	65.3	90.8
Takumi 20% WG (Flubendiamide)	100 g	70.0	97.0	100	59.5	79.7	91.9	58.7	77.3	90.7	20.0	78.0	79.7	22.0	57.1	63.2
Emox 2% EC (Emamectin benzoate)	100 ml	80.0	95.0	99.0	71.7	91.9	100	74.2	87.6	88.6	56.0	72.0	71.7	38.0	55.1	59.1
Summer Season 2014																
Coragen 20% SC (Chlorantraniliprole)	60 ml	80.0	91.9	100	78.0	93.0	99.0	69.0	85.8	87.8	61.0	81.0	88.0	54.0	68.6	87.8
Takumi 20% WG (Flubendiamide)	100 g	61.0	85.8	98.9	61.0	76.0	96.0	58.0	76.7	80.8	34.0	75.0	75.0	28.0	49.4	65.6
Emox 2% EC (Emamectin benzoate)	100 ml	79.0	91.9	98.9	65.0	98.0	98.0	64.0	80.8	81.8	58.0	71.0	69.0	40.0	48.4	49.4

Histopathological studies:-

Histopathological changes of the muscles and mid gut 6th larval instars of *S. littoralis*, resulting from 4th larval instar fed on fresh castor bean leaves dipped in LC_{50}s of Coragen, Takumi and Emox were illustrated in plates 1 and 2.

Histological structure of muscles

The skeletal muscles (m) are made up of elongated contractile fibers lying parallel to each other or converging upon the point of insertion (Plate 1.A). Most of insect muscles form of striated fibers; each fiber consists of a number of parallel fibrillae or sarcostyles laid down in nucleated plasma. The fibrils are cylindrical in shape. Each fiber is ensheathed in a relatively tough structure less membrane, the sarcolemma, and the nuclei of the sarcoplasm are either scattered throughout the substance of the fiber or disposed immediately beneath the sarcolemma. Each fibril is composed of alternating light and dark bands, so that the fiber shows as alternation of light and dark discs (Plate 1.B). The attachment of muscles to the body wall by tonofibrillae (tf) was observed (Plate 1.A).

Histpathological examination of treated larvae with sublethal dose (LC_{50}) of coragen showed that, the attachment of muscles to the body wall by tonofibrillae was severely affected owing to disruption of these tonofibrillae (Plate 1.C). Also, the muscles grouped into masses, complete destruction, shrinkage of myofibrils and complete destruction of sarcolemma and sarcoplasm were noticed. Also, there were strong fissures. The chromatin of nucleus condensed and form pycnosis (Plate 1.D). This histopathological effect may be due to the release and depletion of intracellular calcium stores from the sarcoplasmic reticulum of muscle cells. These findings are in agreement with that obtained by **(Cordova *et al.*, 2006)** who stated chlorantraniliprole stimulates the release and depletion of intracellular calcium stores from the sarcoplasmic reticulum of muscle cells, causing impaired muscle regulation, paralysis and ultimately death of sensitive species. Also, treated larvae with LC_{50} value of Takumi in several parts, attachment of muscles to the body wall by tonofibrillae was severely affected owing to disruption of these tonofibrillae (Plate 1.E). In addition, this treatment caused shrinkage of myofibrils that grouped into masses separated by vacuoles and fissures. The muscles appeared irregular in shape and the chromatin of nucleus became condensed (Plate 1.F). Histpathological examination of treated larvae with LC_{50} of Emox caused noticeable changes on larval muscles compared to that of the control ones as shown in (plates G&H). The attachment of muscles to the body wall by tonofibrillae, which extend through the hypodermis and the cuticle, was not affected (Plate 1.G). But causes shrinkage of myofibrils that separated by vacuoles and fissures (Plate 1.H).

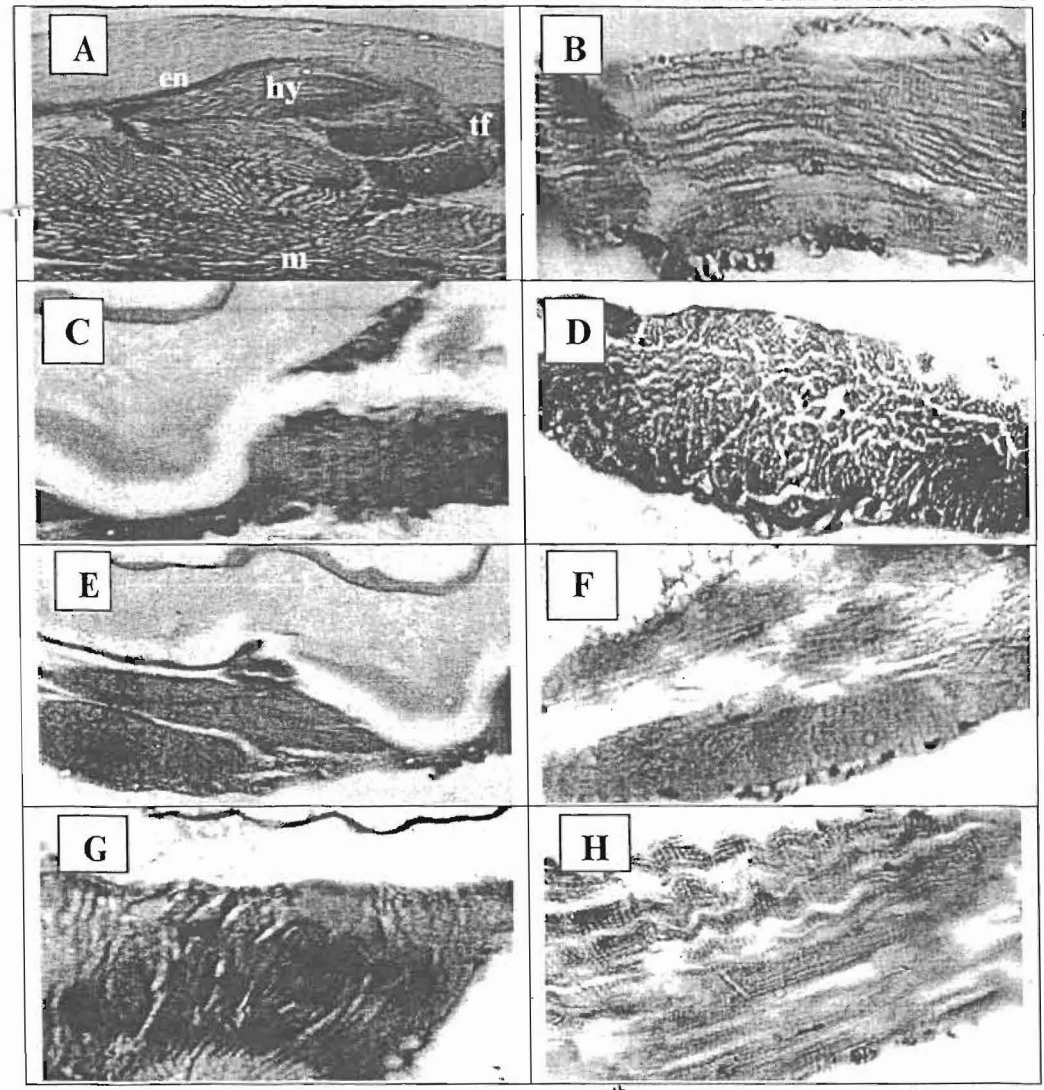


Plate (I): Histopathological changes in *S. littoralis* 6th larval instars muscles which treated as 4th instar with tested compounds.

Histological structure of midgut:-

The histological structure of normal midgut of 6th instar larva consists of a single cellular layer resting upon a basement membrane, which is surrounded externally by the circular (cm) then by the longitudinal muscles (lm). The single cellular layer consists of columnar epithelium cells each of them has a large nucleus (n). It is the major cell type in the midgut of *S. littoralis* larvae. Microvilli cover densely the distal surface of the columnar epithelial cells. The goblet cells are invaginated (inv) from the gut lumen to the level of their nuclei. Columnar epithelial cells are interspersed with small regenerative cells (rc) that rest also upon the basement membrane. Each regenerative cell contains a relatively large nucleus and strong basophilic cytoplasm. The peritrophic membrane (pm) is a thin layer slightly separated from the epithelial layer to inner side (PL.2.A).

Histopathological effect of Coragen, Takumi and Emox on treated larvae with LC_{50}s shown in (plate 2.B, 2.C and 2. D), respectively. The cytoplasmic matrix was released in the space between the epithelium and the peritrophic membrane. Also, some cells elongated and showed chromatin clumping or lysis. The cytoplasm appeared to be cracked probably because of the loss of its elasticity which led to vacuoles occurrence Plate 2.B.

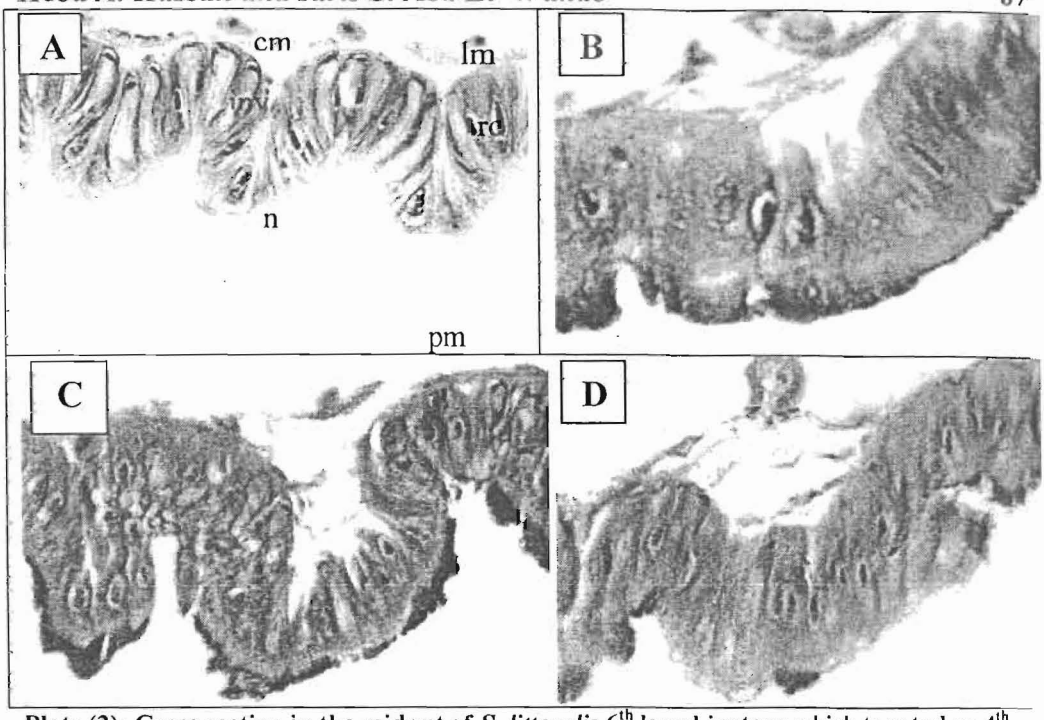


Plate (2): Cross section in the midgut of *S. littoralis* 6th larval instars which treated as 4th instar with tested compounds.

The midgut epithelium increased in thickeness in several parts and this due to cell proliferation. In many parts, the epithelial cells were separated from the basement membrane, degeneration of basement and peritrophic membranes was observed. Also, the cells showed abnormal architecture and many cells were destructed and degenerated. The chromatin material became condensed, forming cell pycnosis Plate 2.C.

Plate.2.D showed abnormal elongation of the cells represented by large intercellular spaces. Many cells were destructed and degenerated. In addition, the muscles lose their normal appearance.

Elongation of midgut epithelial cells of the 6th larval instar of *S. littoralis* may be a result of swelling of the epithelial cells, which caused by hypersecretory activities. Vacuoles may occur as a result of cell elongation as the cytoplasm loses its elasticity and cannot stretch uniformly or vacuoles may be formed as a result of the appearance of excessive fat droplets, which dissolved during fixation and dehydration processes. Shedding of the cytoplasm, fading of cell boundaries, and degeneration may be considered as several steps in the process of degeneration of the midgut epithelia. These results are accordance with that reported by **(Toppozada *et al.*, 1968)** in the midgut of *S. littoralis* after treatment of the larvae with different insecticides. **(Hussein *et. al.*, 2002)** studied the histological and histochemical changes of vertimec and neemazal-natural product on *Pectinophora gossypiella* and *Erias insulana* in laboratory conditions. They found that the two compounds caused destruction for the midgut epithelial cells. The histochemical studies revealed that both compounds affected on the polysaccharides in midgut epithelincluum.

It could be conclude that, the results demonstrated that the three tested compounds were effective to control cotton leaf worm on potato plant. Whereas, coragen is considered the most a valuable option for IPM strategies followed by Emox and then Takumi. Insecticides which have new mode of action should be used in rotation program to decrease resistance development as occurred with the use of many older insecticides. In addition, further field work

is needed to understand the most appropriate timing for applications of these insecticides in order to minimize the rate of application and to maximize their effectiveness in various cropping systems.

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الاثر المتبقى لكل من مستقبلات الريانودين (دياميد) و منشط قنوات الكلوريد (أفيرميكتين)
على التغيرات البيولوجية والنسيجية لدودة ورق القطن.

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مبيدات Chlorantraniliprole و Flubendiamide من مجموعة الدياميد و Emamectin benzoate احدى مشتقات الافيرميكتين ينتمون الى فئة تم تطويرها مؤخرا من المبيدات الحشرية فمجموعة الدياميد تعمل على تعطيل مستقبلات الريانودين و التي لها وظيفة مهمة في التقلص العضلي ووظيفة العصب بينما مشتقات الأفيرميكتين تعمل كمفعّل لقنوات الكلور معطلة الإشارات العصبية عند المفصليات. لذلك كان أهداف هذه الدراسة تسجيل التركيز المميت للنصف ليرقات العمر الرابع لدودة ورق القطن في المختبر و تقييم فعاليتها فى الحقل وتحديد فعالية الاثار المتبقية لهذه المبيدات الحشرية التي تم تطويرها حديثا. وبالإضافة إلى ذلك، تم تسجيل التغيرات النسيجية لكلا من العضلات والمعى المتوسط ليرقات العمر السادس الناتج من معاملة العمر اليرقى الرابع. وقد تمت دراسة النشاط الحيوى باستخدام طريقة غمس الأوراق المستخدمة كغذاء للعمر اليرقى الرابع وقد كان التركيز المميت للنصف لمركب الكوراجين تحت الاسم الشائع Chlorantraniliprole 0,11جزء من المليون ولمركب التاكومى Flubendiamide 0,33 جزء من المليون ولمركب الايموكس Emamectin benzoate 0,29 جزء من المليون وعندما تم دراسة النشاط البيولوجي للتركيز المميت للنصف لكل من الكوراجين و التاكومى و الايموكس على الطور اليرقى الرابع لدودة ورق القطن أوضحت الدراسة أن كل المركبات المختبرة لها تأثير سلبي على اليرقات الحية ونسبة التعذر و وزن العذارى و نسبة خروج الطور اليافع وقد كانت مركبات الدياميد الاكثر تأثيرا على وزن العذارى كما اتجهت النسبة الجنسية الى الاناث فى حالة المعاملة بهذة المركبات بينما اتجهت النسبة الجنسية جهة الذكور فى حالة المعاملة بمركب الايموكس وقد أجريت التجربة الحقلية في محطة بحوث قها، منطقة طوخ بمحافظة القليوبية خلال موسمى الزراعة 2013 و 2014 على نبات البطاطس العروة الصيفية. وقد تم استخدام التقنية شبه الحقلية لتحديد النشاط الأبادى الفورى والمتبقي للمبيدات الحشرية المختبرة ضد العمر اليرقى الثانى والرابع لدودة ورق القطن. وقد سجلت تجربة وقت الصفر نسبة موت لليرقات تراوحت بين 100 و98،9٪ بعد 120 ساعة (5 يوم) لجميع المركبات التى اخضعت للاختبار خلال الموسمين المتعاقبين. من ناحية أخرى، أشار الأثر المتبقي للمبيدات المختبرة والتي قدرت على فترات من 3 و 5 و 7 و 10 أيام بعد التطبيق الحقلى أن العمر اليرقى الثانى اكثر حساسية للمركبات المختبرة عن العمر اليرقى الرابع. وسجل مركب الكوراجين أعلى معدل موت على مدار كل الفترات الزمنية للتجارب حيث سجل نسبة موت تراوحت بين 100و 87،8٪ لكل الأعمار اليرقية المختبرة يلية مركب الايموكس ثم مركب التاكومى. وقد أدت المعاملة بالمركبات المختبرة إلى وجود خللا" واضحا" في التركيب العضلي حيث ظهر انكماش وفجوات وشروخ في هذه الطبقة وتفكك الروابط التي تصل العضلات بجدار الجسم كما أظهرت الدراسة الهيستوباثولوجية للمعى المتوسط ليرقات الطور السادس الناتج عن معاملة الطور اليرقى الرابع بالتركيز المميت للنصف لكل من المركبات المختبرة إلى حدوث استطالة للخلايا وانفجار للسيتوبلازم وتمركزه بين طبقة الخلايا الطلائية والغشاء البريتونى. أما في مركبى لكوراجين والتاكومى فقد لوحظ وجود انقسامات عديدة وسريعة في الخلايا المجددة للمعى المتوسط .كما أدت المعاملة بهذه المركبات إلى حدوث تشققات في السيتوبلازم. وكان من الملاحظ أيضا" أن الكوراجين له تأثير أكبر على التركيب الهيستولوجى للمعى المتوسط.