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Effect of IGIs and IGRs on Embryonic development stages of *Agrotis ipsilon* (Hufn.) (Lepidoptera: Noctuidae).

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Abstract

Histological study was conducted in order to add to the previous studies concerning studied the effect of LC_{50} of pyriproxyfen and tebufenozide as insect growth inhibitors (I.G.Is) and hexaflumuron, flufenoxuron and lufenuron as insect growth regulators (I.G.Rs) on different ages of *Agrotis ipsilon* (Fam. Noctuidae) eggs. The effect of LC_{50} for these compounds were studied on the embryonic development. The treated deposited eggs showed acute effect causing decomposition to yolk content and defects in early stages such as germ band. In advanced stage after treatment, the embryonic development may be stopped and defective embryos were found. Histological examination of pyriproxyfen treated *A. ipsilon* eggs early showed various effects of treatment on the embryonic development such as a clear shortening of the formed germ band and great blockage of the embryonic development at the gastrulation stage. Eggs treated with tebufenozide, the embryonic development was inhibited showing the formation of undifferentiated of tissues and showed a clear invagination in the germ band. Application of flufenoxuron caused separation of the embryo components and the eggs became without strong chorion. Hexaflumuron caused undifferentiation tissues was apparent. On the other hand, the weakest effect was found when lufenuron was used at 24- 48 hrs. old of *A. ipsilon* eggs where a slight inhibition of embryonic development was occurred.

Key words: IGI, IGR, Histological, *Agrotis ipsilon*, eggs

Introduction

The development of insect growth regulators and insect growth inhibitors during the recent years has increased the option available for control of economically important insects.

Infestation by the greasy cutworm *Agrotis ipsilon* Fam. Noctuidae can cause severe losses in the yield. A number of non-insecticidal methods for controlling the greasy cutworm have been developed and can be effective, especially when considered in integrated pest control system. Many of the requirements of integrated pest control were demonstrated by FAQ (1966). Steiner, (1974) and Metcalf and Luckmann (1975) studied the use of certain insect growth regulators (IGRs). The compounds of this group of insecticides are active against many lepidopterous species.From such compounds, diflubenzuron, triflumuron, flufenoxuron, chlorfluazuron, teflubenzuron, and tebufenozide were studied by Rizk and Radwan (1975) Asher and Nemny (1976) Asher *et. al*., (1979) Becher *et al*., (1983) Moawad *et al*., (1990), Haga and Nishiyama(1982) Becher *et al*., (1983) Moawad *et al*., (1990) Chandler *et al*., (1992) Fesk *et al.,* (1993) and Shalaby and Tawfik (2001).

Many researchers showed that treatment of the eggs of different insect species with IGR compounds could bring about an arrest of embryonic development (Matolin,1970), Rohdendrof and Sehnal (1973), Srivastava and Shukla (1982) observed that if eggs was treated early, embryonic development could be terminated at any stage from cleavage to embryonic moulting.

Materials & Methods

The effects of pyriproxyfen 10% EC, tebufenozide 24% FL as insect growth inhibitors and hexaflumuron 10% EC, flufenoxuron 10% EC, and lufenuron 5% EC as insect growth regulators were tested in the laboratory on the different ages of the greasy cutworm *Agrotis ipsilon* eggs .

1-Insect rearing technique:

Large number of *Agrotis ipsilon* were collected from the field and reared successfully under laboratory condition of 25± 1 C^5 and 65±5 R.H., using the technique described by Gesraha (1993).

2- Material used:

A:-Insect growth inhibitors (IGIs):

- Common name: Pyriproxyfen
Trade name: Admiral10% EC
Chemical name: (RS)-1-(4-phenoxyphenxy (RS)-2-(2-pyridyloxy) propane.
- Common name:-Tebufenozide
Trade name:-Mimic 24% FL
Chemical name: -3, 5-dimethylbenzoic acid 1-(1,1dimethylethyl)-2-(4-ethybenzoyl) hydrazide

B- **Insect growth regulators (IGRs.):**

- Common name: Hexaflumuron
Trade name:-Consult 10% EC
Chemical name: 1-(3, 5-dichloro-4-(1, 1, 2, 2-tetrafluoroethoxy)
- Common name: Flufenoxuron
Trade name: Cascade 10% EC.

Chemical name: 1-(4-(2-chloro-&, &-&-trifluoro-p-tolyoxy)-2-fluorophenyl)-3-(2, 6-difluorobenzoyl) urea
- Trade name: Match 5% EC
Chemical name: (*RS*)-1-[2, 5-dichloro-4-(1, 1, 2, 3, 3, 3-hexafluoropropoxy) phenyl]-3-(2, 6-difluorobenzoyl) urea

Egg treatment:
The collected eggs were divided into three groups; one, two and three days old eggs.

Treatment of the different ages of eggs with LC_{50} values of the tested compounds was carried out according to El sappagh (2006) by dipping the eggs for 30 sec.

Table 1. LC_{50} for I.GIs. and I.G.Rs on two and three days old eggs of *Agrotis ipsilon*, according to El Sappagh 2006

Tested compounds	LC_{50}	
	Two days old eggs	Three days old eggs
I.G.Is		
Pyriproxyfen	**1788**	**3107**
tebufenozide	**4947**	**5695**
I.G.Rs		
hexaflumuron	**52.2**	**60.4**
flufenoxuron	**711**	**988.1**
lufenuron	**2958**	**4679**

Histological effects of IGIs and IGRs on *A. ipsilon* eggs treated at different ages:

This study was conducted to complete previous studies concerned with the effect of LC_{50} of tested compounds on the embryonic development in *Agrotis. ipsilon* eggs after treatment by different IGI and IGRs i.e. Pyriproxyfen, tebufenozide, hexaflumuron, flufenoxuron and lufenuron at LC_{50} values using dipping technique of eggs on the embryological developmental stages until hatching of *A. ipsilon*. Selected eggs were treated at two different stages of development i.e. (two and three days old eggs).

The inhibition of the embryonic development after the application of juvenile hormone analogues were described by several authors (Chen *et al* 1993, 1998, Slama and Williams, 1966, Riddiford and Williams, 1967, Novak, 1969 and Matolin, 1970).

1- Histological effects of IGI on two days old egg of *A. ipsilon*:

Data on the eggs of two and three days old (i.e. 24-48 and 48-72 hrs old) which were treated with IGIs pyriproxyfen and tebufenozide at LC_{50} values showed a great inhibition in different stages of development. These stages included invigilation of the germ band, blastokinesis and segmentation of the embryo.

1.1- pyriproxyfen:

Histological examination of pyriproxyfen treated *A. ipsilon* egg masses early showed various effects of treatment on the embryonic development. A clear shortening of the formed germ band (Fig.2A), great blockage of the embryonic development at the gastrulation stage (Fig. 2B) as well as formation of cavities of grooves inside the formed embryo's body (Fig. 2C).

Fig. (2A) clears a cross section of pyriproxyfen treated egg showing shortening of the germ band.

Embryonic study:

From the LC_{50} concentration potency, specimens of eggs which hatched at the end of the normal incubation period about 4 days were used for the emberyonic study. Eggs were dechorionized in 1% sodium hypochlorite for 5 min. and washed 3 times with distilled water. The eggs were fixed in aqueous Bouin's solution for dehydrated through a series of ethanol dilutions, placed in xylene for clearing and finally the eggs were embedded in paraffin blocks and sectioning took place at 5µm Ribbons obtained from sectioning were adhered onto thoroughly cleaned slides, and stained with Erich's hematoxylin and counter stained with Eosin. These preparations were used for microscopic examination and photomicrographs were taken. To compare the histological changes, specimens from the check and treatment were sampled and slides were prepared as previously described, Shalaby (2002).

Results and Discussion

The description of the normal embryonic development of lepidoptrous insect eggs was detailed by Johannsen and Butt (1941) and Hagan (1951) as follows:
Maturation at 30-45 min., blastoderm formation at 10 hours, germ band and serosa are distinguishable at 16 hours, Gastrulation at 22 - 24 hours after egg deposition, invagination of the germ band which attains its greatest length formation of head and its appendages (antennae and mouth parts) at 37 hours. Completion of blastokinesis took place at 42 hours and completion of segmentation at 55 hours (Fig. 1 A, B&C).
Complete formation of the embryo and hatching of the first instar larvae occurred at 56-60 hours after egg deposition.

hexaflumuron. A clear undifferentiated tissues were apparent (Fig. 5A) and disorganization of cells could be also seen in few numbers of eggs Fig. 5 B and C.
Fig. 5 (A; B &C): Transverse section showing the clear effects LC_{50} of hexaflumuron on 24- 48 hrs. eggs.

2.3- lufenuron:
The weakest effect of tested used IGRs was found when lufenuron was used on 48 hrs *A. ipsilon* egg masses. A slight inhibition of embryonic development occurred. (Fig. 6 A & B).

Administration of IGI and IGRs to three days old eggs of *A. ipsilon*:

Histological studies on three days old of *A. ipsilon* fixed before hatching cleared that, nearly all eggs had undifferntiaated embryos. This agree with (Riddiford 1970) who detailed that when Juvenile hormone is applied after germ band formation, it has progressively less effect on embryonic development and more effect subsequently appears, in post embryonic life.
The inhibition of embryonic development after the application of IGRs has been described as the follows:

Embryological development blocked during blastokinesis of a miniature embryo which also sunk into the yolk for three days old eggs treated with LC_{50} value of pyriproxyfen (Fig.7A). Also found that destroyed embryo in blastokinesis (Fig. 7B,) a syminetric defect of certain structure for eggs treated with tebufenozide (Fig. 8). While, *A. ipsilon* eggs tested by LC_{50} value of flufenoxuron caused, the embryological development ceased during segmentation (Fig.9).

Also, the embryological development was blocked during blastokinesis of aminiaturer embryo occurred when treated eggs of *A. ipsilon* were treated with hexaflumuron after 48 hours of deposition (Fig.10).On the other hand, eggs treated with lufenuron after 48 hours of deposition caused disorganized bunches of cells (Fig.11).
The explained results on the effect of IGRs on the embryonic development of *A. ipsilon* are complete confirmation to the conclusion that previously recorded on two days old eggs which indicates that older eggs were less sensitive for IGRs than freshly deposited eggs.

Fig. (2B) Longitudnal section of pyriproxyfen treated egg showing blockage (BL) of the embryonic development at the gastrulation stage. Fig. (2C): Transverse section showing blockage of the embryonic development at the gastrulation stage, the first evidence of gastrulation is seen in mid ventral immigration of the presumptive mesoderm, associated with formation of a cavity (v).

1.2- Tebufenozide:

Eggs treated with tebufenozide revealed that the embryonic development was inhibited showing the formation of undifferentiated tissues (Fig. 3A). A clear invagination in the germ band also appeared (Fig. 3B).In addition tebufenozide at LC_{50} sometimes caused that the embryo seemed sunk into the yolk (Fig. 3C).
Fig. (3A) longitudinal section of tebufenozide treated *A. ipsilon* egg showing the formation of undifferentiated tissues. Fig. (3B):.Transverse section showing inhibited embryological development during invagination of the germ band. Fig. (3C) Transverse section of the tebufenozide treated *A. ipsilon* egg showing the embryo sunk in the yolk.

2- Histological effects of IGRs on two days old egg of *Agrotis ipsilon*:

2.1- flufenoxuron:

Dipping of eggs in flufenoxuron caused a clear shortening of the germ band, other eggs showed no embryological development with large gaps. Cavities occurred in same two days old eggs treated with this IGR at LC_{50} value. The most deleterious effect appeared as separation of the embryo components and the eggs became without strong chorion (Fig. 4, A,B &C).
Fig. 4 (A,B&C) : Cross section showing the effect of flufenoxuron on 24-48 hrs old eggs of *Agrotis. ipsilon* as follows:
A- Numerous morphological defects and abnormalities.

B- Clear disruption and distorted with large gap cavities (v).
C- Separation of the embryo components and the eggs without strong chorion.

2.2- hexaflumuron:

The inhibition of embryonic development after application was also noticed when 48 hrs old egg masses of *A. ipsilon* were treated with the LC_{50} of

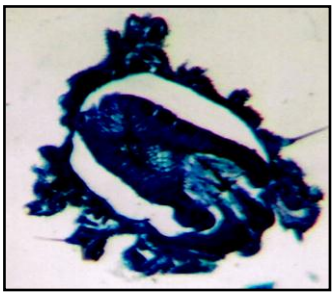
Fig.1A

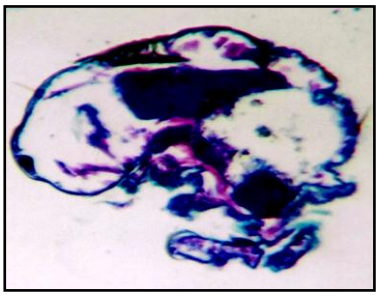
Fig.1B

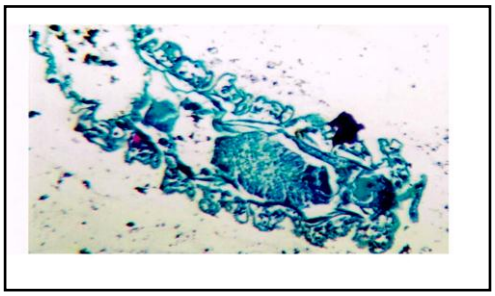
Fig.1C

Fig. (1 A, B & C): Cross sections through untreated *A. ipsilon* eggs showing the serial steps of normal embryonic development as the follows:
A: Germ band, gastrulation formed and invagination of the germ band (30 hrs); B: Completion of blastokinesis (42 hrs); C: Completion of the alimentary tract, segmentation of the body and the embryo will hatch soon.

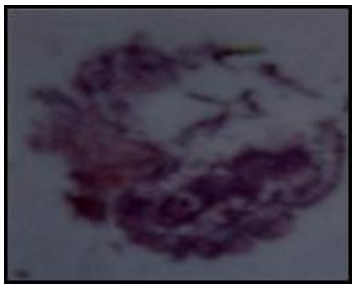
Fig.2 A 10X

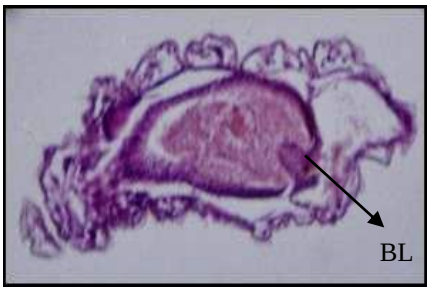

Fig.2 B 10X

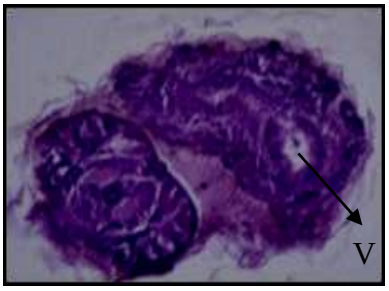

Fig.2 C 10X

Fig. (2 A, B & C) show the effect of pyriproxyfen on the two days old eggs of *A. ipsilon.*
A: Cross section showing shortening of the germ band; B: Longitudinal section showing blockage (BL) of the embryonic development at the gastrulation stage; C: Transverse section showing blockage of the embryonic development at the gastrulation stage.

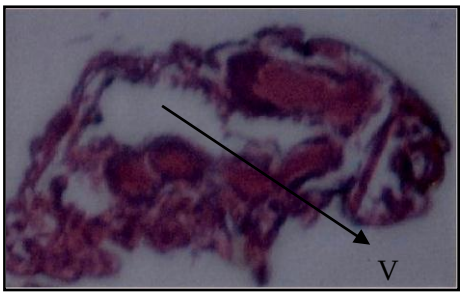

Fig. 3 A 10X

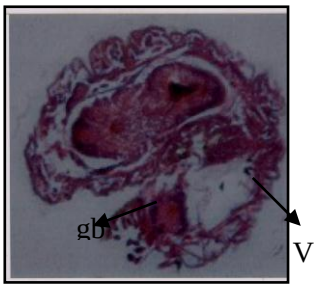

Fig. 3 B 10X

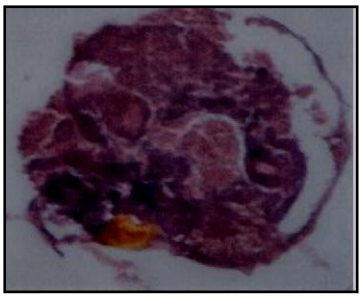
Fig. 3 C 10X

Fig. (3 A, B & C): show the effect of tebufenozide on the two days old eggs of *A. ipsilon*.
A: Longitudinal section showing the formation of undifferentiated tissues; B: Transverse section showing inhibited embryological development during invagination of the germ band (gb); C: Transverse section showing the embryo seemed sunk into the yolk.

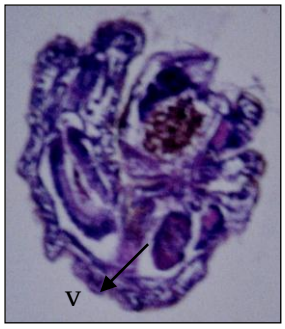

Fig. 4 A 10X

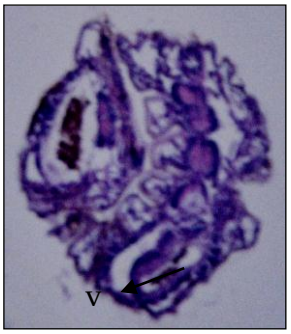

Fig. 4 B 10X

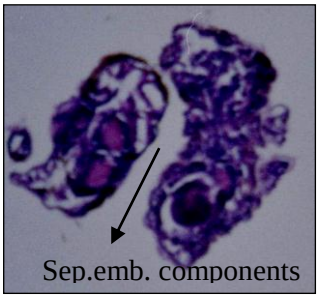

Fig. 4 C 10X

Fig. (4 A, B & C): Cross sections showing the effect of flufenoxuron on two days old eggs of *A. ipsilon*.
A: Numerous morphological defects and abnormalities; B: A clear disruption and distorted with large gaps (cavities)v; C: Separation of the embryo components and the eggs without strong chorion

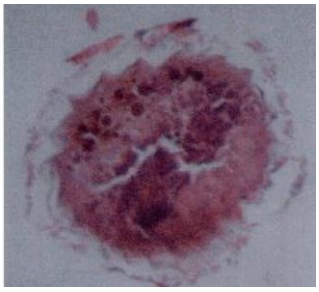

Fig.5 A

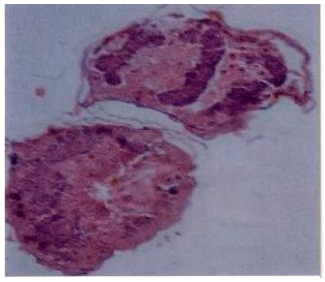

Fig.5 B

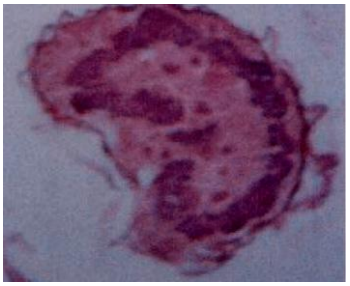

Fig.5 C

Fig.(5 A, B & C): Transverse sections showing the clear effects of hexaflumuron LC_{50} on 24-48 hrs egg masses.
A: A clear undifferentiated tissues were apparent; B and C: Disorganization of cells could be also seen in few numbers of eggs.

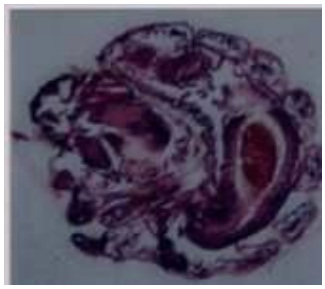

Fig. 6 A

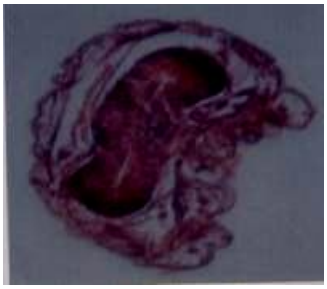

Fig. 6 B

Fig.(6 A, B): Cross sections showing the effect of lufenuron on two days old eggs of *A. ipsilon* showed a slight inhibition of embryonic development

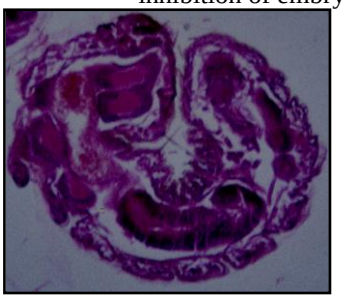

Fig 7A

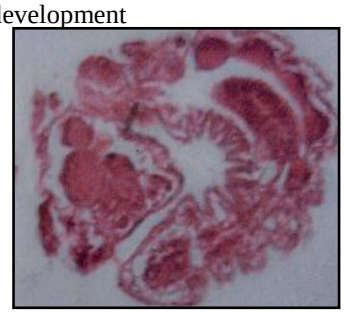

Fig 7B

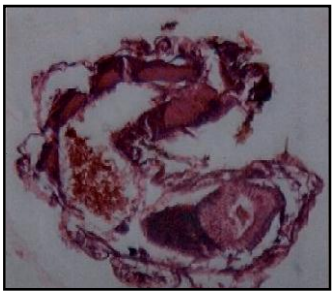

Fig 7C

Fig. (7 A, B & C): Sections in *A. ipsilon* egg treated after 48 hrs. of deposition by LC_{50} of pyriproxyfen showed that:
A: The embryological development blocked during blastokinesis of a miniature embryo which also sunk into the yolk; B&C: Sections through egg treated showing destroyed in blastokinesis

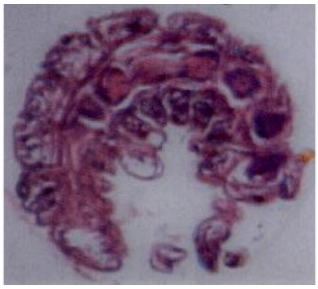

Fig.(8):Section showing the effect of tebufenozide on three day old eggs of *A. ipsilon*, a symintric defects of certain structure.

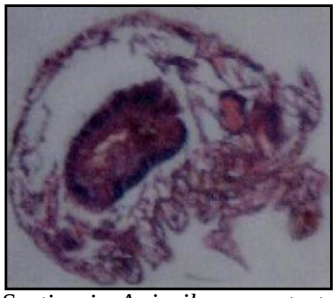

Fig. (9): Section in *A. ipsilon* eggs tested by LC_{50} value of flufenoxuron showing, the embryological development ceased during segmentation.

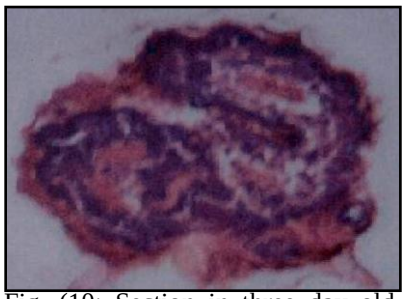

Fig. (10: Section in three day old eggs treated with LC50 value of hexaflumuron showing the embryological development was blocked during blastokinesis of miniature embryo.

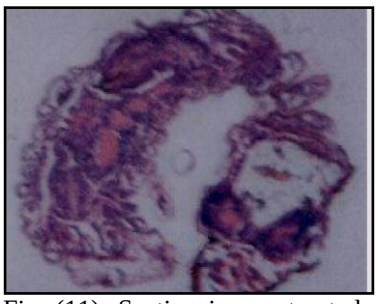

Fig. (11): Section in egg treated after 48-72 hrs. by lufenuron ,showing disorganized bunches of cells.

References

Ascher,K.R.S. and Nemny, N.E. (1976): Toxicity of the chitin synthesis inhibitors, diflubenzuron and its dichloro analogue to *Spodoptera littoralis* larvae. Pestic.Sci., 7 :1-9.

Ascher,K.R.S.; Nemny, N.E.; Eliyahu, M. And Ishaya,I.(1979): The effect of BAY SIR 8514 on *Spodoptera littoralis* (Boisduval) eggs and larvae. Phytoparasitica,7: 177-181.

Becher. H.M.; Becker, P.;Procjic-Inmmel, R. and Wirtz, W. (1983): CME- 134, a new chitin synthesis inhibiting insecticides. Proc. 10th Int. Congr. Prot.(Brighton, 1983),/: 408-415.

Chandler, D.; Pair, S.D, and Harrison, W.E. (1992): RH-5992, a new insect growth regulator active against corn earworm and fall armyworm (Lepidoptera; Noctuidae). J. Econ. Entomol., 85: 1099-1103.

Chen, P.; H. F., Gong; J. Z.,Wang ; J. Y., Ding and Z.S., Wang (1993)**:** Mechanism of chemosterilization by diflubenzuron in armyworm. Acta-Entomologica Sinica., 36: 396-408.

El Sappagh, I.A. (2006): Studies on the effect of some growth regulators on the eggs of some insects. Ph. D. Thesis, Fac. of Agric., Benha Univ.Egypt

FAO (1966): Proceedings of the FAO Symposium on integrated Pest Control (Rome, 1965). FAO Rome.

Fisk, T.; Cooper, J. and Wright, D.J. (1993): Control of *Spodoptera spp*. Using ULV formulations of the acylurea insect growth regulator, flufenoxuron; field studies with *S. exempta* and effect of toxicant concentration on contact activity. Pestic. Sci., 39: 79-83.

Gesraha, M.A.M. (1993): Ecological and biological studies on the hymenpterous parasitoids *Meteorus spp*.(Braconidae:Hymenoptera). Ph. D. Thesis, Fac.of Agric., Cairo Univ.Egypt.

Johanson, O. A. & Butt, F. H. (1941). Embryology of Insects and Myriapods. New York: McGraw-Hill.

Matolin, S. (1970): Biochemical of a juvenile hormone analogue on embryogenesis in *Pyrrhocoris opterus* L. Acta Etomol. Bohemoslov., 67:b9-12.

Metcalf, R.L. and Luckmann, W. (1975): Introduction to insect pest management (Ed.), Jhon Wiley & Sons, 587 p.

Moawad, G.M.; Rashad Amira M.; Shalaby, M.A.M. and Gadallah, A.L. (1990): Effect of some insect growth regulator on the larvae of the pink bollworm *P. gossypiella* (S.) (Lepidoptera; Gelechiidae). Bull.Entomol. Sci. Egypt, Econ. Ser., 18:149.

Novak, V.J.A. (1969): Morphological analysis of the effect of juvenile hormone analogues and other morphogenetically active substances on embryos of *Schistocerca gregaria* (Forskal).J.Embryol. Morph., 21:1021.

Riddiford, L.M. (1970): Prevention of metamorphosis by exposure of insect eggs to

juvenile hormone analogues. Science., 167: 287-288.

Riddiford, L.M. and C.M., Williams (1967): The effects of juvenile hormone analogues on the embryonic development of silkworms. Proc. Nat. Acad. Sci. U.S. 57: 595-601.

Rohdendrof, E.B. and Sehnal, F. (1973): Inhibition of reproduction and embryogenesis in the firebrat, *Thermobia domstica* by juvenile hormone analogues. J. Insect Physiol., 19: 37-56.

Rizk., G.A.M. and Radwan, H.S.A. (1975): Potencey and residuality of two antimoulting compounds against cotton leafworm and bollworms. Z.angew. Entomol., 79: 136-140.

Slama, K. and Williams, C. M. (1966) 'Paper factor' as an inhibitor of the embryonic development of the European bug, Pyrrhocoris apterus. Nature, Lond. 210, 329-330.

Shalaby, M.A.M.and Twfik,S.M. (2001): Some tests on the effects of tebufenozide Mimic, on bollworm *Pectinophora gossypiella* (Saund.) and *Erias insulana* (Boisd.). Egypt, J. Appl. Sci., 16: 233-244.

Shalaby, M.A.M (2002): Effect of flufenoxuron Cascade on embryogenesis of *Pectinophora gossypiella* (SAUND). J. Acad. Soc. Environ Develop. (A. Entomology), 2: 53-66.

Sirvastava, U.S. and Shukla, K.M. (1982): Effect of a juvenoid on embryonic development of *Earias fabia* (Stoll) (Lepidoptera; Noctuidae). J.Insect Physiol., 28:299-304.

Steiner, H.(1974): Richtlinien fur den integriertea pflanzenschutz. Landesanatalt fur pflanzenschutz, Stuttgart.

تاثير مانعات الانسلاخ ومنظمات النمو الحشرية على مراحل النمو الجنينى للدودة القارضة

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اجريت دراسة هستولوجية استكمالا لدراسات سابقة حيث تم دراسة التركيز المميت ل 50 % من الافراد المعاملة لكل من البيريبروكسيفين، التبيوفينوزايد كامانعات للانسلاخ و الهكسافلومورون، الفلوفينوكسيرون و اللوفينيرون كمنظمات للنمو الحشرى على الاعمار المختلفة لبيض الدودة القارضة.

وقد اظهرت النتائج ان البيض الموضوع حديثا عند معاملتة بالتركيز النصف مميت للمركبات قد ادى الى تاثير حاد على المحتوى المحى للبيض وخلل فى المراحل المبكرة للنمو كما حدث للشريط الجرثومى اما عند المعاملة فى مراحل النمو المتقدمة فقد لوحظ توقف النمو الجنينى كما ادت المعاملة بتلك المركبات الى ظهور الاجنة المشوهه.

كما أظهرت النتائج ان المعاملة بالتركيز المميت ل 50 % من الافراد المعاملة للبيريبروكسيفين ادى الى ظهور تاثيرات مختلفة على مراحل النمو الجنينى ، كما أدت ان المعاملة بالتركيز النصف مميت للتبيوفينوزايد الى ظهور انسجة غير مميزة، كما ادت المعاملة بالتركيز النصف مميت للفلوفينوكسيرون الى اختزال واضح للشريط الجرثومى و فصل مكونات الجنين ، كما أدت المعاملة بالتركيز المميت ل 50 % من الافراد المعاملة للهكسافلومورون الى ظهور انسجة غير مميزة.

من ناحية اخرى وجد أان أضعف تاثير غلى التمو الجنينى للدودة القارضة كان عند استخدام التركيز المميت ل 50 % من الافراد المعاملة لمركب اللوفينيرون.