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Effect of insect growth regulators combined with nucleopolyhedrovirus on certain biological and histological aspects of *Spodopera littoralis.*

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ABSTRACT

Five insect growth regulators (IGR's) were tested for increasing the susceptibility of the cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) to its homologous nucleopolyhedrovirus (*Spli*MNPV). *S. littoralis* MNPV was tested alone or in combination with IGR's at LC_{10} level against the 2nd instar larvae of the pest. An increased viral infection rate was detected in the mixture treatment in the case of using Chlorfluazuron, Flufenoxuron, Triflumuron, Hexaflumuron or Teflubenzuron at 10%. The LC_{50} value of the virus alone treatment $1x10^7$ PIB's was reduced to $4.3x10^6$, $9.9x10^4$, $4.9x10^4$, $3.1x10^5$ and $1.69x10^6$ PIB's, with the tested five IGR's, respectively. It was observed that Flufenoxuron and Triflumuron mixtures slightly prolongated larval duration compared either untreated control or IGR's alone treatments. The highest rate of decrease in the pupation percentage (47%) was recorded in case of Triflumuron mixed with *Spli*MNPV followed by Flufenoxuron and Chlrofluazuron mixes. On the other hand there is no significance difference in pupal weight for all treatments. Changes Adult longevity was increased for all treatments compared to untreated control. Addition Flufenxuron (LC_{10}) to *Spli*MNPV (LC_{50}) showed histopathological effects to mid gut *e.g*. loss of the compact appearance of the muscularis layer, vaculation and exfoliation of the columnar cells.

Keywords: Insect growth regulators (IGR's), Nucleopolyhedrovirus (NPV), *Spodoptera littoralis*, biological and histological aspects.

INTRODUCTION

The extensive use of insecticides to control *Spodoptera littoralis* (Boisd.) larvae has led to its resistance to various classes of insecticides (Tabashink *et al.,* 1987), residual toxicity and environmental pollution (Frank *et al*., 1990) and negative effects on non-target organisms (Franz, 1974). Numerous studies have been undertaken for unconventional control agents owing to the hazards of conventional pesticides. Among such agents are the baculoviruses. Several efforts have been made to enhance the baculoviruses efficiency by increasing insect host susceptibility to virus by using certain additives such as Fluorescent brighteners (El-Salamouny, 2004). Flufenoxuron (IGR) promoted infection of the silkworm *Bombyx mori* 5th instar larvae by *B. mori* nucleopolyhedrovirus (*Bm*NPV) which could be due to interference with chitin synthesis of peritrophic membrane (Arakawa, 2002). Therefore, the present study is undertaken to further investigate the combined effect of the mixture of IGR and NPV. Certain IGR's were evaluated as additives to increase the susceptibility of *S. littoralis* larvae to its homologous nucleopolyhedrovirus (*Spli*MNPV).

MATERIALS AND METHODS

Materials:-

Used compound: I-Nucleopolyhedrovirus (*Spli*MNPV): The Egyptian isolate of *Spodoptera littoralis* multiple embedded nucleopolyhedrovirus (*Spli*MNPV) was used (Abul Nasr, 1956). The different concentrations of polyhedra inclusion bodies (PIB's) were prepared in distilled water and number of PIB's was determined by haemocytometer.

II- Insect growth regulators (IGR's), (Chitin synthesis inhibitors): Five insect growth regulators were evaluated (Chlorfluazuron (IKI-7899, 10% EC), Flufenoxuron (10% DC), Hexaflumuron (Consult, 10% EC), Teflubenzuron (15% EC), and Triflumuron (48% SC) and their concentrations were prepared in distilled water.

Methods:-

1- Bioassay and follow up:

The cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) was maintained at the "Insect Virology Unit", Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University, under laboratory conditions of 25±2°C and 60±5% RH. The standard test insect used in the bioassay of all experiments was three to four days old 2^{nd} instar larvae. Newly moulted 2^{nd} instar larvae were treated with a mixture of the LC_{10} of the tested (IGR's) mixed with the LC_{50} of *Spli*MNPV. Treated larvae were examined daily to determine the post treatment effects on those insects survived the treatments, (*e.g.*, the larval duration, pupation %, pupal weight and adult emergence %). These parameters were compared with the untreated control larvae.

2- Histopathological study:

The Histopathological study was undertaken on the mid gut of late 6^{th} larval instars treatments as 2^{nd} instars with the mixture of IGR + *Spli*MNPV. The tested tissues were fixed in aqueous Bouin's solution for 24 hr. The normal paraffin wax embedding procedure was followed. The sections were cut 6µ thick and stained with heamatoxylin and eosin for microscopic examination. Control sections of non-treated larvae were also carried out.

3-Statistical analysis:

Statistical analysis (ANOVA) of the obtained data was performed using COSTAT program, which runs under WIN. Also, the difference between means was conducted by using Duncan's multiple range test (Duncan, 1955). Mortality-concentration response was estimated according to Finney (1971).

RESULTS AND DISCUSSION

Effect of IGR's at LC_{10} concentration, mixed with *Spli*MNPV at LC_{50} value.

The results presented in table 1 indicate that mixing SpliMNPV with IGR reduced the LC_{50} of SpliMNPV from $1x10^7$ BIP/ml to $4.3x10^6$, $9.9x10^4$, $4.9x10^4$, $3.1x10^5$, $1.69x10^6$,when mixed with Chlorfluazuron, Flufenoxuron, Triflumuron, Hexaflumuron, Teflubenzron, respectively.

The use of IGR_s is based on its effect on the chitin synthesis process. The obtained result that the insect growth regulator "Flufenoxuron" proved to be a synergistic additive to viral pesticides of *Spodoptera littoralis* is similar to that found in case of the silkworm, *Bombyx mori* by Arakawa (2002). However, the rate of enhance-ment of 204.08 fold with IGR additive is much less compared to 2000 fold in case of the silkworm. This could be attributed to differences among insects in the rate

of interference with chitin synthesis of peritrophic membrane (PM). This explanation agrees with the theory of enhancement of baculovirus by protease in the enhancing protein as indicated by Lepore *et al*. (1996). Increasing the rate of mortality which is reflected by decreasing the LC_{50} value of *Spli*MNPV in present results could be due to facilitating the virus invasion through the midgut epithelial cells by IGR's.

Table 1: Viral mortality among *Spodoptera littoralis* 2nd instar larvae treated with LC_{10} of IGR's mixed with different concentrations of *Spli*MNPV.

Tested IGR additive at LC_{10} concentration	Larval mortality % at the indicated concentration of (*Spli*MNPV) mixed with IGR's additive at LC_{10}				Virus LC_{50} in the mixture	Fold
	10^7	10^6	10^5	$4.3x10^4$		
Chlrofluazuron	57.1 (49)♦	41.6 (48)	6.00 (50)	6.25 (48)	$4.30 x10^6$	2.3
Flufenoxuron	72.0 (50)	48.9 (49)	16.0 (50)	2.00 (50)	$9.90 x10^4$	101
Triflumuron	75.57 (49)	64.0 (50)	62.0 (50)	36.0 (50)	$4.90 x10^4$	204.08
Hexaflumron	84.0 (50)	78.0 (50)	20.0 (50)	18.0 (50)	$3.10 x10^5$	32.25
Teflubenzuron	68.0 (50)	46.9 (49)	16.0 (50)	18.0 (50)	$1.69 x10^6$	6.25
None	51.1 (47)	29.7 (49)	18.3 (50)	4.40 (49)	$1.07 x10^7$	-

* Mortality is due to virus infection, ♦Total tested larvae.

2. Effect of either single or combined treatments of tested IGR's and *Spli*MNPV (PIB's), on some biological aspects of the host.

The mean larval duration in Table (2) recorded highest value for both Flufenoxuron, Triflumuron alone (27.3 ± 0.17 and 27 ± 0.29 days) compared to (25 ± 0.58 and 25.5± 0.16) days for the mixture. The virus alone treatment recorded 25.5 ± 0.12 days compared to the untreated control (24.5± 0.29 days). The larval weight demonstrated in Table (2) recorded insignificant differences between treatments and the untreated control.

Table 2: Rate of pupation, adult emergence and adult longevity of *S. littoralis* treatment as 2nd instar larvae with either insect growth regulators; *Spli*MNPV or their mixture.

Tested treatment	Larval duration days)	Pupal weight (gm)	Pupation %	Adult emergence %
Control	$24.5^c \pm 0.29$	0.33 ± 0.02	100	100
Virus(10^7)alone	$25.5^b \pm 0.12$	0.30 ± 0.03	100	100
Teflubenzuron alone	$25.5^b \pm 0.23$	0.33 ± 0.01	100	87
Teflubenzuron+*Spli*MNPV	$25^{bc} \pm 0.06$	0.34 ± 0.02	90	97
Flufenoxuron alone	$27.3^a \pm 0.17$	0.29 ± 0.02	100	73
Flufenoxuron+ *Spli*MNPV	$25.0^{bc} \pm 0.58$	0.33 ± 0.02	80	88
Triflumuron alone	$27.0^a \pm 0.29$	0.34 ± 0.01	100	47
Triflumuron+ *Spli*MNPV	$25.5^b \pm 0.16$	0.35 ± 0.03	47	100
Chlrofluazuron alone	$25.5^b \pm 0.34$	0.30 ± 0.01	100	100
Chlrofluazuron +*Spli*MNPV	$25.3^{bc} \pm 0.05$	0.33 ± 0.01	80	100
F value	10.421^{***}	1.064^{ns}		
L.S.D.	0.801	-		

Means with the same letter are not significantly different ($p<0.05$).

Obtained results agree with Abd-El Wahed *et al.,* (2010) as *Spli*MNPV reduced larval duration as well as adult emergence of *S. littoralis* . Results about the reduction in pupation due to viral infection go in line with those obtained by Dutton *et al.* (2003). Also, the data in Table (2) demonstrate that the pupal weight recorded

insignificant result for either *Spli*MNPV alone, IGR's alone or the mixture of them .The data presented in Table (2) demonstrate that, the highest reduction in the pupation percentage (47%) was recorded in case of Triflumuron mixed with *Spli*MNPV followed by Flufenoxuron & Chlrofluazuron (LC_{10}) mixed with *Spli*MNPV (LC_{50}) (80%) compared to 100% in case of both the untreated control and IGR's alone. The adult emergence (Table 3) was remarkably reduced in case of Triflumuron alone (47%) followed by Flufenoxuron alone (73%), Teflubenzuron alone (87%), Flufenoxuron + *Spli*MNPV (88%) and Teflubenzuron+ *Spli*MNPV (97%),while it recorded 100% for the other treatments.

4. Histpathological effect on mid gut of treated larvae:

The histological structure of mid gut in normal larvae (fig.1) is well documented (Chapman, 1988). The light microscope examination of *S. littoralis* treated with LC_{50} *Spli*MNPV alone in (Fig.2) Shows vacuolization of the columnar cells. The peritrophic membrane was considerably deteriorated. On the other hand, the larvae treated with the IGR Flufenxuron alone (Fig 3) Show exfoliation and vacuolization of the midgut epithelium. The peritrophic membrane was completely disrupted. However, treatment with the mixture *Spli*MNPV (LC_{50}) + Flufenxuron (LC_{10}) (Fig. 4) Shows a loss of the compact appearance of the muscular layer, vacuolation and exfoliation of the columnar cells. Many of the histological alterations reported in the present study for the midgut of *S. littoralis* larvae treated with IGR are similar to those reported by Thabit *et al*. (2010). Also, Federici (1993) found that the ingestion of toxicant by the insects releases a toxic peptide which binds to sites on the microvillar membrane of the mid gut causing cytolysis, which leads to paralysis and subsequently death of the insect.

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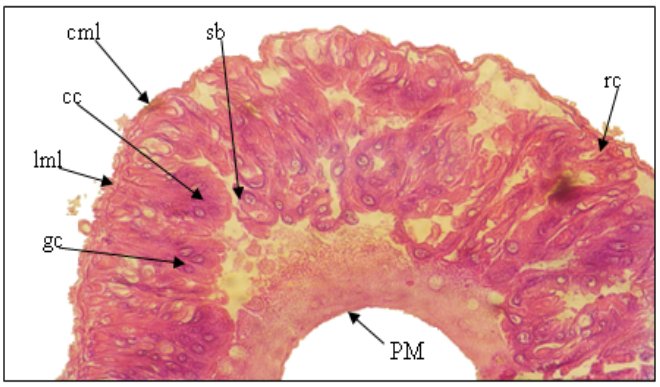

Fig. 1: Photomicrograph of longitudinal section in the mid gut of untreated late 6th instar larvae of *S. littoralis* (X400). cc: Columnar cell. pm: Peritrophic membrane. cml: Circular muscle layer. rc: Regenerative cell. gc: Goblet cell. sb: Striated boarder iml: Longitudinal muscle layer.

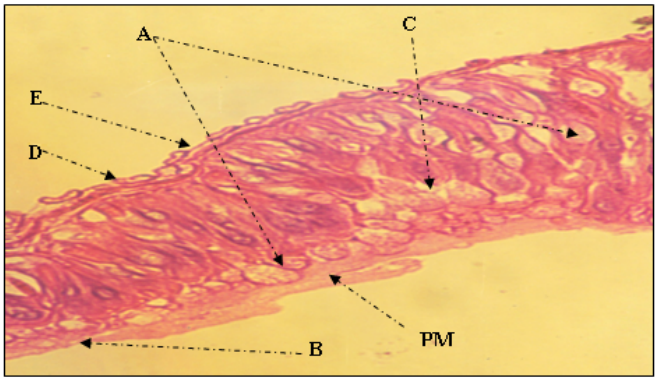

Fig. 2: Photomicrograph of longitudinal section in the mid gut of late 6th larval instar of *S. littoralis* treated as 2nd instar larvae with LC_{50} of *Spli*MNPV(X400). A: Vaculization of columnar cells. B: Shrinking of the peritrophic membrane. D: longtuidinal muscle layer L.M.L. C: Loss of striated border of epithelial cell. E: circular muscle layer C.M.L.

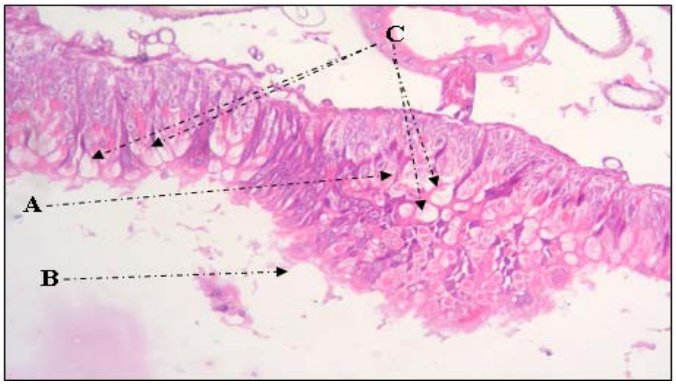

Fig. 3: Photomicrograph of longitudinal section in the mid gut of late 6th larval instar of *S. littoralis* treated with LC_{50} of Flufenoxuron as 2nd instar larvae (X400). A: The colomnar cell lost its compact appearance. B: Completely disrupted peritrophic membrane. C: Vaculization of midgut epithelial.

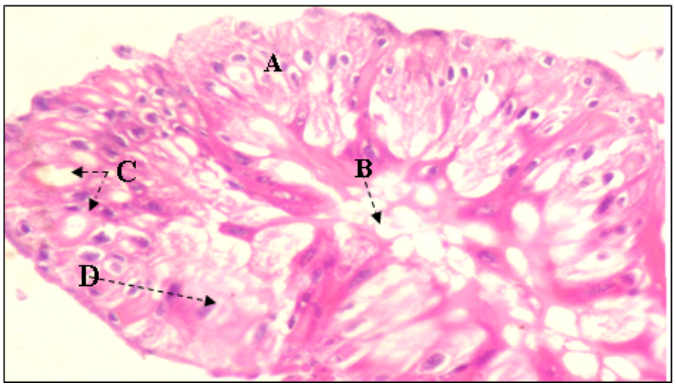

Fig. 4: Photomicrograph of longitudinal section in the mid gut of late 6th larval instar of *S. littoralis* treated as 2nd instar larvae with the mixture (LC_{50} of NPV and LC_{10} of IGR) (X400). A: Loss of the compact appearance of muscular layer. B: Lumen with punched epithelial cells. C: Vaculation. D: Exoflation the culamar epithili layer.

ARABIC SUMMARY

تأثير منظمات النمو الحشريه مخلوطا مع المعامله بالفيروس علي بعض الجوانب البيولوجيه والهستولوجيه لدودة ورق القطن.

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تم اختبار خمسه من منظمات النمو الحشريه بهدف زيادة حساسيه دودة ورق القطن اتجاه nucleopolyhedrovirus وذلك من خلال الفيروس بمفرده او مخلوطا بمنظمات النمو الحشريه عند تركيز LC_{10} اتجاه العمر اليرقي الثاني لهذه الأفه ادي المعامله بخلط الفيروس ومنظمات النمو الحشريه إلي نقص $1.69x10^6$,$4.3x10^6$, $9.9x10^4$, $4.9x10^4$, $3.1x10^5$ الي $1x10^7$التركيز المميت للنصف للفيروس من Chlorfluazuron, Flufenoxuron, Triflumuron, Hexaflumuron و PIB'sلكل علي التوالي . كما ادت المعامله بالمخلوط إلي نقص في كلا من نسبه التعذير وفتره طول Teflubenzuron عمر اليرقات . واثبت النتائج عدم وجود فروق معنويه في وزن العذارى . كما ادت المعامله إلي وجود تغيرات هستولوجيه في المعي الأوسط للحشرة