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ENHANCEMENT OF *Spodoptera littoralis* (Boisd.) SUSCEPTIBILITY TO NUCLEOPOLYHEDROVIRUS (*Spli* MNPV) COMBINED WITH INSECT GROWTH REGULATORS (IGR's)

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ABSTRACT

Five insect growth regulators (IGR's) were tested to increase the susceptibility of the cotton leaf worm, *Spodoptera littoralis* (Boisd.) **(**Lepidoptera: Noctuidae) to its homologous nucleopolyhedrovirus **(***Spli*MNPV). *Spodoptera littoralis* MNPV was tested alone or in combination with IGR's at LC_{10} level against second instar larvae of the pest. An increased infection rate was detected in the mixture containing *Spli*MNPV+IGRs in the case of using Chlorfluazuron, Flufenoxuron, Triflumuron, Hexaflumuron or Teflubenzuron at 10%. The LC_{50} value for the virus alone treatment was $1x10^7$ PIB's reduced to $4.3x10^6$, $9.9x10^4$, $4.9x10^4$, $3.1x10^5$ and $1.69x10^6$ PIB's, with the five IGR's, respectively.

***Key words**: insect growth regulators, IGR's, nucleopolyhedrovirus, NPV, Spodoptera littoralis.*

1. INTRODUCTION

Baculoviruses are promising biocontrol agents in plant protection due to their host specificity, thus safety to the environment. Several studies showed an increase of host susceptibility to virus by using certain additives such as fluorescent brighteners (Shapiro and Dougherty, 1994; and El Salamouny, 2004). The mechanism of increase in the susceptibility by brighteners was confirmed as causing disruption in the midgut defense system in insects (the peritrophic membrane) which lines the midgut (Wang and Granados, 2000). Lepidopteran insects are most susceptible to IGR's that cause molting disturbances (Smagghe *et al*., 2001; Smagghe and Degheele, 1992 and 1994) and the difference in susceptibility depends on the larval age.

Based on the work published by Arakawa (2002), 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 the peritrophic membrane.

In this study, five insect growth regulators (IGRs) were tested to increase the susceptibility of *Spodoptera littoralis* to its homologous nucleopolyhedrovirus (*Spli*MNPV) in order to select the best enhancement effect.

2. MATERIALS AND METHODS

2.1. Test insect

The cotton leaf worm, *Spodoptera littoralis* larvae were raised on the semi-synthetic diet of Shorey and Hale (1965). The test insect in all experiments was the 2nd instar larvae.

2.2.Virus

The Egyptian isolate of *Spodoptera littoralis* multiple embedded nucleopolyhedrovirus (*Spli* MNPV) was used. Virus suspension was prepared in distilled water. Serial viral concentrations ranged from $4.3x10^4$ to 10^7 PIBs/ml were used to determine the lethal concentrations (LC) values.

2.3. Insect growth regulators

Five insect growth regulators (IGR's), were used as synergistic additives to *Spli*MNPV inocula. The tested additives were Chlorfluazuron (IKI-7899, 10% EC, Atabron™), Flufenoxuron (10% EC, Cascade™), Triflumuron (Systeine, SIR 8514™), Hexaflumuron (Consult™) and Teflubenzuron (15 % EC, Nomoult™). All tested IGR's were freshly prepared in distilled water before each test. The concentrations were adjusted as part per million (ppm). To determine the LC values, serial concentrations ranged from 0.0078 to 8 ppm were used.

2.4. Virus purification

To get enough virus inocula for the experiments,

propagation of *Spli*MNPV in the third instar *S. littoralis* larvae was done. The obtained dead larvae were homogenized in distilled water, then the suspension was filtered and centrifuged at 600 rpm for 10 minutes. The collected supernatant was centrifuged at 4000 rpm for 15 minutes and PIB's particles were separated as a supernatent. The final stock suspension of approximately 2.8 $\times 10^9$ PIB's/ml, was stored frozen until usage.

2.5.Bioassay

The diet bioassay technique (Huber, 1986) was used for the second instar test larvae. Fifty ml of diet (synthetic diet without the formaldehyde) were poured in a special bioassay plate measuring 14 x 7 x 2 cm, contains fifty cells. A standard volume suspension of 2 ml per plate was pipetted evenly on the surface of 50 ml of the diet, and left to air dry. One second instar larva was placed into each cell, and each plate was covered with two layers of tissue paper and a 14.5 x 7.5 cm glass cover fixed with rubber bands. Three replicates were tested in each treatment and all incubated at 25 ± 2 °Ċ and 60-70° RH for 14 days during which mortality was daily recorded.

2.6.Statistical analysis

Analysis of variance (ANOVA) of the obtained data was performed by using COSTAT program, which runs under Microsoft Windows. Also, the means were compared by using Duncan's multiple range test (Duncan, 1955) Mortality-concentration response was estimated according to Finney (1971).

3. RESULTS

Data presented in Table (1) and illustrated in Fig. (1), show the susceptibility of the 2nd instar larvae of *S. littoralis* towards the tested IGRs. Based on the obtained LC_{50} values, the toxicity of the IGR's ranked in the following descending order: Hexaflumuron, Chlorfluazuron, Flufenoxuron, Teflubenzuron and Triflumuron.

Table (1): LC_{10} and LC_{50} of different insect growth regulators (IGRs) and the *Spli*MNPV, each tested separately.

Tested material	LC_{10}	LC_{50}	Toxicity line Slope ± S.E.
Hexaflumuron (ppm)	0.0017	0.0039	3.638± 0.915
Chlorfluazuron (ppm)	0.0636	0.1179	4.22± 0.088
Flufenoxuron (ppm)	0.0571	0.140	3.28±0.180
Teflubenzuron (ppm)	0.0877	0.171	4.425±0.915
Triflumurone (ppm)	1.431	3.6002	3.251±0.212
*Spli*MNPV(PIB's/ml)	$4.5x10^4$	$1.07x10^7$	0.82±0.2

The results presented in Table (2) indicate that mixing *Spli*MNPV with IGR's reduced the LC_{50} of *Spli*MNPV from $1x10^7$ PIB's/ml to $4.3x10^6$, $9.9x10^4$, $4.9x10^4$, $3.1x10^5$ or $1.69x10^6$ PIB's/ml when mixed with Chlorfluazuron, Flufenoxuron, Triflumuron, Hexaflumuron or Teflubenzuron, respectively.

Initial tests showed no, enhancement effect when *Spli* MNPV and IGR's were mixed each at the level of LC_{10}. When the five tested IGR compounds were mixed at the LC_{10} level with the virus at LC_{50} level ($1x10^7$ PIB's/ml) enhancement effect on larval mortality could be detected. The average of mortality in the virus alone treatment (25.33%) increased to 52.02, 75.33, 57.14, 70.94 and 86.23 %,by adding the IGR compounds

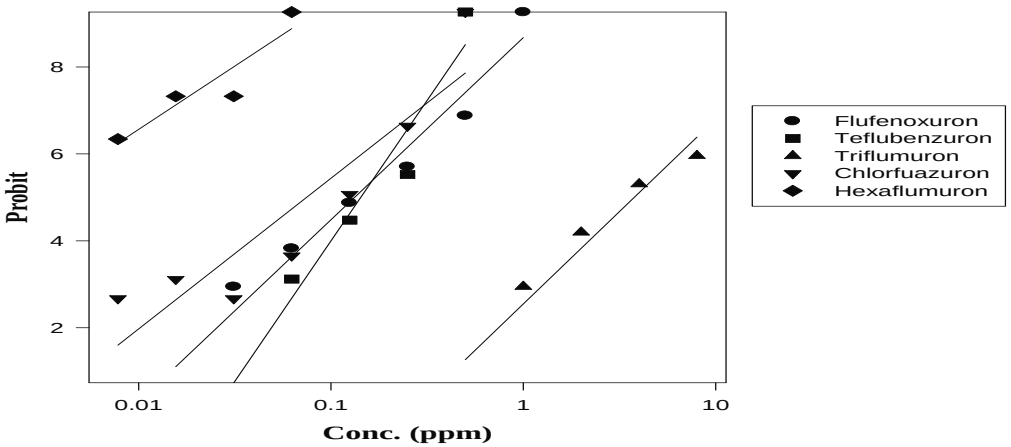

Fig. (1): Toxicity lines of insect growth regulators on *Spodoptera littoralis* **second instar larvae.**

Table (2): Larval mortality (%) at the different *Spli*MNPV concentrations mixed with the insect growth regulators (IGRs) at LC_{10} .

IGRs	Larval mortalities (%) at LC_{10} of IGRs mixed with the indicated *Spli*MNPV concentrations				Resulted LC_{50} value PIB's
	10^7	10^6	10^5	$4.3x10^4$	
Chlorfluazuron	57.1	41.6	6	6.25	4.30×10^6
Flufenoxuron	72	48.9	16	2	9.90×10^4
Triflumuron	75.5	64	62	36	4.90×10^4
Hexaflumron	84	78	20	18	3.10×10^5
Teflubenzron	68	46.9	16	18	1.69×10^6
Virus alone	51.1	29.7	18.3	4.4	1.00×10^7

Chlorfluazuron, Triflumurone, Hexaflumuron, Teflubenzuron or Flufenoxuron, respectively (Table, 3 and Fig., 2). A decrease in the lethal time needed (LT_{50} value) for viral mortality in virus alone treatment varied from 8.8 days to 8.54, 8.278, 7.242, 8.41 or 8.21 days was achieved by adding the above-mentioned IGR the products, respectively. However, in the case of total mortality, a significant decrease in the LT_{50} value was detected (Table, 3 and Fig., 3)

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Table (3): Viral mortality (%) and LT_{50} value (Days) among *Spodoptera littoralis* test larvae resulting from mixing the five IGR's at LC_{10} with *Spli*MNPV at LC_{50} level ($1x10^7$ PIBs/ml).

Treatment	Mortality (%)	LT_{50} (Days)
NPV alone ($1x10^7$ PIBs/ml)	**25.33 f**	**8.8 a**
NPV+Chlorfluazuron	**52.02 e**	**8.493 a**
NPV+ Triflumurone	**75.33 b**	**7.187 bc**
NPV+Hexaflumuron	**57.14 d**	**7.084 bc**
NPV+Teflubenzuron	**70.94 c**	**7.865 ab**
NPV+Flufenoxuron	**86.23 a**	**6.`121 c**
F value	**474.49*** **	**8.780** **
L.S.D	**3.039**	**1.03272**

Means with the same letter are not significantly different ($p<0.05$).
all dead larvae were individually smear tested to confirm *Spli*MNPV causal agent

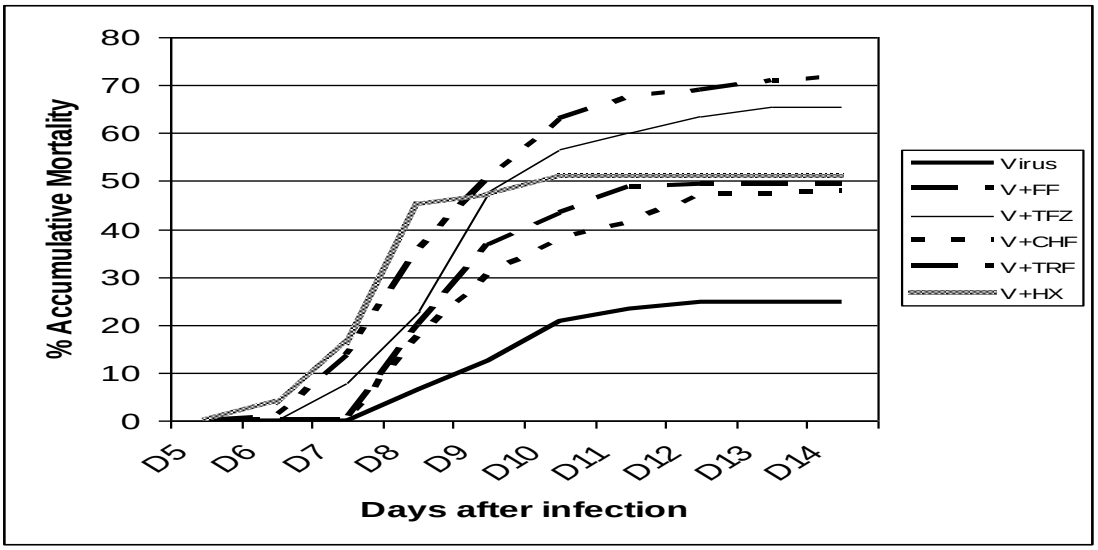

Fig. (2). Accumulated viral mortality of *Spodoptera littoralis* larvae treated with *Spli*MNPV at LC_{50} (10^7 PIBs/ml) alone or mixed with insect growth regulators. V= **Virus** FF= **Flufenoxuron,** TFZ=**Teflubenzuron,** CHF= **Chlorfluazuron,** TRF= **Triflumuron** HX= **Hexaflumron**.

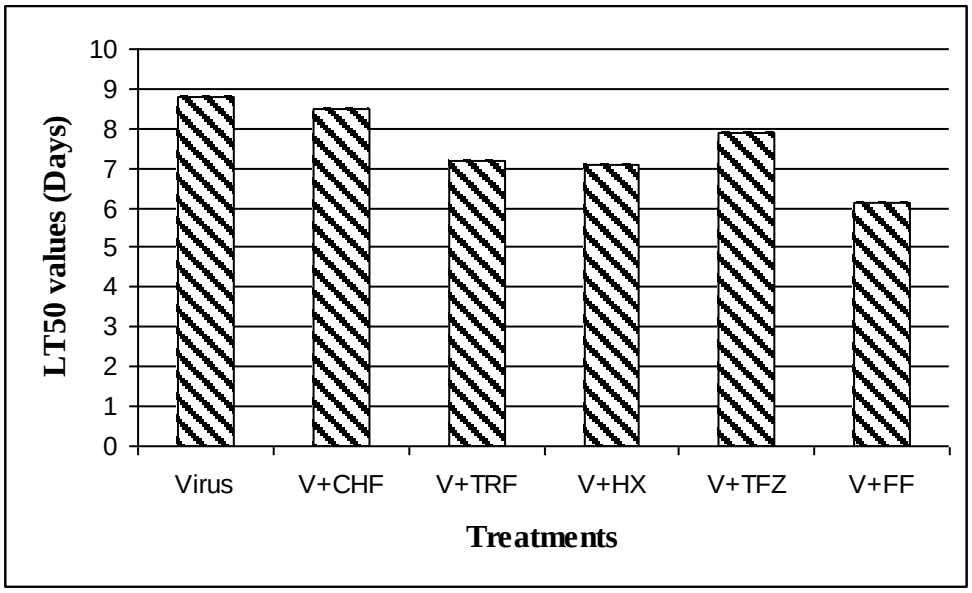

Fig. (3): LT_{50} values (in days) of viral mortality at 10^7 PIB's/ml *Spli*MNPV either in virus alone treatment ,or with IGRs at LC_{10}:

V= **Virus**FF= **Flufenoxuron,** TFZ=**Teflubenzron,** CHF=**Chlorfluazuron,** TRF= **Triflumuron,** HX= **Hexaflumron**

4. DISCUSSION

In the present investigation, the mortality response of the larvae caused by the tested IGRs is similar to that found by Abdel Aal (2003) and Ali (2005).

Previous studies showd the mechanism of increasing the susceptibility to NPV in the absence of peritrophic membrane (PM) in tortricid insects (El Salamouny, 2009). The enhancement effect can be explained by damaging the chitin or protein in PM (Shapiro *et al*., 1987 and Lepore *et al*., 1996).

The highest rate of enhancement obtained in the case of flufenoxuron was less than that found by Arakawa (2002). The obtained increase in mortality rate could be explained by the role played by IGR in facilitating the virus infection invasion of the midgut.

The results support the possibility of using sublethal IGRs to enhance baculoviruses activity in an Integrated Pest Management Program.

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زيادة حساسية دودة ورق القطن للإصابة بفيروس البوليهيدروسز النووى(*Spli* MNPV) بالخلط مع منظمات النمو الحشرية

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ملخص

تم اختبار خمسة مركبات من منظمات النمو الحشرية (IGRs) لزيادة حساسية دودة ورق القطن *Spodoptera littoralis* ممثلة بالعمر اليرقي الثانى تجاه فيروس النيكلوبوليهيدروسز النووي المعزول منها. تم اختبار الفيروس بمفرده أو خليطا مع تركيز مستوي (LC_{10}) لمنظمات النمو الحشرية. حدث زيادة في معدل العدوى فى الخليط الذي احتوى علي الفيروس + منظمات النمو حيث انخفض التركيز نصف المميت للفيروس (LC_{50}) من 1×10^{7} بوليهدرا إلى 4.3×10^{6}، 9.9×10^{4}، 4.9×10^{4}، 3.1×10^{5}و 1.69×10^{6} بوليهدرا في حالة المعاملة بالخليط المحتوي علي الكلوروفلوزورون، الفلوفينوكسرون، الترافلومورون، الهكسافلومورون، التيفلوبنزرون، على التوالي. وتقترح الدراسة استخدام منظمات النمو الحشرية في تحسين معاملة الفيروس ضد يرقات دودة القطن.

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