ENHANCEMENT OF Spodoptera littoralis (Boisd.) SUSCEPTIBILITY TO NUCLEOPOLYHEDOVIRUS (SpLMNPV) 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 (SpLMNPV). Spodoptera littoralis MNPV was tested alone or in combination with IGR's at LC₅₀ level against second instar larvae of the pest. An increased infection rate was detected in the mixture containing SpLMNPV+IGRs in the case of using Chlorfluazuron, Flufenoxuron, Triflumuron, Hexaflumuron or Teflubenzuron at 10%. The LC₅₀ value for the virus alone treatment was 1x10⁶ PIBs reduced to 4.3x10⁵, 9.9x10⁴, 4.9x10⁴, 3.1x10⁵ and 1.69x10⁵ PIBs, 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 (BmNPV) 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 (SpLMNPV) 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 (SpLMNPV) was used. Virus suspension was prepared in distilled water. Serial viral concentrations ranged from 4.3x10⁴ to 10⁵ 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 SpLMNPV 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, Nomoul™). 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 SpilMNPV in the third instar S. littoralis larva 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 supernatant. The final stock suspension of approximately 2.8 x10^8 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 °C 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 LC50 values, the toxicity of the IGR’s ranked in the following descending order: Hexaflumuron, Chlorfluazuron, Flufenoxuron, Teflubenzuron and Triflumuron.

Table (1): LC10 and LC50 of different insect growth regulators (IGRs) and the SpilMNPV, each tested separately.

<table>
<thead>
<tr>
<th>Tested material</th>
<th>LC10</th>
<th>LC50</th>
<th>Toxicity line Slope ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexaflumuron (ppm)</td>
<td>0.0017</td>
<td>0.0039</td>
<td>3.638± 0.915</td>
</tr>
<tr>
<td>Chlorfluazuron (ppm)</td>
<td>0.0636</td>
<td>0.1179</td>
<td>4.22± 0.088</td>
</tr>
<tr>
<td>Flufenoxuron (ppm)</td>
<td>0.0571</td>
<td>0.140</td>
<td>3.28±0.180</td>
</tr>
<tr>
<td>Teflubenzuron (ppm)</td>
<td>0.0877</td>
<td>0.171</td>
<td>4.42±0.195</td>
</tr>
<tr>
<td>Triflumuron (ppm)</td>
<td>1.431</td>
<td>3.6002</td>
<td>3.251±0.212</td>
</tr>
<tr>
<td>SpilMNPV (PIB’s/ml)</td>
<td>4.5x10^6</td>
<td>1.07x10^7</td>
<td>0.82±0.2</td>
</tr>
</tbody>
</table>

The results presented in Table (2) indicate that mixing SpilMNPV with IGR's reduced the LC50 of SpilMNPV from 1x10^7 PIB’s/ml to 4.3x10^6, 9.9x10^6, 4.9x10^5, 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 Spil MNPV and IGR’s were mixed each at the level of LC10. When the five tested IGR compounds were mixed at the LC10 level with the virus at LC50 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.](image-url)
Enhancement of *Spodoptera littoralis* susceptibility to 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Fig. (3): LT<sub>50</sub> values (in days) of viral mortality at 10<sup>7</sup> PIB’s/ml Spli/MNPV either in virus alone treatment ,or with IGRs at LC<sub>10</sub>: 
V= Virus FF= Flufenoxuron, TFZ=Teflubenzon, 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 showed 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.

5. REFERENCES


Enhancement of Spodoptera littoralis susceptibility to...


Shapiro M. and Dougherty E. M. (1994). Effect of neem extract upon the gypsy moth (Lepidoptera: Lymantriidae) and its nuclear polyhedrosis virus. J. Econ. Entomol. 87:356-360.


