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Efficacy of the Pyrethroid, S-fenvalerate and Nucleopolyhedrovirus Combinations against the Cotton Leaf Worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae)

Magda Khattab[*] and Hanan F.Abdel-Hafez[**]

[*]Dep. of Cutworms and Mole Cricket, PPRI, Agric. Res. Center, Dokki, Giza, Egypt

[**] Dep. of Cotton Pesticides Research, PPRI, Agric. Res. Center, Dokki, Giza, Egypt

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ABSTRACT

Laboratory tests were conducted to evaluate the efficacy of sequential treatments of the cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lep.: Noctuidae) nucleopolyhedrovirus (*Spli*NPV) and a synthetic pyrethroid, S-fenvalerate (Sumi-alpha) on the larvae of *S. littoralis*. The results indicated that all the tested concentrations of *Spli*NPV and pyrethroid produced potontiation effect. The total mortality increased when S-fenvalerate was applied to larvae 5 to 7 days previously infected with the tested virus compared to the virus or the insecticide when used alone. The most promising potentiation occurred when LC_{25} of pyrethroid was applied 7 days after *Spli*NPV infection.

Key Words: *Spodoptera littoralis*, Nucleopolyhedrovirus, Pyrethroid, Joint action.

INTRODUCTION

The cotton leaf worm, *Spodoptera littoralis* is considered one of the most serious and destructive lepidopterous pests, not only to cotton plants but also to other field crops and vegetables. Control of this pest is primarily dependent upon foliar application of certain broad spectrum synthetic organic, chemical insecticides against larval stage (Croft 1990).

However, continuous use of these insecticides created serious problems such as environment contamination and toxicity to non-target organisms. These problems increase by the increasing costs of discovery, development, and registration of new insecticides. In addition, an increasing number of insect species have become resistant to most of the available insecticides.

Baculoviruses are pathogens that attack insects and other arthropods and are mostly species-specific pathogens. They have been shown to have no negative impacts on plants, mammals, birds, or even on non-target insects. Different viruses were isolated from *S. littoralis* in Egypt; *S. littoralis* NPV (*Spli*NPV) (Abul-Nasr, 1956), was known by its acute pathogenicity to the 1^{st} instar larvae, a granulovirus *S. littoralis* GV (*Spli*GV) (Abol-Ela *et al.*, 1994), which acted strongly against late instars (Narayanan, 1985), and densovirus (Parvoviridae) and picornavirus (Picornaviridae) were isolated from natural field population of *S. littoralis* (Fédiére *et al.*, 1999).

In order to increase larval mortality, and alternatively decrease the quantities of conventional insecticides used, the idea of combining viruses and insecticides has been proposed and reviewed (Benz 1971, Jaques and Morris 1981). Several investigators have documented a positive interaction between classical insecticides and microorganisms (Ignoffo and Montoya 1966).

The present study aimed to evaluate the role of pyrethroid, S-fenvalerate in the enhancement of *Spli*NPV activity against *S.littoralis* larvae. In other words, the efficacy and sequential treatments of nucleopolyhedrovirus *Spli*NPV and the synthetic pyrethorid were tested against *S. littoralis* larvae in order to decrease the quantities of insecticidal applications to minimize the environmental hazards and costs.

MATERIALS AND METHODS

All laboratory procedures were performed at 25 ± 2°C and 65 ± 5% R.H. To determine the degree of potentiation provided by different concentrations of the pyrethroid, S-fenvalerate and *Spli*NPV, a laboratory bioassay based on the technique described by Cisneros *et al.*, (2002) was performed.

Insect: *S. littoralis* was reared on semi synthetic diet described by Shorey and Hale (1965) under laboratory conditions at 25 ± 2°C and 65 ± 5% R.H.

Pyrethroid insecticide: S -Fenvalerate (Sumi-alpha 5% EC), (S) - cyano-3-phenoxybenzyl (S)-2-(4-chlorphenyl)-3 methylbutyrate, was used at the application rate of 600cm^3/feddan.

Virus: The multiple embedded polyhedrovirus isolate *S. littoralis* NPV (*Spli*NPV) was used in the present study. The source of the virus was the Oxford isolate - originally obtained from Egypt.

Serial dilutions of the stock virus suspension were made in Tris buffer pH 8, in addition to 2.5% Teepol as a wetting agent.

Bioassay: Laboratory bioassay tests were performed using diet-surface contamination technique described by Cisneros *et al.* (2002). A special bioassay plates (L1CEFA, Bad-Salzuflen, Germany) were used. The plate, measuring 14x7x2 cm, contains 50 cells, was filled with artificial diet (50 ml) to form a 5 mm thick layer. When solidified, a 2.0 ml volume of virus suspension was spread over the diet surface (9313 mm^2) using fine-pipette. Into each cell a neonate larva of *S. littoralis* was placed. The plates were covered with tissue paper and 14x7 cm glass cover was fixed with rubber bands. All treatments were incubated at 25±2°C. Mortality due to virus infection was recorded daily and up to 16 days (until death of larvae or pupation). The larval mortality in untreated larvae (control) was determined. All virus treatments included 2.5% wetter- sticker (Triton X-100) to reduce clumping of occlusion bodies (OB's).

Mean infection concentrations of virus: To determine the LC_{25} and LC_{50} of *Spli*NPV, four concentrations of virus were prepared suspended in Tris buffer pH 8. Fifty neonate larvae taken from the laboratory culture were used for each concentration. A similar number of control larvae were placed in a plate containing only 2.5% Triton X-100 and diet.

Median lethal concentrations of pyrethroid: The toxicity lines (median lethal concentrations, LC_{25} and LC_{50} values) of the tested insecticide compound were determined as follows: a series of six concentrations of S-fenvalerate was prepared by diluting the formulated compound in distilled water. The larvae of *S. littoralis* were placed upon flatland diet surface in cups (52.78 cm^2) sprayed with each concentration then left for one hour to dry. Fifty 2nd and 3rd (5 day-old and 7 day-old) larvae were put in each treated cup. All the treatments were replicated 3 times. Mortality percentage was recorded after 24 hours. The average percent mortality of the tested larvae was calculated and corrected using Abbott's formula (1925) if necessary. The corrected percent mortalities were statistically analyzed according to Finney (1971).

Joint action and sequential treatments of Pyrethroid, S-fenvalerate on larvae treated with *Spli*NPV: To examine the interaction between pyrethroid and *Spli*NPV newly hatched larvae of *S. littoralis* were placed upon diets treated with two concentrations, LC_{25} and LC_{50} values of *Spli*NPV alone. Then three possible sequential treatments of pyrethroid and *Spli*NPV (LC_{25} of virus and LC_{25} of pyrethroid), (LC_{50} of virus and LC_{25} of pyrethroid) and (LC_{25} of virus and LC_{50} of pyrethroid) were applied; larvae infected with virus were left for 5 and 7 days, then they were transferred to new diet-trays sprayed with 2 ml. concentrations of pyrethroid, S-fenvalerate (Sumi-alpha) causing 25% and 50% mortality (LC_{25} and LC_{50}). Fifty larvae were placed on untreated diet as control. Mortalities were calculated after 24 and 48 hrs. of insecticide treatment and compared with controls. The synergistic effect was calculated as suggested by Mansour *et al.*, (1966) for the calculation of the joint action of insecticide-virus mixtures, to differentiate the final effect of the combinations between synergism, antagonism and additive effect. A positive factor of 20 or more is considered potentiation, a negative factor of 20 or less means antagonism, while a value between (-20 and +20) is considered additive effect.

Statistical analysis: Data of bioassay results were subjected to probit analysis using the method described by (http://www.-1). The relative potencies of the treatments were calculated according to the changes in LC_{50} value.

RESULTS AND DISCUSSION

The concentration - mortality responses of *Spli*NPV against *S.littoralis* neonate larvae using the surface contamination diet technique were 6, 22, 50 and 80.95 % for the tested virus concentrations, 0.897, 8.97, 8.97x10 and $8.97x10^2$, respectively (Table 1). The LC_{25} and LC_{50} values were 1.18x10 and 8.1x10 PIB^s/mm^2 diet surface, respectively. The slope of the regression line was 0.809 (Table 2).

Also, the data demonstrated that the concentration-mortality response of pyrethroid against *S. littoralis* (5-day-old) larvae using treated diet were 24, 36, 48, 60, 64 and 80 % for the tested concentrations, 1, 2.5, 5, 10, 15 and 20 ppm., respectively (Table 1). The LC_{25} and LC_{50} values were estimated by 1.217 and 5.203 ppm, respectively. The slope of the regression line was 1.069 (Table 2).

Pyrethroid concentrations (50, 100, 200, 300 and 400 ppm.) against 7-day old *S.littoralis* caused mortalities of 16.7, 32.5, 45, 60 and 77.5%, respectively (Table 1). The LC_{25} and LC_{50} values were estimated by 78.907 and 191.787 ppm, respectively. The slope of the regression line was

Table (1): Concentration-mortality response, of *Spli*NPV and S – fenvalerate against *Spodoptera littoralis* larvae.

Treatment	Larval age	Tested concentration	% Mortality
*Spli*NPV	Neonate	0.897 PIB/mm^2	6.00
		8.97 PIB/mm^2	22.00
		8.97x10 PIB/mm^2	50.00
		8.97x10^2 PIB/mm^2	80.95
S-fenvalerate (Sumi-alpha)	5 day-old	1.00 ppm	24
		2.50 ppm	36
		5.00 ppm	48
		10.0 ppm	60
		15.0 ppm	64
		20.0 ppm	80
	7 day-old	50 ppm	16.7
		100 ppm	32.5
		200 ppm	45.0
		300 ppm	60.0
		400 ppm	77.5

1.749 (Table 2).

Interaction between pyrethroid and virus: *S. littoralis* NPV and S-fenvalerate were applied at their LC_{25} and LC_{50} values alone and in sequential treatments against *S. littoralis* larvae on diet. Estimated LC_{25} and LC_{50} values for *Spli*NPV produced 31.9, 54 % (after 5 day) and 36, 62% (after 7 day) actual mortalities, respectively, against neonate larvae. No mortality was recorded in the control treatments. Application of pyrethroid at the rate of estimated LC_{25} and LC_{50} values produced 23.8 and 47.6 % actual mortalities, respectively, against 5-day old larvae, and produced 20 and 44% actual mortalities, respectively, against 7-day old larvae.

Larval mortality was increased by sequential treatment of *Spli*NPV and S-fenvalerate at (LC_{25} of virus and LC_{25} of pyrethroid), (LC_{50} of virus and LC_{25} of pyrethroid) and (LC_{25} of virus and LC_{50} of pyrethroid) than in treatments with either agent alone. Table (3) shows the effect of applying pyrethroid to *Spli*NPV- infected larvae after 5-day (sequential treatment). Observed mortality increased to 80, 95 and 100% compared to the expected mortalities 55.7, 77.8 and 79.5% for the above three sequential, respectively. Table (3) shows the effect of applying pyrethroid to *Spli*NPV- infected larvae after 7 days (sequential treatment). The observed mortality was increased to 93.5, 100, 96.9% compared to the expected mortalities 56, 82 and 80% for the above three sequentials, respectively. The most promising potentiation occurred when pyrethroid (LC_{25} of pyrethroid and LC_{50} of *Spli*NPV) was applied 7 days after virus infection. This result is in agreement with that obtained by (Savanurmath and Mathad, 1981). They reported that one of the most promising synergisms occurred when insecticidal treatment was applied 7 days after treatment with the virus (LC_{50} of NPV and LC_{25} of fenitrothion) against the armyworm *Mythimna (Pseudaletia) separata.*

Also, the data indicated that interaction between *Spli*NPV and pyrethroid was of potentiation effect in all treatments. The observed mortality was higher than in treatments with either agent alone. The most promising synergism occurred when LC_{25} pyrethroid

Table (2): Slope of regression line, LC_{25} and LC_{50} values of tested *Spli*NPV and S–fenvalerate against *Spodoptera littoralis* larvae.

Treatments	Larval age	Time after spraying	LC_{25}	LC_{50}	Slope
*Spli*NPV	neonate	16 days	1.18x10 PIB/mm^2	8.1x10 PIB/mm^2	0. 809
S-fenvalerate (Sumi-alpha)	2nd	24 hr.	01.217 ppm	05.203 ppm	1.069
	3rd	24 hr.	78.907 ppm	191.787 ppm	1.749

Table (3): Expected and observed mortality (%) in all *Spli*NPV and S–fenvalerate combination against *Spodoptera littoralis* larvae.

S-fenvalerate (Sumi-alpha)	*Spli*NPV	Expected mortality % at		Observed mortality % at		C.F. at		Potentiation at	
		5 days*	7 days*	5 days*	7 days*	5 days*	7 days*	5 days*	7 days*
LC_{25}	LC_{25}	55.7	56	80	93.5	+ 43.6	66.96	+	+
LC_{25}	LC_{50}	77.8	82	95	100	+ 22.1	+ 21.95	+	+
LC_{50}	LC_{25}	79.5	80	100	96.9	+ 25.8	+ 21.13	+	+

*Days after virus treatment C.F. = Co- Toxicity Factor + = Potentiation

was applied 7 day after *Spli*NPV treatment. This finding is in accordance with that previously described by Savanurmath and Mathad (1981 and 1982), Mohamed *et al.* (1983 a, b) and Erling and Belloncik (1984). The most common situation providing synergism was in assays involving endosulfan applied 7 days after the virus NPV against *M. separata* (Savanurmath and Mathad, 1982). Mohamed *et al.* (1983 a) tested the commercial formulations of *Heliothis* NPV (Elcar) under laboratory conditions in combination with 16 chemical pesticides against 1st and 3rd instar larvae of *Heliothis virescens* (F.) on diet. All such tests involved simultaneous challenges and most combinations resulted in additive responses. However, several resulted in either antagonism or synergism. The combination of NPV with methoprene, fentinhydroxide, benomyl or thiabenadazole gave synergism responses when evaluated at 7 days post treatment. In second test, the same authors (1983 b) utilized 8 pesticides in combination with *Heliothis* NPV on field-gathered cotton leaves which were then fed to larvae in the laboratory. Again, several, particularly using 1st instar larvae, resulted in antagonism or synergism response.

A significant increase in total mortality occurred when synthetic pyrethroid, permethrin (Ambush), was applied to larvae of *Euxoa scandens* previously infected for a period of 4 to 7 days with virus NPV or cytoplasmic polyhedrovirus (CPV). The toxicity of permethrin increased by 1.51-fold for CPV-infected larvae after 6 days and 4.18-fold for NPV-infected larvae (Erling and Belloncik, 1984).

Chemical-baculovirus combinations provide several distinct advantages for insect pest management programs. As noted by several authors (Krieg, 1971, Benz, 1971 and Harper, 1980), these include the potential for reducing amounts of each agent used. Such reduction would mean potentially lower costs, lower environmental impacts, less damage to beneficial organisms, and reduced selection pressure leading to the development of resistance to each agent. When pests in addition to the pathogen host are present, the chemical can provide control (Harper, 1987).

McGarr and Ignoffo (1966) reported that control of *Heliothis* spp. on cotton using combinations of *H. zea* NPV and azinphosmethyl was better than the insecticide used alone. The susceptibility of *Trichoplusia ni* larvae treated with NPV sublethal doses (less than 10% mortality) to endrin, endosulfan and trichlorfon was significantly increased if such chemicals were applied 24 or 48 hr after the virus. This increase was not noted when the two agents were applied simultaneously. Their data were analyzed by comparing LD_{50} values for each chemical used alone, simultaneously, or at 24 or 48 hr post virus treatment (Girardeau and Mitchell 1968). Also chemical insecticides have been shown to be compatible with viruses. The *Trichoplusia ni* NPV or *Pieris rapae* GV with low doses of endosulfon or methomyl were as effective as full rates of chemical alone on cabbage (Jacques, 1973). Luttrell *et al.*, (1979) demonstrated simple additively between Elcar commercial formulation of (*Heliothis* NPV) and permethrin, EPN-methyl parathion, and methomyl when the chemicals were administered simultaneously or at 24 or 48 hr after the virus. The combinations of viral preparations and diflubenzuron were compatible and highly effective (95-99%) against gypsy moth *Lymantria dispar* (Mihalache and Simionescu, 1987). The mixtures of *Autographa californica* NPV or *Pieris rapae* GV with low rates of permethrin were as effective in control of *Trichoplusia ni* and *Pieris rapae* on cabbage as high rates of permethrin alone (Jacques, 1988). Also, NPV enhanced the action of endosulfon and cypermethrin when used in combination for the control of *H. armigera* under laboratory conditions and in cotton field (Rajasekhar *et al.*, 1996). Combined NPV with low concentrations of several conventional insecticides, especially those act on the insect nervous system (*e.g*, pyrethroids and ACHE inhibitors) showed a positive interaction (decrease in the median lethal time (LT_{50}) compared to either of them (Mccutchen *et al.*, 1997). Synergistic effects were detected in combinations the chitin synthesis with *Spodoptera litura* NPV (*Sl*NPV) against 5th instar larvae of *S. litura* at doses of 0.05 and 0.025 µg/insect and additive effect at doses of 0.1 and 0.2 µg. Furthermore, the time required for *Sl*NPV to kill larvae was significantly reduced by chlorfluazuran at all tested doses (Guo *et al.*, 2007).

It appears that insects under virus stress are more susceptible to the insecticides than healthy ones. Histopathological studies in *L.dispar* larvae showed that Dimilin destroyed the cuticle tissue, stimulating infection by *L.dispar* NPV (*Lydi*NPV) (Mihalache and Simionescu, 1987). Mccutchen *et al.* (1997) suggested that a simultaneous exposure of *Autographa californica* NPV and pyrethroids resulted in a synergistic interaction at the insect sodium channel.

Scanning electron microscopy (ESEM) showed that the peritrophic matrices (PM_S) of *S. litura* exposed to chlorfluazuron, were markedly disrupted.

Obvious ruptures on the outer surfaces of the PM of virions through the matrix (Guo *et al.*, 2007).

The present study of *S. littoralis* nucleopolyhedrovirus (*Spli*NPV)-Pyrethroid, S-fenvalerate, sequential treatments suggests that, the use of sublethal doses of *Spli*NPV and pyrethroid 5 to 7 days after virus treatment would were observed, which potentially facilitated the passage reduce the amount of pyrethroid and virus needed. Also, it would reduce the costs of insecticides for controlling *S. littoralis* and reduce the environmental pollution. Further studies are needed to determine their persistence in the field.

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