Bull.Fac, Agric.,Cairo Univ.,61:437-449 (2010).

EFFICACY ENHANCEMENT OF FOUR BIO-CONTROL AGENTS AGAINST *Spodoptera littoralis* (Boisd.) BY FLUORESCENT BRIGHTENER

(Received: 1.7.2010)

By

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ABSTRACT

Efficacy enhancement of four biocontrol agents; Spintor 24% SC (Spinosad), Neemix 4.5% EC (Azadirachtin), Protecto 10% WP (*Bacillus thuringiensis*) and *S. littoralis* nucleopolyhedrovirus (*Spli*NPV) against *S. littoralis* larvae using Fluorescent Brightener-28 (FB) was investigated. These biocontrol agents were arranged according to LC_{50} values in the following descending order; Spintor 24% SC (0.097 µg/cm^2), Neemix 4.5% EC (0.119 µg/cm^2), Protecto 10% WP (0.262µg/ cm^2) and *Spli*NPV (1469.388 PIB's/mm^2). There was no enhancement in the efficacy of Spintor 24% SC or Protecto 10% WP at LC_{10} and LC_{25} levels when FB was added at 0.01, 0.1 and 1.0%. On the contrary, Neemix 4.5% EC and *Spli*NPV were enhanced by combination with of FB concentrations. The highest enhancement effect tested was found with *Spli*NPV at LC_{25} value with 1.0% FB, where the co-toxicity factor reached 348.8, 650 and 129.9 5, 6 and 7 days after application, respectively. The results indicated that there was a positive correlation between activity enhancement of Neemix 4.5% EC & *Spli*NPV and rate of increasing FB concentration. The estimated LT_{50} value of tested larvae decreased from 7.01 days in the virus alone to 6.91, 6.30 and 5.70 days when FB at 0.01, 0.1 and 1.0% was added to the virus, representing percentage reductions in time which reached 1.4, 10.1 and 18.7%, respectively. Generally, the addition of FB reduced the LC_{10} and LC_{25} values of Neemix or *Spli*NPV and hastened the death of *S. littoralis* larvae. Also, it was found that the addition of 1% FB to *Spli*NPV at LC_{10} caused a decrease in larval weight of *S. littoralis* reached by 44.1%.

Key words*: activity enhancement, Flouresecent Brightener, Neemix 4.5% EC, nucleopolyhedrovirus, Protecto 10% WP, S. littoralis, Spintor 24% SC .*

1. INTRODUCTION

The Cotton leaf worm, *Spodoptera littoralis* (Boisd.) is one of the most destructive insect pests which attack certain vegetable and field crops such as cotton, tomatoes, and cabbage in Egypt and other countries across the region. Extensive use of chemical insecticides to control the insect has led to the development of resistance and pollution of the environment (Koul, 1982). Therefore, recent investigations have been aimed to reduce dependency on chemical pesticides and to use safe alternatives in pest control programs. Spinosad is an ingredient of a natural pesticide, SpinTor® 24% SC is derived from actinomycetes *Saccharopolyspora spinosa* and has been shown to be active against several lepidopterous pests (Thompson, 1996). Spinosad showed high efficiency against large numbers of insects, such as *Helicoverpa zea, Heliothis virescen, Spodoptera exigua* and *Lobesia botrana* (Obando-Rodriguez *et al*., 1998 and Tosi *et al*., 1999).

Numerous studies have reported the effect of neem extract on lepidopterous larvae (Ascher *et al.*, 1987, Simmonds *et al*., 1990, and El-Maghraby and Kelany, 1992). Neem plant extract (Neemix 4.5% EC) has become the most famous plant extract used to control insect pests when considering the preservation of the environment and the safety of natural enemies (Chari *et al.*, 1996 and Saucke *et al.*, 2000).

Recently, attempts have been made to use microbial control, which has become one of the potential components of an integrated control strategy. The microbial insecticide *Bacillus thuringiensis* is used world wide. It has shown considerable promise in the control of several important lepidopterous pests and has no adverse effect on beneficial species (Farrag, 1992, Fahmy,

1994 and Ibrahim and Farrag 1997). In Egypt, Abul Nasr, (1956) found a nucleopolyhedrosis virus disease on cotton leaf worm, *Prodenia litura* for the first time. Significant control of *S. littoralis* using this virus was achieved when tested under field conditions (El Sheikh, 1984).

Optical or Fluorescent brighteners (stilbene disulfonic acid derivatives) were evaluated as synergistic adjuvants for baculovirus formulations (Shapiro, 1992). In attempts to increase the susceptibility of the insect host, Shapiro and Robertson (1992) reported the enhancement effect of the F. brightener on the gypsy moth nucleopolyhedrovirus. Further studies showed an enhancement effect of NPV on *S. littoralis*, *Autographa californica*, *Mamestra brassicae* and *Agrotis ipsilon* (El Salamouny *et al*., 1997; Anthony *et al.*, 2001 and El Salamouny, 2004). It was suggested that F. brighteners significantly lowered the LC_{50} and LT_{50} values in a variety of (NPV)-insect host systems (Dougherty *et al.*, 2006).

Therefore, the aim of the present study was to evaluate the efficacy enhancement of the biocontrol agents; Spintor 24% SC, Protecto 10% WP, Neemix 4.5% EC and *S. littoralis* nucleopolyhedrovirus (*Spli*NPV) against *S. littoralis* using F. brightener under laboratory conditions.

2. MATERIALS AND METHODS

2.1. Test insect

A culture of the cotton leaf worm, *S. littoralis* was obtained from the Plant Protection Research Institute, Dokki, Giza, Egypt. The stock culture was maintained in the laboratory under controlled conditions of 25 ±2 ºC, 60-65% relative humidity, and a photoperiod of 14:10(L:D).

Larvae were fed on a semi synthetic diet for several generations as described by (Shorey and Hale, 1965). The eggs were collected on tissue paper and kept in sterilized plastic cups. The newly hatched larvae were transferred into the surface of the plastic cups lined with a layer of diet until reaching the second instar. Larvae were left until pupation in each cup. The pupae were desinfected with chlorax 10%, rewashed with tap water and kept to dry, then transferred into plastic boxes till adult emergence. Five pairs of moths (males and females) were kept in each glass cage provided with 10% sugar solution for mating and egg laying.

2.2. Used virus

The method described by El-Salamouny *et al.*, (2005) was followed to propagate the nucleopolyhedrovirus (*Spli*NPV). A concentration of Polyhedral Inclusion Bodies (PIB's): 1 X 10^8 PIB's/ml was used in the virus propagation using *S. littoralis* third instar larvae. In the second day of the treatment, each larva was transferred into a pot containing 5 ml of the diet. All the pots were kept at 25 ºC and observed daily to collect the dead larvae, which were kept in the incubator at -20 ºC for further purification. Virus purification was performed as described by Khattab, (2003). The frozen larvae were thawed, then blended in Tris/HCl buffer at pH 8. The suspension was filtrated through several layers of muslin cloth and the filtrate was then centrifuged at 1000 rpm for 5 min. The supernatant was taken and centrifuged twice for 20 min. at 4000 rpm. The pellet containing the purified polyhedra was collected, re-suspended in distilled water and stored at -4 ºC for further dilution. The PIB's were counted using a haemocytometer slide (B.S. 748 Weber England, Neuberger depth 0.1 min, 1/400 mm^2).

S. littoralis nucleopolyhedrovirus (*Spli*NPV) was isolated by Abul Naser (1956).

2.3. Tested materials

- SpinTor® 24% SC (Spinosad) is produced by Sigma/Aldrich and provided by Dr. Martin Shapiro, CREC, Clemson University, USA.
- Neemix® 4.5% EC (Azadirachtin) is the principal insecticidal ingredient of neem seed (*Azadirachta indica*) extracts which contain a variety of limonoids, such as nimbolide, nimbin and salannin. Azadirachtin was initially found to be active as a feeding inhibitor and growth disruptor, it possesses considerable toxicity toward insects and very low toxicity to mammals.
- Protecto 10% WP *(Bacillus thuringiensis* var. *kurstaki)* produced by Kafr El Zayat company under supervision of the Plant Protection Research Institute, ARC, Dokki, Giza, Egypt.
- Fluorescent brightener 28 (FB) (Tinopal LPW) is produced by Sigma/Aldrich and provided by Dr. Martin Shapiro, CREC, Clemson University, USA.

2.4. Standard laboratory bioassay of biocontrol agents

The activity of the four tested biocontrol agents was evaluated using the diet surface treatment bioassay technique (Huber, 1981). The diet was poured into plastic plates "Licifa" Germany (14 X 7 X 2 cm) immediately after preparation then kept for 15-20 min. to get solidified. Different concentrations of each tested biocontrol agent were prepared for toxicity

evaluation against *S. littoralis* larvae.A 2 ml of each concentration was overlaid on the diet surface of the diet in the plates. Spintor was tested at the concentrations of 0.016, 0.032, 0.064, 0.128, 0.255 and 0.51 µg/cm^2, while the concentrations of Neemix were 0.026, 0.051, 0.102, 0.204, 0.408 and 0.816 µg/cm^2. The used concentrations of Protecto were 0.038, 0.077, 0.153, 0.306, 0.612 and 1.225 µg/cm^2. The concentrations of *Spli*NPV were 1.02 X10, 1.02X10^2, 1.02X10^3, 5.02X10^3 and 1.02X10^{4}PIB's/mm^2. After dryness of the treated agents, the diet in the plates was divided to 50 wells. A number of 50 second instar larvae was added into each plastic plate. Each concentration of each tested agent was replicated four times, in addition to the control treatment.

2.5. Enhancement bioassay with different FB concentrations

Three different concentrations of (FB) 0.01, 0.1 and 1.0% (wt/vol) were tested to determine the optimum concentration for enhancing the biocontrol agents against *S. littoralis* larvae. The calculated values of LC_{10} and LC_{25} of the tested agents were mixed with the three FB concentrations. Mixtures of each bicontrol agent with FB were aliquoted onto the surface of the diet in the plastic plates, allowed to dry and then plates were exposed to test larvae. Fifty second instar larvae were applied into each plate.

2.6. Statistical analysis

Alive and dead larvae were counted daily for ten days and mortality percentage was corrected by Abbott's formula (1925). Mean percentage mortalities after correction were plotted on a probit scale against the log of concentration. According to Finney (1971), the regression lines, Ld-p lines were drawn. The slope and median lethal concentrations were calculated.

Each concentration of each tested agent was replicated four times, in addition to the control. Alive and dead larvae were counted daily for ten successive days and mortality percentage was corrected by Abbott's formula (1925). For estimation of the lethal times (LT_{50} and LT_{90}), the bioassay technique of El- Salamouny (2004), was used, where the elapsed time for 50 or 90% kill of tested larvae was determined for accumulated mortality from Neemix and *Spli*NPV alone or combined with the of FB concentrations. The LC_{50} and LT_{50} values were calculated by probit analysis (Finney, 1971). Also, the larval weight was estimated in the treatments of Neemix and *Spli*NPV at the values of LC_{10} and LC_{25} alone or incorporated combined with the of FB concentrations.

The joint action of the tested agents with FB was expressed as the co-toxicity pathogenicity factor (CF), estimated according to the equation given by Mansour *et al.* (1966) as follows:

$$\text{Co-toxicity pathogenicity factor} = \frac{\text{Observed inhibition \% - Expected inhibition\%}}{\text{Expected inhibition\%}} \times 100$$

The Co-toxicity pathogenicity factor differentiates the results into three categories. A positive factor of 20 or more is considered synergism, a negative of 20 or more is considered antagonism and intermediate values between -20 and +20 indicate additive effect.

3. RESULTS

3.1. Toxicity of the tested biocontrol agents to *S. littoralis* larvae

The results in Table (1) show the efficiency of the tested bio-control agents against *S. littoralis* larvae. These agents were arranged according to LC_{50} values in the following descending order; Spintor (0.097 µg/cm^2), Neemix (0.119 µg/cm^2), Protecto (0.262µg/ cm^2) and *Spli*NPV (1469.388 PIB's/mm^2). The corresponding LC_{90} values were 0.322, 0.315, 2.693 µg/cm^2 and 1.0X10^6 PIB's/mm^2, respectively. Concerning the slope values of the regression lines, *Spli*NPV showed the steepest line (Slope = 0.401). The toxicity lines of Spintor, Neemix and Protecto had approximately the same slope value and ranged between 0.115 and 0.203.

Table (1): Comparative activity of the four biocontrol agents tested against *S. littoralis* larvae.

Determined parameter / Tested biocontrol agent	LC levels of tested biocontrol agents (µg/cm^2)				Slope	Activity Index at LC_{50}
	LC_{10}	LC_{25}	LC_{50}	LC_{90}		
Spintor 24% SC	0.029	0.052	0.097	0.322	0.188	100
Neemix 4.5% EC	0.045	0.072	0.119	0.315	0.203	81.51
Protecto 10% WP	0.026	0.080	0.262	2.693	0.115	37.02
*Spli*MNPV*	0.955*	30.816*	1469.388*	1.0X10^6*	0.401	---

* = PIB's/mm^2

3.2. Efficacy enhancement of the tested bio-control agents by Fluorescent Brightener

The mortality percentages among *S. littoralis* larvae treated with LC_{10} or LC_{25} values of the biocontrol agents with or without FB at three concentrations are reported in Table (2). None of the FB concentrations was toxic to *S. littoralis* larvae when tested alone at 0.01, 0.1 and 1.0%. The data indicated that there was no enhancement in the efficacy of Spintor and Protecto at LC_{10} and LC_{25} values when FB was added at the tested concentrations. On the contrary, both Neemix and *Spli*NPV were affected by combination with of FB concentrations.

Table(2): Percentage mortality among *S. littoralis* larvae treated with any of the four tested biocontrol agents at LC_{10} or LC_{25} alone or combined with different concentrations of F. brightener throughout 10 days after treatment.

LC+FB / Tested Biocontrol agent	LC_{10}	LC_{10} + FB			LC_{25}	LC_{25} + FB		
		0.01%	0.1%	1.0%		0.01%	0.1%	1.0%
Spintor 24% SC	10	10	10	8	20	20	17	22
Neemix 4.5% EC	10	25	40	62	25	17	27	43
Protecto 10% WP	12	8.2	10	8	28	25	26	30
*Spli*MNPV	17	6	26	30	35	52	55	80

Larval mortality increased from 10% for the treatment of Neemix without FB to 25, 40 and 62% in the treatments with 0.01, 0.1 and 1.0% FB, respectively. The same trend was found in the case of *Spli*NPV alone, (17%) increased to 26 and 30% in the treatments mixed with 0.1 and 1.0% FB, respectively. Whereas larval mortality was reduced to 6% with FB at 0.01%. Using Neemix or *Spli*NPV at LC_{25} value in combination with FB at 0.01, 0.1 and 1.0%; the mortality percentage increased in Neemix alone from 25% to 27& 55% or *Spli*NPV when mixed with 0.1 and 1.0% FB, respectively. In general, larval mortality increased with increasing of FB concentration. Although FB at 1.0% did not cause mortality to *S. littoralis* larvae but mixing it with Neemix at LC_{10} value increased this application rate from 0.045 to more ≈ 0.119 µg/cm^2, the percentage mortality reached 62%. The same trend was also found on mixing *Spli*NPV at LC_{25} value with FB. The produced percentage mortality indicated that the actual application rate was $\approx 1.0X10^6$ PIB's/mm^2 instead of 30.816 PIB's/mm^2. The combined treatments with FB resulted in the highest larval mortality when FB was used at 1.0%. According to the above mentioned results, the enhancing effect of FB on both Neemix and *Spli*NPV was noticed precisely and the results are illustrated in Tables (3-8).

The data presented in Table (3) show the effects of Neemix at LC_{10} and LC_{25} values and their mixtures with FB concentrations; against *S. littoralis* larvae throughout 10 days after application. Neemix at the LC_{25} level was faster than that of LC_{10} level where the mortality caused by LC_{25} and LC_{10} started at the second and fifth day, respectively, while both levels caused complete mortality in the ninth day of treatment. The accumulated percentage larval mortality at LC_{10} and LC_{25} values were 10.3, 20.0, 40.0, 80.0 & 100% and 50, 60, 65, 70 & 100% after 5, 6, 7, 8 and 9 days of the treatments, respectively. The percentage mortality in the 5th day of application at LC_{10} increased from 10.3% in the Neemix alone treatment to 25, 40 and 61.5% when FB was added at 0.01, 0.1 and 1.0%, respectively. Also, the accumulated mortality at the 7th day of application increased from 40% with the Neemix alone to 80, 80 and 92.3% on mixing with FB at 0.01, 0.1 and 1.0%, respectively.

The enhancement results of Neemix at LC_{25} value combined with FB concentrations are presented in Table (3). The results showed that Neemix combined with 0.01% FB did not increase the percentage mortality. On the contrary, the other FB concentrations enhanced efficacy of Neemix against tested larvae. The percentage mortality in the 4th day of Neemix application increased from 25.0 to 27.3 and 42.7% when FB was added at 0.1 and 1.0%, respectively. The FB at 0.1% caused moderate potentiation when added to LC_{25} value of Neemix. The percentage mortality in the Neemix alone was 50, 60, 65 and 70% increased to 61.3, 66.7, 77.3 and 96.0% in the mixed treatment of Neemix with 1.0% FB determined at the 5th, 6th, 7th and 8th day of application, respectively.

Table (3): Accumulated (%) mortality among *S. littoralis* larvae treated with Neemix 4.5%EC at LC_{10} or LC_{25} alone or combined with different concentrations of Fluorescent brightener throughout 10 days after treatment.

Days after treatment	Neemix at LC_{10}	Neemix (at LC_{10}) + FB at the indicated conc.			Neemix at LC_{25}	Neemix (at LC_{25})+ FB at the indicated conc.		
		+0.01%	+0.1%	+1.0%		+0.01%	+0.1%	+1.0%
2	-	-	-	2.6	5.0	5.5	-	6.7
3	-	-	-	10.3	15.0	16.7	22.7	34.7
4	-	-	-	43.6	25.0	16.7	27.3	42.7
5	10.3	25.0	40.0	61.5	50.0	38.9	50.0	61.3
6	20.0	40.0	50.0	63.3	60.0	50.0	63.6	66.7
7	40.0	80.0	80.0	92.3	65.0	50.0	73.6	77.3
8	80.0	100	100	95.0	70.0	72.2	78.2	96.0
9	100	-	-	100	100	100	100	100

Table (4) presents the effect of joint action between Neemix at LC_{10} value and FB at 0.01, 0.1 and 1.0% concentrations. The results clearly showed that all the combined treatments exhibited a potentiation effect on *S. littoralis* larvae at the 5^{th}, 6^{th} , 7^{th} and 8^{th} day of application, except for 1.0% FB mixture at the 8^{th} day, which gave an additive effect. Also, the co-toxicity factor increased when the concentration of FB in the mixture increased, thus indicating that there was a positive relationship between the potentiation action and increasing concentration. of FB.

Table (5) shows that there was no enhancement effect scored using FB at the lowest concentration (0.01%) combined with Neemix at LC_{25} value. Slight enhancement was recorded using the mixture of Neemix (0.072 µg/cm^2) and FB (0.1%) with co-toxicity factor ranging between 0.0 to 13.2 from the 4^{th} to 8^{th} day after application, which represents an additive effect (-20 to + 20) of joint action categoryA. Potentiation effect in this mixture was found only after three days of application. The combined treatment of Neemix +1.0% FB gave 34.7, 42.7, 61.3 and 96.0% mortality, however the expected mortality of 15.0, 25.0, 50.0 and 70% exhibited potentiation effect on *S. littoralis* larvae after 3, 4, 5 and 8 days of application, respectively. An additive effect was obtained at the 6^{th} and 7^{th} day of application when Neemix was mixed with 1.0% FB.

Generally, the data indicated that there was a positive correlation between enhancement of

Table (4): Toxicity of Neemix 4.5% EC at LC_{10} value (0.045 µg/cm^2) combined with three concentrations of FB against *S.littoralis* larvae throughout10 days after treatment.

Days after application	Neemix at LC_{10} value (0.045 µg/cm^2) + FB at three conc.	% Mortality		Co-toxicity factor	Joint action category
		Observed	Expected		
5	+0.01%	25.0	10.3	142.7	Potentiation
	+ 0.1%	40.0		288.4	Potentiation
	+ 1.0%	61.5		497.1	Potentiation
6	+0.01%	40	20	100.0	Potentiation
	+ 0.1%	50		150.0	Potentiation
	+ 1.0%	63.3		216.5	Potentiation
7	+0.01%	80	40	100.0	Potentiation
	+ 0.1%	80		100.0	Potentiation
	+ 1.0%	92.3		130.8	Potentiation
8	+0.01%	100	80	25.0	Potentiation
	+ 0.1%	100		25.0	Potentiation
	+ 1.0%	95		18.8	Additive effect

Neemix against the tested larvae and increasing FB concentration. As mentioned in Tables (4&5) the co-toxicity factor was increased as the concentration of Neemix in the mixture decreased, indicating that the potentiation action of Neemix increased with the decrease of its concentration in the mixture. It was found that the high potentiation effect was found in the combined treatment of Neemix at LC_{10} or LC_{25} values with FB at 1.0% after five and three days of application with co-toxicity factors 497.1 and 131.3%, respectively.

both 0.1 and 1.0% FB after six and seven days of application, respectively. The percentage mortalities reached 89.0, 89.5 and 93.2% when *Spli*NPV treatment was combined with FB at 0.01, 0.1 and 1.0% after 8 days of application, respectively; the virus alone treatment gave only 83% mortality. The same trend of results was found after nine days of application, where larval percentage mortality increased from 92.5% in the virus alone treatment to 94.4, 98.6 and 100% in the combined treatments with FB at 0.01, 0.1 and 1.0%, respectively.

Table (5): Toxicity of Neemix 4.5% EC at LC_{25} value (0.072 µg/cm^2) combined with three concentrations of FB against *S. littoralis* larvae throughout 10 days after treatment.

Days after application	Neemix at LC_{25} value(0.072 µg/cm^2) + FB at three conc.	% Mortality		Co-toxicity factor	Joint action category
		Observed	Expected		
3	+ 0.01%	16.7	15.0	11.3	Additive effect
	+ 0.1%	22.7		51.3	Potentiation
	+ 1.0%	34.7		131.3	Potentiation
4	+ 0.01%	16.7	25.0	-33.2	Antagonism
	+ 0.1%	27.3		9.2	Additive effect
	+ 1.0%	42.7		70.8	Potentiation
5	+ 0.01%	38.9	50.0	-22.2	Antagonism
	+ 0.1%	50.0		0.0	Additive effect
	+ 1.0%	61.3		22.6	Potentiation
6	+ 0.01%	50.0	60.0	-16.7	Additive effect
	+ 0.1%	63.6		6.0	Additive effect
	+ 1.0%	66.7		11.2	Additive effect
7	+ 0.01%	50.0	65.0	-23.1	Antagonism
	+ 0.1%	73.6		13.2	Additive effect
	+ 1.0%	77.3		18.9	Additive effect
8	+ 0.01%	72.2	70.0	3.1	Additive effect
	+ 0.1%	78.2		11.7	Additive effect
	+ 1.0%	96.0		37.1	Potentiation

The data presented in Table (6) show the potency of *Spli*NPV at of LC_{10} and LC_{25} values and their mixtures with FB at 0.01, 0.1 and 1.0% against *S. littoralis* larvae throughout 10 days after application. The accumulated mortality percentages of *S. littoralis* larvae at LC_{10} level of *Spli*NPV were 16.7, 50.0, 83.3, 92.5 and 100% after 6, 7, 8, 9 and 10 days of the treatment. The corresponding values at LC_{25} level were 8.7, 34.8, 87.0, 95.6 and 100% from 6 to 10 days after the treatment, respectively. In this study, F. brightener enhanced *Spli*NPV the activity against *S. littoralis* larvae. Larval mortality increased from 16.7 & 50% for *Spli*NPV (LC_{10}value) alone treatment to 25.7 & 29.8 and 54.0 & 62.7% for the virus with

Also, the enhancement effect of FB combined with *Spli*NPV at LC_{25} value was detected Table (6). The percentage mortality after six days of application increased from 8.7% in the virus alone treatment to 19.1, 38.4 and 65.3% when FB was added at 0.01, 0.1 and 1.0%, respectively. The corresponding values after seven days of application were 52.4, 55.3 and 80% at 0.01, 0.1, 1.0 % FB respectively; only 34.8% recorded in the virus alone treatment. Larval mortality increased from 87.0 & 95.6% for the treatment of virus alone to 90.1 & 98.6 and 95.5 & 100% for the treatments with both 0.1 and 1.0% of FB after eight and nine days of application, respectively.

Table (6): Accumulated (%) mortality among *S. littoralis* larvae treated with *Spli*NPV at LC_{10} or LC_{25} values alone or combined with different concentrations of Fluorescent brightener.

Days after treatment	*Spli*NPV at LC_{10}	***Spli*NPV (at LC_{10}) + FB at the indicated conc.**			***Spli*NPV at LC_{25}**	***Spli*NPV (at LC_{25}) + FB at the indicated conc.**		
		+ 0.01%	**+ 0.1%**	**+ 1.0%**		**+0.01%**	**+ 0.1%**	**+ 1.0%**
4	-	-	-	0.6	-	-	5.3	4.0
5	-	-	2.9	2.7	4.3	4.8	13.4	19.3
6	16.7	5.5	25.7	29.8	8.7	19.1	38.4	65.3
7	50.0	50.0	54.0	62.7	34.8	52.4	55.3	80.0
8	83.3	89.0	89.5	93.2	87.0	85.7	90.1	98.6
9	92.5	94.4	98.6	100	95.6	85.7	95.5	100
10	100	100	100	-	100	100	100	-

The enhancement effect of FB at different concentrations added to *Spli*NPV at LC_{10} or LC_{25} values against *S. littoralis* larvae was calculated and illustrated in Tables (7 & 8). All the combinations between *Spli*NPV at LC_{10} or LC_{25} values + FB at 0.01 or 0.1 or 1.0% exhibited an additive effect on *S. littoralis* larvae after 8 and 9 days of application. On the contrary, the mixing treatments of *Spli*NPV at LC_{25} value with FB at 0.01 or 0.1 or 1.0% exhibited a potentiation effect on *S. littoralis* larvae after 5, 6 and 7 days of application except the combined treatment of *Spli*NPV and FB at 0.01% in the fifth day, which gave an additive effect only.

enhancement effect occurred in the mixing treatment of *Spli*NPV at LC_{10} with FB at 1.0%, of which the co-toxicity factor reached 78.4 and 25.4 after 6 and 7 days of application. Generally, the co-toxicity factors increased when the concentration of FB in the mixture increased indicating that there was positive relationship between the potentiation action and increasing the rate of FB. Slight enhancement was recorded using the mixture between *Spli*NPV at LC_{10} value and FB at 0.1% with co-toxicity factor ranging between 53.9 to 8.0 after 6 and 7 days of application, which represented potentiation and additive effects, respectively.

Table (7): Percentage mortality among *S. littoralis* larvae treated with *Spli*NPV at LC_{10} value (0.955 PIB's/mm^2) combined with three different concentrations of FB throughout 10 days after treatment.

Days after application	*Spli*NPV at LC_{10} value (0.955 PIB's/mm^2) + FB at three conc.	% Mortality of *S. littoralis* larvae		Co-toxicity factor	Joint action category
		Observed	Expected		
6	+0.01%	5.5	16.7	-67.1	Antagonism
	+0.1%	25.7		53.9	Potentiation
	+1.0%	29.8		78.4	Potentiation
7	+0.01%	50.0	50.0	0.0	Additive effect
	+0.1%	54.0		8.0	Additive effect
	+1.0%	62.7		25.4	Potentiation
8	+0.01%	89.0	83.3	6.8	Additive effect
	+0.1%	89.5		7.4	Additive effect
	+1.0%	93.2		11.9	Additive effect
9	+0.01%	94.4	92.5	2.1	Additive effect
	+0.1%	98.6		6.6	Additive effect
	+1.0%	100.0		8.1	Additive effect

The highest enhancement effect on *S. littoralis* larvae was found with *Spli*NPV at LC_{25} value with FB at 1.0%, of which the co-toxicity factor reached 348.8, 650 and 129.9 after 5, 6 and 7 days of application, respectively. On the contrary, slight

Generally, studying the activity enhancement of Neemix 4.5% EC and *Spli*NPV at LC_{10} and LC_{25} levels by adding FB at 0.01, 0.1 and 1.0% against *S. littoralis* larvae indicated that there was a positive correlation between activity

enhancement of both insecticides and increasing FB concentration. Mostly, enhancement effect of both insecticides occurred in the first days after application and then decreased as time elapsed. The combination between Neemix at LC_{10} value and FB at tested concentrations gave higher enhancement effect on *S. littoralis* larvae than that obtained with Neemix at LC_{25} in combination. To the opposite, the combined treatments of *Spli*NPV at LC_{25} with FB at tested concentrations were enhanced higher than those at LC_{10} value.

concentrations was obtained with LT_{90} value of tested larvae, as it decreased from 10.04 days Neemix alone to 9.73 and 7.86 days on mixing with FB at 0.1 and 1.0% giving 3.1 and 21.7% reduction in time, respectively. Data showed that elapsed time to kill 90% of the tested larvae was less affected than LT_{50} when mixing Neemix with FB concentrations.

In the case of using *Spli*NPV at LC_{10} value in combination with FB at (1.0%), a slight decrease in LT_{50} and LT_{90} values was found, which gave

Table (8): Percentage mortality among *S. littoralis* larvae treated with *Spli*NPV at LC_{25} value (30.816 PIB's/mm^2) combined with three different concentrations of FB throughout 10 days after treatment.

Days after application	*Spli*NPV at LC_{25} value (30.816 PIB's/mm^2) + FB at three conc.	% Mortality of *S. littoralis* larvae		Co-toxicity factor	Joint action category
		Observed	Expected		
5	+0.01%	4.8	4.3	11.6	Additive effect
	+ 0.1%	13.4		211.6	Potentiation
	+ 1.0%	19.3		348.8	Potentiation
6	+0.01%	19.1	8.7	119.5	Potentiation
	+ 0.1%	38.4		340.2	Potentiation
	+ 1.0%	65.3		650.6	Potentiation
7	+0.01%	52.4	34.8	50.6	Potentiation
	+ 0.1%	55.3		58.9	Potentiation
	+ 1.0%	80.0		129.9	Potentiation
8	+0.01%	85.7	87.0	-1.5	Additive effect
	+ 0.1%	90.1		3.6	Additive effect
	+ 1.0%	98.6		13.3	Additive effect
9	+0.01%	85.7	95.6	-10.4	Additive effect
	+ 0.1%	95.5		-0.11	Additive effect
	+ 1.0%	100.0		4.6	Additive effect

In general, the data in Tables (9 &10) indicate that adding F. brightener at the tested concentrations to LC_{10} or LC_{25} values of Neemix or *Spli*NPV affected the elapsed time to kill 50 or 90% of tested *S. littoralis* larvae. The LT_{50} value of tested larvae decreased from 6.46 days using Neemix at LC_{10} value to 5.70, 5.52 and 4.80 days when FB was added at 0.01, 0.1 and 1.0%, respectively. Representing percentage reduction in time, reached 11.8, 14.6 and 25.7%, respectively. On the contrary, the LT_{90} value of tested larvae using the previous treatments was unaffected by mixing Neemix with all FB concentrations. On testing Neemix at LC_{25} value, the time required to kill 50% of the larvae was 5.22 days but when mixed with FB at 0.1 and 1.0% it decreased to 5.05 and 4.18 days representing reduction percentages of 3.3 and 20.0%, respectively. The same trend of results for the above mentioned

4.1 and 2.1% reduction, respectively. The elapsed time to kill 50% of tested larvae decreased from 7.01 days using FB- free virus at LC_{25} value to 6.91, 6.30 and 5.70 days when adding FB at 0.01, 0.1 and 1.0% representing percentage reduction in time reached 1.4, 10.1 and 18.7%, respectively. On the other hand, the LT_{90} value of tested larvae using the previous treatments was obtained in the mixed treatment of the virus with FB at 1.0%, which gave 14.8% reduction in time. Also, mixing treatments of *Spli*NPV with FB at different concentrations confirmed that the percentage reduction in LT_{90} value of tested larvae was less affected than LT_{50} value. Larvae died most quickly when FB concentrations was added to Neemix or *Spli*NPV at LC_{10} and LC_{25} values High reduction in LT_{50},^{s} and LT_{90},^{s} was obtained at 1% FB in the mixture treatments. These results indicated that the addition of 1% FB to Neemix or

Table (9): Effect of mixing F. brightener at three concentrations to Neemix 4.5% EC or *Spli*NPV at LC_{10} or LC_{25} values on kill speed of *S. littoralis* larvae

Treatment	LC_{10} & LC_{25} values	Elapsed time (days)to kill 50 and 90% of *S. littoralis* larvae							
		LT_{50}				LT_{90}			
		Alone	Mixing with FB at Conc.			Alone	Mixing with FB at Conc.		
			0.01%	0.1%	1.0%		0.01%	0.1%	1.0%
Neemix 4.5% EC	0.045 µg/cm^2	6.46	5.70	5.52	4.80	7.33	7.63	11.18	8.10
	0.072 µg/cm^2	5.22	5.71	5.05	4.18	10.04	11.16	9.73	7.86
*Spli*NPV	0.955PIB's/mm^2	6.92	7.07	6.64	6.65	8.21	8.25	8.25	8.04
	30.816PIB's/mm^2	7.01	6.91	6.30	5.70	8.50	8.74	8.42	7.24

LT_{50} and LT_{90} for Neemix 4.5% EC and *Spli*NPV were used as the standard and all other LT_{50}'s and LT_{90}'s were compared to the standard (Table 10).

Table (10): Reduction percentage in kill speed (LT_{50} and LT_{90}) of *S. littoralis* larvae in the combined treatments of Neemix 4.5% EC or *Spli*NPV at LC_{10} or LC_{25} values with F. brightener at three concentrations

Treatment	LC_{10} & LC_{25} values	Reduction% in kill speed at LT_{50} and LT_{90}					
		LT_{50} in mixture treatments			LT_{90} in mixture treatments		
		Mixing with FB at indicated Conc.			Mixing with FB at indicated Conc.		
		0.01%	0.1%	1.0%	0.01%	0.1%	1.0%
Neemix 4.5% EC	0.045 µg/cm^2	11.8	14.6	25.7	--	--	--
	0.072 µg/cm^2	--	3.3	20.0	--	3.1	21.7
*Spli*NPV	0.955PIB's/mm^2	--	4.1	4.1	--	--	2.1
	30.816PIB's/mm^2	1.4	10.1	18.7	--	0.9	14.8

*Spli*NPV hastened the death of *S. littoralis* larvae.

Effect of mixing Neemix or *Spli*NPV with the three FB concentrations on larval weight is shown in Table (11). Neemix treatments at LC_{10} and LC_{25} decreased larval weight 43 and 19 mg/larva against 187 mg/larva for the control treatment, representing 77.0 and 89.8% reduction in larval weight, respectively. This could be due to the antifeedant effect of the neem extract. Addition of F. brightener to Neemix resulted in more decrease in the larval weight. In the case of using Neemix alone at LC_{10} value, the larval weight was 43 mg/larva and on mixing with FB at 0.01, 0.1 and 1%, the larval weight decreased to 18, 19 and 12 mg/larva representing percentage reductions of 55.8, 58.1 and 72.1%, respectively. Increasing Neemix concentration to LC_{25} value resulted in a high decrease in the larval weight to 19 mg/larva, which became 17, 15 and 2 mg/larva after mixing it with 0.01, 0.1 and1% FB with percentage reduction reached 10.5, 21.1 and 89.5%, respectively. Generally, the larval weight of *S. littoralis* was less affected to all virus treatments. Detectable decrease in the larval

Table (11): Effect of mixing F. brightener at three concentrations to Neemix 4.5% EC or *Spli*NPV at LC_{10} or LC_{25} values on larval weight of *S. littoralis.*

Treatment	**LC_{10} & LC_{25} values**	**Larval weight (mg/larva)**				**%Reduction in larval weight**		
		Alone	**Mixing with FB at Conc.**			**Mixing with FB at Conc.**		
			0.01%	0.1%	1.0%	0.01%	0.1%	1.0%
Neemix 4.5% EC	0.045 µg/cm^2	43	18	19	12	55.8	58.1	72.1
	0.072 µg/cm^2	19	17	15	2	10.5	21.1	89.5
*Spli*NPV	0.955PIB's/mm^2	179	168	171	100	6.2	4.5	44.1
	30.816PIB's/mm^2	173	193	181	---	-11.6	- 4.6	---

-Larval weight in the control treatment of Neemix 4.5% EC = 187 mg/larva

-Larval weight in the control treatment of *Spli*NPV = 198 mg/larva

weight was recorded when 1% FB was added to *Spli*NPV at LC_{10}.

4. DISCUSSION

The differences in toxicity of these compounds are due to the intrinsic toxicity of these compounds. Considerable attention has been given to enhance of biocontrol agents efficacy by increasing host susceptibility.

The enhancement effect of F. brightener on efficacy of biocontrol agents against *S. littoralis* larvae on synthetic diet in the laboratory was reported in the present study.

The current study evaluated the efficacy enhancement of Spintor, Neemix, Protecto and *Spli*NPV against *S. littoralis* larvae at levels of LC_{10} and LC_{25} using FB at 0.01, 0.1 and 1.0%. The results indicate that there was no enhancement in the efficacy of Spintor and Protecto at LC_{10} and LC_{25} values when FB was added at the three tested concentrations. On the contrary, , Neemix and *Spli*NPV were enhanced by combination with FB concentrations. Mostly, enhancement effect of both insecticides occurred in the first days of application and then decreased as time elapsed. The combination treatments of *Spli*NPV at LC_{25} with FB at three concentrations were enhanced higher than those at LC_{10} value against *S. littoralis* larvae.

Several studies reported that FB increase the efficacy of certain nuclear polyhedrosis viruses by decreasing LC_{50} (Zou and Young 1996). In the present study, the combined treatments of *Spli*NPV at LC_{25} value with FB at 0.01 or 0.1 or 1.0% exhibited an enhancement effect on *S. littoralis* larvae after 5, 6 and 7 days of application except the combined treatment of *Spli*NPV and FB at 0.01% on the fifth day, which gave an additive effect only. The highest enhancement effect on *S. littoralis* larvae was found with *Spli*NPV at LC_{25} value with 1.0% FB, where the co-toxicity pathogenicity factor reached 348.8, 650 and 129.9 after 5, 6 and 7 days of application, respectively. These results are in agreement with those previously noticed on the effect of FB as an enhancer for the activity of NPV (Shapiro and Robertson 1992, Shapiro and Dougherty 1994, Shapiro and Vaughan 1995, Vail *et al*., 1996, Zou and Young 1996, Farrar and Ridgway 1997, Shapiro, 2000 and El-Salamouny *et al*., 2005). Results of enhancement for Neemix and *Spli*NPV at LC_{10} and LC_{25} levels by adding FB at 0.01, 0.1 and 1.0% against *S. littoralis* larvae indicated that there was a positive correlation between the activity enhancement of both insecticides and increasing FB concentration. Also, the current study indicated that the co-toxicity factors increased when the concentration of FB in the mixture increased indicating that there was positive relationship between the potentiation action of *Spli*NPV. These results are comparable with those reported by Hamm and Shapiro, (1992), Shapiro and Robertson (1992), Zou and Young (1996) and Vail *et al*., (1996).

On the other hand, the LT_{50} or LT_{90} values using *Spli*NPV were influenced by FB concentration. The LT_{50} of tested larvae decreased from 7.01 days using virus alone at LC_{25} value to 6.91, 6.30 and 5.70 days when FB was added at 0.01, 0.1 and 1.0%, representing percentage reduction in time reached 1.4, 10.1 and 18.7%, respectively. Also, mixing *Spli*NPV with FB at different concentrations confirmed that the percentage reduction in LT_{90} value was less affected than LT_{50} value. These results indicated that the addition of 1% FB to *Spli*NPV hastened the death of *S. littoralis* larvae. Reducing the LT_{50} value as a result of adding FB to virus agrees with the finding by Shapiro and Robertson (1992), Adams *et al*., (1994), Zou and Young (1996), Li and Otvos (1999 a & b), Shapiro and Hamm (1999), Shapiro (2000). Also, it was found in the present study that addition of 1% FB Wang and Granados (2000), Shapiro and Argauer (2001) and El-Salamouny (2004).to *Spli*NPV at LC_{10} decreased larval weight of *S. littoralis* by 44.1%. This finding is in agreement with El-Salamouny *et al*., (2005) and El-Salamouny (2004), who reported that the reduction in larval weight was correlated with a high rate of enhancement effect.

The results in the current study indicated that the combination between Neemix 4.5% EC at LC_{10} value and FB at different concentrations gave high potentiation effect on *S. littoralis* larvae than those at LC_{25} value in combination. There was a positive correlation between activity enhancement of Neemix against *S. littoralis* larvae and increasing FB concentration. Also, adding FB at different concentrations to LC_{10} or LC_{25} values of Neemix affected the LT_{50} or LT_{90} values of tested *S. littoralis* larvae. The obtained data showed that elapsed time to kill 90% of the tested larvae was less affected than LT_{50} when mixing Neemix with different concentrations of FB. Neemix treatments at LC_{10} and LC_{25} decreased larval weight by 43 and 19 mg/larva against 187 mg/larva in the control treatment representing 77.0 and 89.8% reduction, respectively. This could be due to the antifeedant effect of the neem plant extract (Schmutterer, 1995). Addition of the FB to

the Neemix resulted in more decrease in the larval weight. There are no previous investigations about enhancing the Azadirachtin activity using F.brightener.

Acknowledgement

I would like to thank Dr. Martin Shapiro, CREC, Clemson University, Charleston, USA for providing the Fluorescent brightener sample used in present study. Also, I appreciate the help of Prof. Salah Elnagar and Prof. Mohamed El Sheikh, Faculty of Agriculture, Cairo University, Egypt for facilitating carrying out the tests.

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زيادة فاعلية أربعة عوامل للمكافحة الحيوية ضد دودة ورق القطن بواسطة Fluorescent Brightener -28

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ملخص

تمت دراسة زيادة فاعلية أربعة عوامل للمكافحة الحيوية وهى سبينتور 24% SC (سبينوساد)، نيمكس 4.5% EC (أذادراخيتين)، بروتكتو 10% WP (باسيلس ثيرونجينسيس) و فيروس البوليهدروسز النووى (*Spli*NPV) ضد دودة ورق القطن بإستخدام الفلوريسينت برايتنر (FB) . رتبت فاعلية عوامل المكافحة الحيوية حسب قيم LC_{50} كما يلى تنازليا : سبينتور 24% SC (0.097ملى جرام لكل سنتيمتر مربع)، نيمكس 4.5% EC (0.119 ملى جرام لكل سنتيمتر)، بروتكتو 10% WP (0.262 ملى جرام لكل سنتيمتر مربع) و فيروس دودة ورق القطن (1469.388 بولى هيدرا لكل ملى متر مربع) .أتضح انه لا توجد زيادة فى فاعلية سبينتور 24% SC و بروتكتو 10% WP عند قيم LC_{10} وLC_{25}عند إضافة الفلوريسينت برايتنر بتركيزات 0.01، 1.0,0.1% . زادت على العكس ، كفاءة كل من النيمكس 4.5% EC و فيروس دودة ورق القطن حيث تأثروا بالخلط مع تركيزات مختلفة من الفلوريسينت برايتنر، وبالإضافة فقد وجد أن أعلى تأثيرمنشط للمبيد على يرقة دودة ورق القطن باستخدام فيروس دودة ورق القطن عند قيمة LC_{25} مع 1.0 % FB ، حيث وصل عامل السمية المشار له إلى 348.8، و650 و 129.9 بعد 6,5 و7 ايام من المعاملة ، على التوالى. أشارت نتائج زيادة الفاعلية إنه توجد علاقة موجبة بين نيمكس و فيروس دودة ورق القطن ومعدل الزيادة فى تطبيق الفلوريسينت برايتنر . قلت قيمة LT_{50} من 7.01 أيام بإستخدام الفيروس عند 30.816 بولى هيدرا لكل ملى متر مربع (LC_{25}) إلى 6.91، 6.30و 5.70 أيام عند إضافة الفلوريسينت برايتنر بتركيزات 0.01، 1.0,0.1% ، حيث يمثل إنخفاض فى النسبة المئوية للوقت الذى تم التوصل إليه إلى 1.4، 10.1و 18.7%، على التوالى . بشكل عام يخفض إضافة الفلوريسينت برايتنر من قيم LC_{10} و LC_{25} من النيمكس أو الفيروس ويسرع من الموت ليرقة دودة ورق القطن. بالإضافة وجد إنه فى الدراسة الحالية إن إضافة 1.0 % من الفلوريسينت برايتنر إلى فيروس دودة ورق القطن عند مستوى LC_{10} قد سبب إنخفاض فى وزن اليرقة وصل إلى 44.1% .

المجلة العلمية لكلية الزراعة – جامعة القاهرة – المجلد (61) العدد الرابع (اكتوبر 2010):437-449.