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Individual and Combined Biological Effects of *Bacillus thuringiensis* and *Multicapsid Nucleopolyhedrovirus* on the Biological Stages of Egyptian Cotton Leafworm, *Spodoptera littoralis* (B.) (Lep.: Noctuidae)

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ABSTRACT

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) , is known as an important and highly polyphagous pest species worldwide. The objective of the present study was to evaluate synergistic effects of *Bacillus thuringiensis* subsp. *kurstaki* and *NucleoPolyhedroVirus* (SpliMNPV) on the 5-day-old larvae (2nd instars) of *S. littoralis* under laboratory conditions. To do this, the larvae of *S. littoralis* were fed on the treated artificial diet containing only one or combination of Bt (8.31×10^5, 2.78×10^7, 9.69×10^8 spore mL^{-1}) and SpliMNPV (5.26×10, 7.03×10^2, 9.39×10^3 OB mL^{-1}). According to the results, the mortality rate for most of the Bt-SpliMNPV combinations (different concentrations) was higher than that in the treatments containing only one of the studied biocontrol agents. The Bt-SpliMNPV combinations showed different types of interactions, including synergistic, additive, or antagonistic effects. The treatment containing 8.31×10^5 spore mL^{-1} of Bt and 5.26×10 OB mL^{-1} of the SpliMNPV was interpreted as synergism effect, as the real mortality (72.41±12.43%) was significantly more than the expected (48.28%). In addition, application of the Bt-SpliMNPV combinations could significantly increase larval and pupal periods, and reduce pupation, pupal weight and the adult emergence rate compared to the control and treatments containing only one of Bt or SpliMNPV. Finally, it could be concluded that co-application of Bt and SpliMNPV can enhance economic and efficient control of *S. littoralis.*

Keywords: Biocontrol agents, Insect virus, Microbial control, Synergistic effects.

INTRODUCTION

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lep.: Noctuidae), is known as a highly polyphagous pest in the world (Alfazairy *et al*., 2013). Considerable damages are recorded on 44 different plant families, including grasses, legumes, crucifers, deciduous fruit trees and some ornamental crops, many of which are of highly economic importance (Robinson *et al*., 2010; Hatem *et al*., 2011).

Common control strategies for *S. littoralis* rely on chemical insecticides. These chemicals have potentially harmful effects on humans, other mammals and non-target species, especially on predators and parasitoids of important pests (Mosallanejad

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and Smagghe, 2009). Intensive uses of chemical insecticides cause resistance in several leafworms populations (Hatem *et al*., 2011; Khedr *et al*., 2015). Therefore, development of environment-friendly microbial pesticides to fight natural disease-causing microorganisms such as viruses, bacteria, nematodes, protozoa and fungi, is of importance (Lacey *et al*., 2001). *Bacillus thuringiensis* (Bt) is the most widely used microorganism for control of insect pests of crops and forests (Lacey *et al*., 2015; Salehi Jouzani *et al*., 2017). Bt is an aerobic and Gram-positive bacterium that produces crystalline proteins (Cry proteins or δ-endotoxins that are encoded by *cry* genes) during sporulation, which are highly toxic to a wide-range of pest insects especially on Lepidoptera, Diptera and Coleoptera (Mansour *et al*., 2012; Marzban *et al*., 2016; Salehi Jouzani *et al*., 2017). Insect resistance to Bt-formulations is develops slower than resistance to chemical insecticides. As for the development of insect resistance against Bt-toxins, several important mutations are required (Ullah *et al*., 2014; Qayyum *et al*., 2015). However, there is still a need to find new efficient biocontrol agents with less possibility of insect resistance to them.

In addition to Bt, Baculoviridae is a large family of entomopathogenic viruses, which have been widely used as biocontrol agents of crop pests. These viruses are safe and selective bio-insecticides, restricted to many invertebrates, particularly Lepidoptera species (Theilmann *et al*., 2005). Virions are protected by the polyhedral occlusion bodies as they can retain infectivity for several years. Previous studies have confirmed that *S. littoralis Nuclear Polyhedrosis Virus* (SpliNPV) causes high mortality in larval populations of this pest (Toprak *et al*., 2007). However, the narrow host range, slow speed of kill that allows larvae to continue thier damage to crop, large dose requirement, and resistance development are some of the limiting factors in use of *baculoviruses* (Magholi *et al*., 2014). Therefore, improvement of *baculovirus* based formulations with enhanced efficiency is of importance. The aims of the present study were: (1) To quantify the lethal activity of SpliMNPV and Iranian Bt. *kurstaki* strain on 2nd instars (5-day old) of *S. littoralis*, (2) To investigate LC_{25}, LC_{50}, and LC_{75} effects of combinations of SpliMNPV-Bt and their individual treatment on larval period, pupal period, pupation rate, pupal weight, emergence rate, (3) To find the most effective combination of SpliMNPV and Bt that has the highest mortality and the adverse effects on the biological stages of this pest.

MATERIALS AND METHODS

Insect Rearing

The *S. littoralis* larvae were collected from cotton fields in Dezful region, Iran, in September 2015. The collected larvae were individually placed in plastic cups (3 cm height, 6 cm diameter) with artificial diet (Novan, 1985) until larvae were pupated. The adults were placed in a plastic cylindrical container (25 cm in height, 17 cm in diameter), and a paper towel strip was hung in it for the egg deposition. Then, eggs were transferred into a clean plastic container for hatching. Adults were fed with a 10% honey solution. Insect culture was maintained at 27˚C and 65% relative humidity under a 16: 8 hour (L:D) photoperiod in a growth chamber. The insects were reared for several generations under these standard rearing conditions, then used for bioassays.

Nuclear Polyhedrosis Virus Preparation

The SpliMNPV was obtained at the concentration 6.67×10^{9} OB mL^{-1} from Professor David Grzywacz, University of Greenwich, England. The viral suspensions ranging from 10^{2} to 10^{6} OB mL^{-1} were prepared by diluting with Tris HCl 50 mM (pH 7.2) (Mahmoud *et al*., 2012; Magholli *et al*., 2013).

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Bt Preparation

A native Iranian Bt subsp. *kurstaki* strain (GON-9) was provided by the Iranian Research Institute of Plant Protection (Marzban, 2002; Marzban and Salehi, 2006). The strain was kept at -80˚C in Freezer (Cryo Freezer Conqueror). The strain was grown on nutrient agar at 27 ˚C. After four days, 3-4 loop of pre-culture were solved to 1 mL of sterile distilled water and added to 99 mL of R_2NB (3 g beef extract, 5 g peptone and 50 g water extract of rice per litre) medium in a 250 mL conical flask, and maintained at 27 ˚C for 48 hours with (100 rpm). Afterwards, it was placed for 1 hour at -4˚C for more separation of crystals and spores. The medium at an initial pH of 7.2 was sterilized at 120˚C for 20 minutes (Alfazairy *et al*., 2013). After final growth, the bacterial suspensions with concentrations ranging from 10^5 to 10^9 spore mL^{-1} were prepared by diluting with Tween 80 (0.4%) (Kalantari *et al*., 2013).

Bioassay

Two bioassay procedures were adopted for the experiment. To perform bioassays, the 5-day-old larvae were placed individually in the white plastic cups (3 cm height, 6 cm in diameter), containing artificial diet (1 cm^3 each). Three replicates, each of 15 larvae, were assayed against each procedure. All cups were maintained under laboratory conditions of 27˚C, 65% relative humidity, and 16: 8 hours (L:D) photoperiod. All the tested larvae were starved for a period of 2-4 hours before feeding to ensure their feeding on treated artificial diets (Burges and Thompson, 1971). Each artificial diet (1cm^3) was treated by means of a micropipette with 0.5 mL per cm^3 of concentration. The surviving larvae were transferred to a clean cup and supplied daily with fresh and untreated artificial diets after 48 hours. The bioassays were continued until the larvae had either died or pupated and adults were emerged.

Procedure 1

At the procedure 1, the LC_{25}, LC_{50}, LC_{75} values for the Bt strain and SpliMNPV were calculated separately. At the first, a bacterial primary bioassay was carried out to obtain a minimum (> 25% mortality) and maximum (< 75% mortality) concentrations required for experiments. Then, seven different concentrations (1.9×10^6, 6.3×10^6, 1.9×10^7, 6.3×10^7, 1.9×10^8, 6.3×10^8 and 1.9×10^9 spore mL^{-1}) were prepared based upon previous tests in a logarithmic fashion using 0.4% Tween 80. Forty eight hours post-treatment, larval mortality was recorded daily for 6 days. The bioassay of virus was performed by the same procedure, except using 50 mM Tris HCl for concentrations preparation (10^2, 2.8×10^2, 8.1×10^2, 2.3×10^3, 6.6×10^3, 1.8×10^4 and 5.2×10^4 OB mL^{-1}). Furthermore, larval mortality was recorded from day 4 to day 10 after treatments. In both assays, the larvae that were unable to move or feed were confirmed dead. The control larvae were fed on the artificial diets treated with sterile distilled water plus Tween 80 for the Bt strain and Tris HCl buffer for SpliMNPV.

Procedure 2

The bioassay procedure included sixteen treatments, the combination of the LC_{25}, LC_{50}, LC_{75} values of Bt and SpliMNPV (8.31×10^5, 2.78×10^7, 9.69×10^8 spore mL^{-1}) and (5.26×10, 7.03×10^2, 9.39×10^3 OB mL^{-1}), respectively, were assayed. In addition, the treatments containing individual Bt and SpliMNPV at the same concentrations and a control (with sterile distilled water plus 0.4% Tween 80 and 50 mM Tris Hcl were studied. The larval were individually placed in plastic cups (3 cm height, 6 cm diameter) with the treated artificial diets. After 48 hours, the survived larvae were transferred to clean cups with untreated diets and observed daily to determine larval period, pupal period, rate of pupal formation, pupal weight and emergence rate.

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Statistical Analysis

One-way ANOVA was performed using SPSS software (1998). Percentage of pupation, larval period, pupal period, and pupal weight were calculated on the basis of the initial number of larvae used in each treatment. The mortality was corrected by the following equation:

M [%]= [(t-c)/(100-c)]×100

Where, M is corrected Mortality, *c* is the mortality in controls and *t* is mortality in treatments (Abbott, 1925; Duffield and Jordan, 2000). The normalization of the data was done in SPSS. Then, mean corrected mortality, pupation rate, larval period, and pupal weight were compared using Duncans test at $P< 0.05$. The equation $CTF= (O_c-O_e) /O_e\times100$ was used to determine the type of interactions between different concentrations, where *CTF* is the Co-Toxicity Factor, O_c is the Observed mortality in the combination and O_e, the expected mortality, is the sum of mortalities caused by each pathogen used in combination. This factor was used to differentiate the results into three categories. A positive factor of 20 or more indicated synergism, while a negative factor of 20 or more stood for antagonism, and any intermediate value (*i.e*. between -20 and +20) was considered as an additive (Mansour *et al*., 1966). LC_{25}, LC_{50}, LC_{75} values were determined by SAS (1999) statistical software.

RESULTS

Bioassay

Procedure 1

Values of LC_{25}, LC_{50} and LC_{75} for the native Bt strain were 8.31×10^5, 2.78×10^7 and 9.69×10^8 spore mL^{-1}, whereas those of the SpliMNPV were 5.26×10, 7.03×10^2 and 9.39×10^3 OB mL^{-1}, respectively (Tables 1 and 2).

Table 1. The results of bioassay of the *Bacillus thuringiensis* strain on the 5-day-old larvae of *Spodoptera littoralis*.

Isolate	LC_{25}	LC_{50}	LC_{75}	Slope	Intercept	χ^2	*df*	Pr> Chi-sq
Bt. *kurstaki*	8.31×10^5 (8.1×10^4-2.9×10^6)	2.78×10^7 (1.1×10^7-6×10^7)	9.69×10^8 (3.6×10^8-5.2×10^9)	0.44	-3.28	1.42	5	0.9222

Table 2. The results of bioassay of the SpliMNPV isolate on 5-day-old larvae of *Spodoptera littoralis*.

Isolate	LC_{25}	LC_{50}	LC_{75}	Slope	Intercept	χ^2	*df*	Pr> Chi-sq
SpliMNPV	5.26×10 (1.3×10-1.2×10^2)	7.03×10^2 (3.6×10^2-1.2×10^3)	9.39×10^3 (4.8×10^3-2.4×10^4)	0.59	-1.71	1.55	5	0.9069

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Procedure 2

The 5-day-old larvae of *S. littoralis* that were fed on the treated artificial diets containing different combinations of Bt-SpliMNPV strains, showed significant variation in terms of their mortality ($F_{14,30}$= 7.87, P< 0.001), larval period (Larval period: $F_{14,30}$= 25.58, P< 0.001), pupal period ($F_{14,30}$= 3.36, P< 0.003), pupation rate ($F_{15,32}$= 9.03, P< 0.001), pupal weight ($F_{14,30}$= 2.29, P< 0.028), and adult emergence ($F_{15,32}$= 12.12, P< 0.001).

The mortality rate for most of the Bt-SpliMNPV combinations (different concentrations) was higher than treatments containing only one of the studied biocontrol agents (Table 3). The treatment containing combination of the Bt strain (with concentration 8.31×10^5 spore mL^{-1}) and the SpliMNPV strain (5.26×10^1 OB mL^{-1}) was interpreted as synergism effect, as the observed mortality was significantly more than the expected. Other Bt-SpliMNPV combinations showed additive effects, however, exceptions were observed for Bt (2.78×10^7 sporeml^{-1})-SpliMNPV (9.39×10^3 OB mL^{-1}) and Bt (9.69×10^8 spore mL^{-1})-SpliMNPV (7.03×10^2 OB mL^{-1}), which showed antagonism effects (Table 3).

The larval and pupal periods increased significantly in combinations and single treatments of Bt and SpliMNPV compared to the control (Table 4). The longest duration of larval period (24.67 day) was observed in the treatments containing Bt-SpliMNPV combinations at concentrations 8.31×10^5 spore mL^{-1} and 5.26×10 OB mL^{-1} and 8.31×10^5 spore mL^{-1} and 7.03×10^2 OB mL^{-1}, respectively. The longest pupal period was recorded in larvae treated by Bt-SpliMNPV combination (2.78×10^7 spore mL^{-1} and 9.39×10^3 OB mL^{-1}). In addition, the pupal rate and pupal weight were significantly decreased in the treatments containing combinations and single forms of

Table 3. Mortality of 5-day-old larvae of *Spodoptera littoralis* exposed to the single or combined *Bacillus thuringiensis* and SpliMNPV. [a]

Bt (Spore ml^{-1})	SpliMNPV (OB ml^{-1})	Actual mortality (%)[b, c]	Expected mortality [c]	Co-toxicity factor	Type of interaction [d]
8.31×10^5	5.26×10	72.41 ± 12.43^{abcd}	48.28	49.97	Syn.
	7.03×10^2	62.06 ± 3.45^{cde}	75.86	-18.19	Add.
	9.39×10^3	86.21 ± 3.44^{abc}	100	-13.79	Add.
2.78×10^7	5.26×10	65.52 ± 6.89^{bcde}	65.51	0.01	Add.
	7.03×10^2	79.31 ± 15.80^{abc}	93.09	-14.8	Add.
	9.39×10^3	93.10 ± 6.89^{ab}	117.23	-20.58	Ant.
9.69×10^8	5.26×10	82.75 ± 6.89^{abc}	93.1	-11.11	Add.
	7.03×10^2	86.21 ± 3.44^{abc}	120.68	-28.56	Ant.
	9.39×10^3	100^{a}			
8.31×10^5	0	24.14 ± 12.43^{f}			
2.78×10^7	0	41.37 ± 3.44^{ef}			
9.69×10^8	0	68.96 ± 11.49^{abcd}			
0	5.26×10	24.14 ± 6.89^{f}			
0	7.03×10^2	51.72 ± 3.45^{de}			
0	9.39×10^3	75.86 ± 9.12^{abcd}			
0	0	-			
DF		14			
F		7.88			
P		0.001			

[a] The 5-day-old larvae of *S. littoralis* were fed on artificial diets with *B. thuringiensis* and SpliMNPV suspensions. [b] The data in the table are means (±SE). Means within the same column followed by different letters are significantly different at P< 0.05, Duncan test. [c] Mortality rates were corrected by Abbott's formula. [d] Abbreviation: Add= Additive, Syn= Synergism, Ant= Antagonism.

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Table 4. The debilitating effects of single or combination forms of *Bacillus thuringiensis* and SpliMNPV on *Spodoptera littoralis.* [a, b]

Bt (spore ml^{-1})	SpliMNPV (OB ml^{-1})	Larval period (Day)	Pupal period (Day)	Pupal rate (%)	Pupal weight (mg)	Emergence rate (%)
	5.26×10	24.67±0.67^{a}	11.76±0.46^{de}	36.67±6.67^{def}	26.67±1.76^{bcd}	26.67±12.01^{defg}
8.31×10^{5}	7.03×10^{2}	24.67±0.33^{a}	11.87±0.32^{de}	46.67±12.01^{cde}	24±1^{d}	36.67±3.33^{cde}
	9.39×10^{3}	20.33±0.33^{def}	12.18±0.42^{cde}	30±5.8^{def}	28.33±2.73^{abcd}	13.33±3.33^{efg}
	5.26×10	22.67±0.89^{bc}	12.08±0.83^{cde}	36.67±3.33^{def}	27.67±0.88^{bcd}	33.33±6.67^{cdef}
2.78×10^{7}	7.03×10^{2}	23±0.01^{ab}	12.43±0.43^{bcde}	26.67±17.64^{defg}	28.33±0.33^{abcd}	20±15.27^{defg}
	9.39×10^{3}	19.67±0.67^{efg}	13.83±0.17^{ab}	6.67±6.67^{fg}	30^{ab}	6.67±6.67^{fg}
	5.26×10	22.67±1.2^{bc}	12.33±0.67^{cde}	20±10.01^{efg}	26.67±0.88^{bcd}	16.67±6.67^{efg}
9.69×10^{8}	7.03×10^{2}	21.67±0.33^{bcd}	14±0.29^{a}	13.33±3.33 ^{fg}	26.67±0.67^{bcd}	13.33±3.33^{efg}
	9.39×10^{3}	-	-	0^{g}	-	0^{g}
8.31×10^{5}	0	16.67±0.58^{ij}	12.72±0.68^{abcde}	73.33±12.01^{abc}	26±2.88^{bcd}	73.33±12.02^{ab}
2.78×10^{7}	0	17±0.01^{ij}	12.93±0.07^{abcd}	56.67±3.33^{bcd}	27.67±1.33^{bcd}	56.67±3.33^{bc}
9.69×10^{8}	0	17.67±0.67^{hi}	13.52±0.13^{abc}	30±11.55^{def}	29^{abc}	30±11.55^{cdef}
0	5.26×10	18±0.01^{ghi}	12.52±0.8b^{cde}	76.67±8.82^{ab}	24.67±2.03^{cd}	73.33±6.67^{ab}
0	7.03×10^{2}	19±0.01^{fgh}	13.43±0.57^{abc}	56.67±3.33^{bcd}	27.33±0.67^{bcd}	46.67±3.33^{cd}
0	9.39×10^{3}	21±1.73^{cde}	13.36±0.25^{abc}	33.33±14.53^{def}	27±0.58^{bcd}	23.33±8.82^{defg}
0	0	15.33±0.58^{j}	11.33±0.33^{e}	100^{a}	32.67±0.58^{a}	96.67±3.33^{a}
DF		14	14	15	14	15
F		25.58	3.36	9.03	2.29	12.12
P		0.001	0.003	0.001	0.028	0.001

[a] The 5-day-old larvae of *S.littoralis* were fed on artificial diets with *B. thuringiensis* and SpliMNPV suspensions. [b] The data in the table are means (±SE). Means within the same column followed by different letters are significantly different at P< 0.05, Duncan test.

Bt and SpliMNPV compared to the control. The highest rate of pupation (76.67%) was observed in the treatment containing the lowest concentration of the Bt strain alone. The lowest pupal weight (24 mg) belonged to the combination of Bt-SpliMNPV with concentration 8.31×10^{5} spore mL^{-1} and 7.03×10^{2} OB mL^{-1}. The adult emergence in all Bt-SpliMNPV combinations and their single treatments were significantly decreased compared to the control. Adult emergence was found inversely related to the pathogenicity of Bt and SpliMNPV. The lowest adult emergence was recorded in the Bt-SpliMNPV combination forms (0%), whereas the highest levels were observed in the control (96.67%) and Bt (8.31×10^{5} spore mL^{-1}) and SpliMNPV (5.26×10 OB mL^{-1}) in single forms (73.33%), respectively. In addition, in the treatments containing Bt-SpliMNPV combination at concentration 8.3×10^{5} spore mL^{-1} and 5.3×10 OB mL^{-1} and Bt in alone (2.78×10^{7} spore mL^{-1}) and SpliNPV in alone (5.26×10, 7.03×10^{2}, 9.39×10^{3} OB mL^{-1}) showed considerable adverse effects on survivors. These effects included deformities in both pupae and moths.

DISCUSSION

The present study was performed to develop a sustainable management strategy for control of S. *littoralis* larvae by using different Bt and SpliMNPV combinations. The results showed that the 50% Lethal Concentration (LC_{50}) of the studied Iranian Bt strain on the *S. littoralis* larvae was about 2.78×10^{7} spore mL^{-1}, which was significantly less than that previously reported by other researchers. For instance, Alfazairy *et al*. (2013) showed that the LC_{50} of Btm27 and Btk66 on *S.littoralis* larvae

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were equal to 0.31×10^8 and 0.89×10^8 spore mL^{-1}. LC_{50} value of Btm27 was similar to LC_{50} of GON-9, but LC_{50} for Btk66 was different. This difference could be due to the presence of more specific *cry* or *vip* genes in the Iranian native Bt strain against the studied pest.

The LC_{50} value of SpliMNPV against *S. littoralis* larvae was about 7.03×10^2 OB mL^{-1} which was significantly less than that previously reported. Shaurub *et al*. (2014) reported that the LC_{50} value of SpliMNPV on fourth instar of *S. littoralis* was 8.43×10^8 OB mL^{-1}. This difference may be due to difference of the assayed instar stage. In another study, Seufi (2008) showed that the LC_{50} value for SpliNPV against 2nd instar of *S. littoralis* was 1.2×10^3 OB mL^{-1}, which was approximately 2 times more than LC_{50} of the SpliMNPV in the present study. These results confirmed that the Bt and SpliMNPV strains used in the present study were more efficient against the studied pest.

Larvae fed on treated diets with Bt started to die from the third day after infection, and most larvae were dead on the fourth or fifth day. The *S. littoralis* larvae treated with combination of Bt-SpliMNPV were dead at the same time as that for the dead larvae treated with Bt alone, but it was 2-3 days earlier than SpliMNPV. Lepidopteran caterpillars were treated by Bt-NPV, after 6 hours, the action of NPV was intensified and, in association with Bt, caused intense vacuolization of the cytoplasm of larval midgut, causing cellular disorganization (Knaak and Fiuza, 2005). Duraimurugan *et al*. (2009) confirmed that combined treatment of NPV and Bt results in suppression of detoxificantion enzymes in *Helicoverpa armigera* (Hübner) and Nouri-Ganbalani *et al*. (2016) showed combination of Bt-Azadirachtin (AZA) on *Plodia interpunctella* (Hübner), reduced the level of digestive enzymes found in midgut. The hypothesis needs to be investigated for *S. littoralis* larvae as well. Although larvae consumed fresh artificial diets during the infection period in this study, larval treated by Bt and SpliMNPV, alone or in combination, ate 1/3 or more of their food according to the concentrations, compared to the control larval that consumed all of their food.

The results showed that combination or single form of Bt and SpliMNPV could extend developmental period and reduce the weight of pupa compared to the control. These results are in consistence with results attained for SpliMNPV on *S. littoralis* by Shaurub *et al*. (2014) and Bt and HaNPV on *H. armigera* by Kalantari *et al*. (2013).

In the present study, the combinations of the lowest concentration of Bt (8.31×10^5 spore mL^{-1}) and SpliMNPV (5.26×10 or 7.03×10^2 OB mL^{-1}) caused longer period than other Bt-SpliMNPV combinations and the control. Therefore, it could be concluded that the non-lethal concentration of the Bt strain can increase larval period in the surviving larvae. Both Bt and SpliMNPV adversely affected the growth and development rates of *S. littoralis*. Prolonged developmental time at any stage would mean greater exposure to natural enemies and environmental stresses, which could reduce the rate of pest population build-up (Sedaratian *et al*., 2013). Furthermore, a longer generation time could result in fewer generations per season. Pupation and adult emergence rate in *S. littoralis* was observed after combinations and single forms of Bt and SpliMNPV in a concentration dependent manner. These results agreed with those of Marzban *et al*. (2009), Kalantari *et al*. (2013), and Qayyum *et al*. (2015) when assayed toxicity against *H. armigera*, and those Magholli *et al*. (2013) when assayed on *Plutella xylostella* (L.).

The data showed that the mortality increased at the lowest concentration of Bt-SpliNPV mixture compared to the mortality due to SpliNPV and Bt alone, and showed a synergistic effect on *S. littoralis* larvae with positive co-toxicity factor of 49.97. Shaurub *et al*. (2014) reported a co-toxicity factor of 58.40 when they studied co-application of SpliMNPV and AZA against *S. littoralis* larvae. Previously, the synergistic effects of different biocontrol agents, such as Bt and

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NPV on *H. armigera* (Matter and Zohdy, 1981), S*I*NPV-Btk on *Culex pipiens* (L.) (Mahmoud *et al*., 2012) and Bt-HaNPV against *P. xylostella* (Magholli *et al*., 2013) were reported. The lowest concentration of Bt might cause delay in the larvae development and damage the cells of the targeted insect gut, and thus enhanced efficacy of SpliNPV (Salama *et al*., 1993) and overall collapse of fitness (Nouri-Ganbalani *et al*., 2016). In the presence of Bt, the number of insects that are able to escape NPV infection is reduced (Hesketh and Hails, 2008). The combination of Bt-SpliMNPV at low concentration significantly caused higher larval mortality and reduced killing time of the pest, which was in accordance with the results of Cook *et al*. (1996). Commonly, at low concentrations of Bt, toxins bind to specific receptors of the targeted insect midgut epithelium, causing the cells to lyse and facilitate the entry of the virus. At high concentrations of Bt, the mode of action of Cry toxins is to damage the cells of the targeted insect gut, and this may inhibit passage of SpliMNPV into the midgut cell. Therefore, Bt-SpliMNPV interactions showed antagonistic effect in some treatments in the present study. Xiaoxia *et al*. (2006) reported antagonistic effect on *H. armigera* at the most combinations of Cry1Ac and HaNPV. Hatem *et al*. (2012a) found that Cry1Ac with SpliNPV show antagonistic effect on *S. littoralis*. Hatem *et al*. (2012b) showed antagonistic effect on *S. littoralis* in the combination of SpliNPV and SpliGV. Kalantari *et al*. (2013) showed antagonistic effect when *H. armigera* larvae were simultaneously infected by HaNPV and Bt at higher concentrations. In the most Bt-SpliNPV combinations, an additive effect occurred. Hatem *et al*. (2012a) reported that additive effect on *S. littoralis* was found in the interaction between Cry1Ac and SpliGV at the low doses. This result was in accordance with the findings of Salama *et al*. (1993), Liu *et al*. (2006) and Marzban *et al*. (2009).

In the present study, when the Bt and SpliMNPV strains were applied alone, to achieve the maximum toxicity and larval mortality of *S. littoralis* larvae, use of higher concentrations of the SpliMNPV and Bt was necessary. The mortality levels of the low concentrations of the Bt-NPV combinations were greater than that of the Bt and NPV strains when they were used in single form. Thus, it could be concluded that reducing the dosage of the biocontrol agents and reduction of killing time will be economically more effective, and can delay the resistance process to the toxic effects of Bt in the target insect populations. Combination of the pathogens may increase their virulence compared with either alone (Marzban *et al*., 2009; Marzban, 2012). Chang *et al*. (2003) have developed an improved *baculovirus* insecticide producing occlusion bodies containing Bt toxin. Therefore, SpliMNPV can be a candidate in combination with Bt for integrated biological control of *S. littoralis*, but it is necessary for further studies to explore new interactions and to carry out the experiments under field conditions.

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اثرات بیولوژیکی جداگانه و ترکیب باکتری *Bacillus thuringiensis* و ویروس *Muiticapsid Nucleopolyhedrovirus* روی مراحل زیستی پروانه برگخوار مصری پنبه *Spodoptera littoralis* (Lep.:Noctuidae) (B.)

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چکیده

پروانه برگخوار مصری پنبه، *Spodoptera littoralis*، به عنوان یکی از گونه های آفات مهم و بسیار پلی فاژ در دنیا شناخته شده است. هدف از مطالعه کنونی، بررسی اثر سینرژیستی باکتری *Bacillus thuringiensis* subsp. *kurstaki* و ویروس *nucleopolyhedrovirus* (SpliMNPV) روی لاروهای پنج روزه آفت در شرایط آزمایشگاهی است. برای انجام این، لاروهای پروانه برگخوار مصری پنبه از غذاهای مصنوعی آلوده حاوی باکتری Bt ($۸/۳۱\times۱۰^{۵}$، $۲/۷۸\times۱۰^{۷}$و $۹/۶۹\times۱۰^{۸}$ اسپور در میلی لیتر) و ویروس SpliMNPV ($۵/۲۶\times۱۰$، $۷/۰۳\times۱۰^{۲}$ و $۹/۳۹\times۱۰^{۳}$ OB در میلی لیتر) به صورت جداگانه و ترکیب تغذیه کردند. در نتایج تیمارها، میزان مرگ و میر لاروی در اکثر ترکیبات Bt-SpliMNPV (غلظت های مختلف) بیشتر از کاربرد جداگانه هر کدام از عوامل کنترل بیولوژیک مورد مطالعه بود. ترکیبات Bt-SpliMNPV انواع مختلفی از برهم کنشها، شامل اثرات سینرژیستی، افزایشی و آنتاگونیستی را نشان دادند. تیمار حاوی $۸/۳۱\times۱۰^{۵}$ اسپور در میلی لیتر باکتری و $۵/۲۶\times۱۰$ OB در میلی لیتر ویروس به عنوان اثر سینرژیستی تفسیر شد که مرگ و میر واقعی(٪ ۱۲/۴۳±۷۲/۴۱)به طور قابل توجهی از مرگ و میر مورد انتظار(٪ ۴۸/۲۸) بیشتر بود. علاوه بر این، کاربرد ترکیبات Bt-SpliMNPV باعث افزایش طول دوره لاروی و شفیرگی و کاهش درصد شفیره شدن، وزن شفیره ها و درصد خروج حشرات کامل به طور معنی دار نسبت به تیمار شاهد

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و تیمارهای حاوی باکتری Bt و ویروس SpliMNPV بصورت جداگانه، شدند. در نهایت، می توان نتیجه گرفت که کاربرد توأم باکتری Bt و ویروس SpliMNPV می تواند کنترل اقتصادی و کارآمد پروانه برگخوار مصری پنبه را افزایش دهد.

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