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Potential of *Bacillus thuringiensis* and Nuclear Polyhedrosis Virus for controlling the Cotton Leaf Worm, *Spodoptera littoralis* (Boisd.)

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Abstract

Laboratory investigations have been carried out pertaining the lethal action of *B. thuringiensis* (Agerin) and nuclear polyhedrosis virus (Helicovex) either separately or in combination on the cotton leaf worm, *S. littoralis* larvae. Results showed that the lethal action (expressed as LC_{50} values) of *Bt* (Agerin) against 2nd, 3rd and 4th instar larvae were 0.05, 0.17 and 0.09% whereas those of NPV (Helicovex) were 0.08, 0.18 and 0.13%, respectively.

The simultaneous application of sublethal concentrations of both Bt (Agerin) and NPV (Helicovex) resulted in an additive lethal effect against 3rd instar larvae and a synergistic effect against 2nd and 4th instar larvae. Results generally indicate that mortality among *S. littoralis* larvae was higher when they were exposed to Bt-NPV sublethal concentrations than when exposed to either pathogen alone. The findings may be helpful and effective for controlling this pest and closely related ones. Yet field tests have to confirm the results and to determine their field performance.

Keywords: *Spodoptera littoralis, Bacillus thuringiensis, nuclear polyhedrosis virus, lethal action.*

1. Introduction

Over reliance on conventional insecticides as a common control strategy and their indiscriminate use have resulted in insect resistance to most insecticides, resurgence, and outbreak of secondary pests as well as residual toxicity [1]. Accelerating public concern about those problems has sparked wide interest in the development of environmentally friendly bioinsecticide alternatives [2, 3, 4]. Since the registration of the first microbial insecticide formulation of *Bacillus thuringiensis* var. *kurstaki* at 1961 and this pathogenic bacterium seems to have potential values in controlling various lepidoptrous insects even alone or combined with other chemicals or entomopathogens [5, 6, 7, 8]. The toxicity of *Bt* is due to the production of crystalline protein protoxins, known as δ-endotoxins [9]. Solubilized protoxins are activated by midgut proteases and bounded with the receptors of the epithelial cells [10]. The entomopathogenic viruses; as one of the microbial control agents; are specific, efficient and safe to non-target organisms. The use of baculoviruses in pest management can be traced back to the 19th century. Baculoviruses, especially nucleopolyhedroviruses (NPV) and granuloviruses (GV), were experimented as more selective and more environementally acceptable agents for the control of insect pests [11]. Interactions among microbial agents may result in co-existence, synergism or antagonism. Antagonism is equivalent to a reduction in virulence whereas synergism enhances virulence as a result of interaction. In principle, synergism and antagonism are possible between different microbial agents as well as between strains of one and the same pathogen [12]. The present study has been undertaken to detect the potency of *Bt* and NPV either separately or in combination on the 2nd, 3rd and 4th instar larvae of the cotton leaf worm, *S. littoralis.*

2. Materials and Methods

2.1. Insect Rearing

The colony of cotton leafworm, *Spodoptera littoralis* was obtained from culture continuously reared under laboratory conditions in Department the division of Cotton Leafworm, Plant Protection

Research Institute, Dokki, Giza, Egypt. Larval stages were reared on castor bean leaves at 27±2°C and 65±5% R.H. as described by [13].

2.2. Microbial Products Tested

The Egyptian, strain *Bacillus thuringiensis aegypti*, (Agerin), a product of Agricultural Genetic Engineering Research Institute (AGERI), containing 32000 International Units per mg (IU/mg) was used, as it is readily available.

A commercial formulation of the nuclear polyhedrosis virus (NPV), named (Helicovex), containing $7.5x10^{12}$ PIB/ml was obtained from Plant Protection Research Institute, Dokki, Giza, Egypt.

2.3. Bioassay Tests

2.3.1. *Bt* bioassay

Leaf-dip bioassay method as described by **Tabashink *et al.*** [14] was followed using castor leaves. The leaves were first washed with distilled water and dipped in solutions of different concentrations of the *Bt* commercial formulation prepared with distilled water. Each leaf was dipped for 5-10 seconds and allowed to air dry for a period of an hour. The leaves were then placed individually into Petri dishes (15cm diameter). 9hours starved twenty five newly molted 2nd, 3rd and 4th instar larvae were released on each dish as a replicate along with three replicates, including control. The concentrations used were 0.025, 0.05, 0.1, 0.2, 0.4 and 0.8% .Larvae were allowed to feed for 48hrs on treated leaves. Then these leaves removed and replaced by untreated ones. Larval mortality was daily recorded and larvae were considered dead if they gave no response to stimulation by touch. The % mortality was corrected according to Abbott's formula [15].

Probity analysis was determined to calculate the median lethal concentration values (LC_{50}) and related parameters according to **Finney** [16] using a software computer program [17].

2.3.2. NPV bioassay:

Larvae used in NPV bioassay were obtained from the laboratory stock with series of 2nd, 3rd and 4th instar larvae groups of *S. littoralis* which were fed on castor leaves. The castor leaves were collected from the field must be washed and kept to dry. Leaf-dip feeding method was employed to treat the various age instars (2nd, 3rd and 4th) of larvae for every experiment. Before treating of NPV, the larvae were kept starved for 9hrs as the instars. Each treatment included 25 larvae and was replicated three times. After 24hr of treatment with NPV, larvae were daily provided fresh castor leaves. Mortality was recorded every day after showing the disease symptoms; then was accumulatively calculated. Data was subjected to probit analysis [16] to determine LC_{50} values and related parameters of NPV for each age group.

2.3.3. *Bt* in combination with NPV bioassay

The values of LC15 and LC25 of both *Bt* and NPV for each instar were calculated and combined to determine the co-toxicity to the tested instars. Leaf-dip feeding technique was followed to treat 2nd, 3rd and 4th instar larvae. Each treatment included 25 larvae and was replicated thrice. Mortality due to action of *Bt* and NPV combined was accumulatively calculated. To test the synergism, antagonism, or the additive action of *Bt* and NPV, the formula of Co-toxicity factor (CTF) was used [18].

$$CTF = (OM-ME)/ ME \times 100$$

Where OM is the observed percentage mortality provided by combination, ME is the expected mortality percentage (i.e. the sum of mortality from each agent).

A= positive factor of 20 or more (synergism).

B= negative factor of 20 or more (antagonism).

C= any value between ± 20 (additive to either effect).

3. Results and Discussion

3.1. Lethal Action of *Bt* (Agerin) on *S. littoralis* Larvae:

Data summarized in table (1) and figure (1) showed the efficiency (expressed as LC_{50} values) of *Bt* (Agerin) against the 2nd, 3rd and 4th instar larvae of *S. littoralis*. These values were 0.0549, 0.171 and 0.09%, respectively. The corresponding toxicity lines were slope values of 1.0094±0.1448, 0.7409±0.1346 and 0.8951±0.1382, respectively. This indicates that the 3rd instar larvae were more tolerant to the pathogen, whereas the 2nd larval instar was more susceptible to Agerin than the 4th one, when the same concentrations were used. Also, from these results it could be concluded that *Bt* is about 3 times as lethal to 2nd instar larvae compared to 3rd ones, while it is about 2 times as lethal to 4th instar

larvae compared to 3rd ones and it is about 1.5 times as lethal to 2nd instar larvae compared to 4th ones. From the present results, it can be concluded that under laboratory conditions, the 3rd instar larvae was less susceptible to Agerin than 2nd and 4th ones as it has higher LC_{50} value than that obtained for both 2nd and 4th ones which indicates that 3rd instar larvae have some mechanisms of the development resistance to *Bt* toxins, while the 2nd instar larvae of *S. littoralis* were more susceptible to Agerin than the 4th one.

Table (1): Efficiency (expressed as LC_{50} values) of *Bt* (Agerin) on 2nd, 3rd and 4th instar larvae of *S. littoralis*

Larval instar	LC_{50}% *	95% fiducial limits		Slope
		Lower	Upper	
2nd	0.0549	0.0342	0.0772	1.0094±0.1448
3rd	0.171	0.112	0.2742	0.7409±0.1346
4th	0.09	0.0588	0.1274	0.8951±0.1382

*LC_{50} values are significant (P < 0.05), whenever fiducial limits do not overlap.

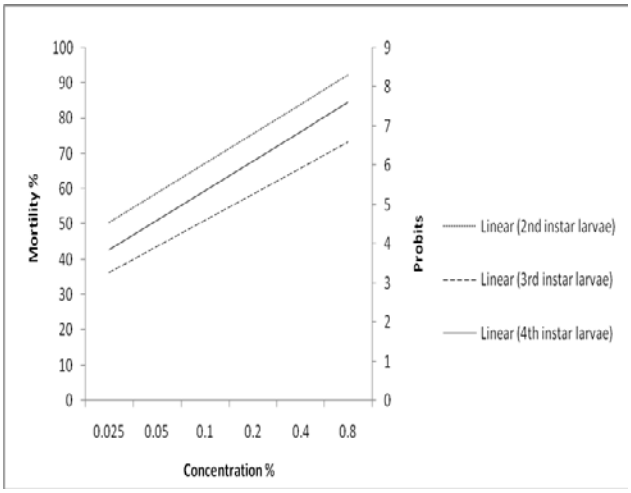


Figure (1): Regression lines of *Bt* against 2nd, 3rd and 4th instar larvae of *S. littoralis*.

These results are in agreement with those of Abd El-Megeed *et al* [19], who applied Dipel 2x to the 2nd instar larvae of *S. littoralis*. As a conclusion, the biocide Agerin was more effective against *S. littoralis*, due to its sensitivity to the *Bt* formulations. This sensitivity may be related to pH of the midgut or the receptor on the brush border membrane of the midgut [20]. It can be concluded that Agerin has the potentials to be used in IPM programs. The 48 hour LC_{50} for Agerin, Dipel 2x and Dipel DF were 0.18, 0.07 and 0.10% against 2nd larval instar of *S. littoralis* for the three formulations, respectively [21]. This result seems disagree with those reported by El-Banna *et al.* [22] who observed that susceptibility of *S. exigua* larvae to *B. thuringiensis* decreased with age. Also, López Lastra *et al.* [23] concluded that third instar of both *S. exigua* and *S. frugiperda* were the most resistance instar to B. thuringiensis endotoxin.

3.2. Lethal Action of NPV (Helicovex) on *S. littoralis* Larvae

The response of larvae to different concentrations of Helicovex by straight regression lines indicating homogeneity. Data presented in table (2) and figure (2) showed that lethal action (expressed as LC_{50} values) of nuclear polyhedrosis virus (Helicovex) against 2nd,3rd and 4th instar larvae of *S. littoralis* were 0.0801, 0.1881 and 0.1312% with slope values of 0.6461±0.1337, 0.5781±0.1323 and 0.7787±0.1352, respectively.

Also, from these results it could be concluded that NPV is about 2 times as lethal to 2nd instar larvae compared to 3rd one, while it is about 1.5 times as lethal to 4th instar larvae compared to 3rd one and it is about 1.5 times as lethal to 2nd instar larvae compared to 4th one.

Table (2): Lethal action (expressed as LC_{50} values) of NPV on 2nd, 3rd and 4th instar larvae of *S. littoralis*

Larval instar	LC_{50} (%)*	95% fiducial limits		Slope
		Lower	Upper	
2nd	0.0801	0.406	0.1286	0.6461±0.1337
3rd	0.1881	0.1098	0.3686	0.5781±0.1323
4th	0.1312	0.0855	0.1979	0.7787±0.1352

*LC_{50} values are significant (P <0.05), whenever fiducial limits do not overlap.

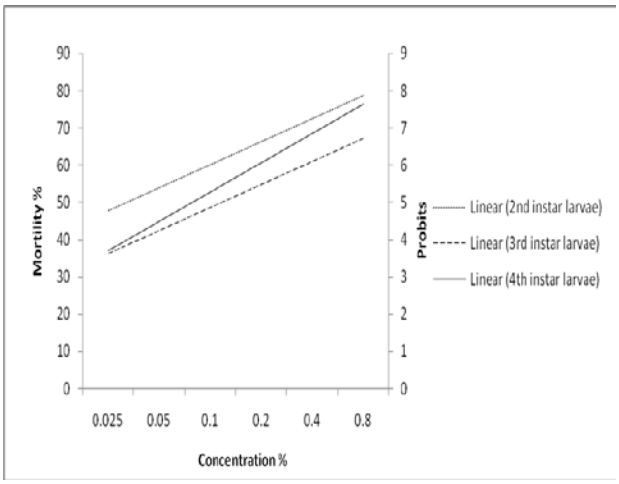


Figure (2): Regression lines of NPV against 2nd, 3rd and 4th instar larvae of *S. littoralis*

These results is supported by Abd El-Aziz [24] and Mahmoud *et al.* [25] who reported that the second larval instar of *S. littoralis* was highly susceptible to NPV than the fourth larval instar. The reason for the increased LC50 value may be due to difference in individual resistance to NPV or due to difference in the larval age. We dealt with late 2nd instar larvae while other investigation dealt with early 2nd instar larvae. The LC_{25}, LC_{50} and LC_{90} needed for infecting 4th instar larvae of *S. littoralis* were $1.1x10^7$, $9x10^8$ and $3.99x10^{12}$ PIB/ml, respectively.

3.3. Combined Lethal Action of *Bt* and NPV on *S. littoralis* Larvae: The interaction between *Bt* and NPV against larvae of *S. littoralis* was investigated in laboratory experiments. Results in table (3) and figures (3 & 4) show that there was a synergistic effect at LC_{25}s which was higher than that obtained at LC_{15}s for *Bt*+NPV for both 2nd and 4th instar larvae. While *Bt* with NPV at both LC_{15} and LC_{25} for 3rd instar larvae produced additive effect which has higher value at LC_{25} than that obtained at LC_{15}. In case of *Bt*+NPV for 2nd instar larvae has positive co-toxicity factor of 23.3 and 24 at LC_{15} and LC_{25} respectively. But in case of *Bt*+NPV for 3rd instar larvae had the additive positive co-toxicity factor were 16.6 and 18 at LC_{15} and LC_{25}, respectively. While in case of *Bt* in combination with NPV for 4th instar larvae had synergistic effect at both LC_{15} and LC_{25} with positive co-toxicity factors of 20 and 30, respectively. Preliminary studies with sublethal dosages with NPV produced higher mortality. There is significant difference between the treatments and virus alone as well as between different treatments at 5% level (Table 3).

Table (3): Combined lethal action of NPV with *Bt* on 2nd, 3rd and 4th instar larvae of *S. littoralis*

Larval instar	Lethal conc.	NPV Conc (%)	Bt Conc. (%)	NPV and Bt combinations %		Co-toxicity factor (%)	Joint action effect
				Expected mortality	Observed mortality		
2nd	LC_{15}	0.023	0.021	30	37.5	23.3	Synergism
	LC_{25}	0.043	0.032	50	62.7	24.0	Synergism
3rd	LC_{15}	0.045	0.047	30	35.9	16.6	Additive
	LC_{25}	0.093	0.049	50	59.2	18.0	Additive
4th	LC_{15}	0.032	0.033	30	36.7	20.0	Synergism
	LC_{25}	0.067	0.053	50	65.3	30.0	Synergism

Figure (3): Combined lethal action of *Bt* (LC15) and NPV (LC15) on S. littoralis

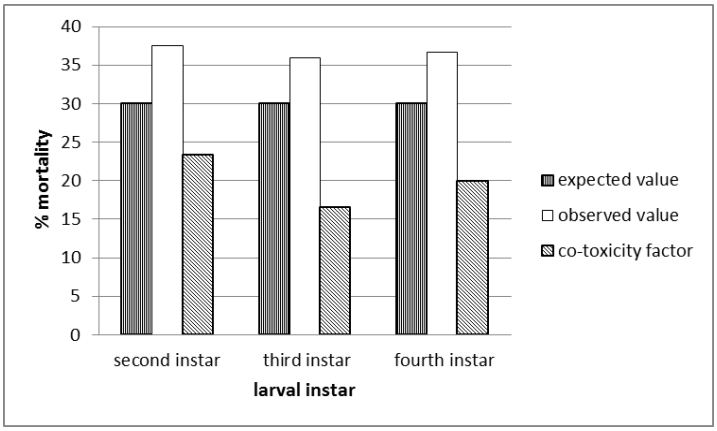
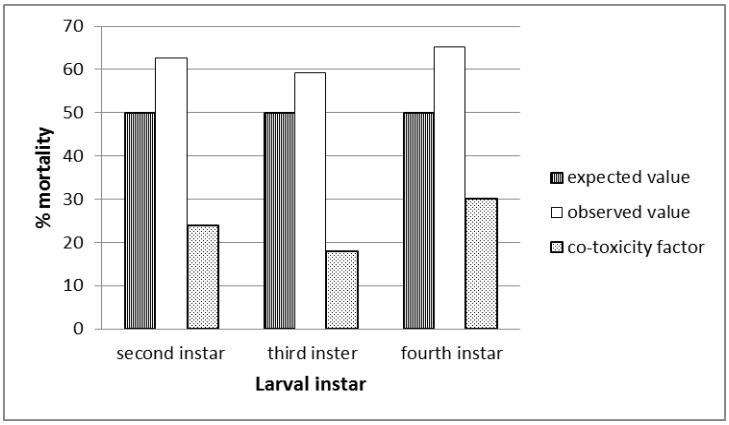


Figure (4): Combined lethal action of *Bt* (LC25) and NPV (LC25) on S. littoralis

Mabrouk [26] found that there is synergism between



the NPV and the *Bt*. Co-infectivity factor of *Sl*NPV isolates/*Bt* against the 2nd instar larvae at $1x10^4$ PIBs/gram diet ranged between +34.56 and +51.82%. Synergistic effect at $5x10^4$ PIBs/gram diet was higher than that obtained at $1x10^4$ PIBs/gram diet. It ranged between +38.99 and +55.52% at

$5x10^4$ PIBs/gram diet. Regarding the LT_{90}s, slight differences in synergistic effect obtained at the two tested virus concentrations for all isolates. As for the 4th instar larvae, synergistic effect at $5x10^6$ PIBs/gram diet with *Bt* was higher than that obtained with virus at $1x10^7$ PIBs/gram diet. Both common NPV+*Bt* and Sh-4+*Bt* revealed slight increase in synergistic effect at $1x10^7$ PIBs/gram diet compared to virus combinations at $5x10^6$ PIBs/gram diet. Raymond *et al.* [27] reported that simultaneous exposure to a mixture of virus and *Bt* toxin reduced the fitness of *Bt*-resistant insects compared with treatments with toxin alone.

Our results seem to agree with that reported by Padua *et al.* [28] who showed that under field conditions a combination of *Bt* and NPV was more effective against *Spodoptera* larvae than NPV alone or *Bt* alone.

4. Conclusions

These results reflect that combination of *Bt* and NPV could be tested in order to increase and enhance the efficacy of microbial products for *S. littoralis* management. Thus, field tests have to confirm the results and to determine field performance of these pathogens.

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