Semi-field Application of a New Formulation Based on *Spodoptera litura* Nuclear Polyhedrosis Virus and *Bacillus thuringiensis* subsp. *mexicanensis* Against Cotton Leaf Worm, S*podoptera littoralis* and Root Knot Nematode, *Meloidogyne incognita* in Egypt

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Abstract

In this study, new biopreparations based on *Spodoptera litura* nuclear polyhedrosis virus (*Slitura*NPV) and *Bacillus thuringiensis* subsp. *mexicanensis* (Btm) were evaluated against two important pests: cotton leaf worm, *Spodoptera littoralis,* and the root knot nematode, *Meloidogyne incognita,* in 2017 and 2018. The mortality of treated *S. littoralis* larvae occurred, with combination treatments of *Slitura*NPV and Btm at two concentrations (20 and 30%), without significant differences between the two tested concentrations (87.9 and 94.0, respectively). Deformities among pupae were the highest in Btm treatments, followed by *Slitura*NPV treatments, then the fewest deformed pupae were recorded in the combined treatment. Increases of the soybean mean seed weights were highly significant with combined treatment when compared to the controls, in both years, and the greatest expansion was seen with combination treatment. The treatment of *M. incognita* with Btm was highly effective for nematode J_2-mortality. Furthermore, the mean numbers of all recorded parameters for tomato showed significant differences.

Keywords: nuclear polyhedrosis virus, bacteria, cotton leaf worm, nematode, field application
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1. Introduction

The Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) is certainly one of the most damaging agricultural lepidopteran pests within the subtropics and tropics [1]. It is a hugely polyphagous pest, which presents in almost the whole of Africa, as well as in parts of the Middle East and southern Europe [2, 3]. This insect uses at least 112 species, in 44 plant families, as host plants, for food or for oviposition. In Egypt, it can attack many economically important crops throughout the year, especially cotton and some vegetables [1, 3, 4]. The level of invasion may reach up to 119,048 egg-mass ha^{-1}, inflicting a great harm to all parts of plants [5-9]. Despite the introduction of several management procedures, this pest is still inflicting significant damage.

Meloidogyne incognita (Kofoid and White) (Nematoda; Meloidogynidae), which is a plant parasitic nematode of the root knot nematode type, is an important pathogen in many crops around the world, such as onion, soybean, rice, olive, cucumber, tomato and potato [10-13]. Yield losses of up to 50% may be incurred from intense infestations of the nematode [14].

Although pesticides still play a crucial role in the control of plant pathogenic nematodes and insect pests such as root-knot nematodes and cotton leaf worm, the massive-scale application of such chemicals causes many environmental problems that cannot be ignored. Furthermore, many pests have developed resistance to the pesticides used, and an example of this is the case of *S. Littoralis,* which has developed resistance to many pesticides [15]. The use of pesticides poses acute and chronic hazards to human and non-target organisms. Such chemicals can pollute the environment, challenge wildlife populations, and cause serious public health and food safety problems [7, 16].

Biological control is a successful method that can be used to manage damage from many pests. In fact, biopesticides production and use is growing at an annual rate of 10-20%, a rate that shows it is outpacing the chemical pesticide products industry [17]. Several studies have revealed much about the insecticidal and nematicidal effects of *Bacillus thuringiensis* (Bt) against economically important insect pests and phytoparasitic nematodes. This bacterium represents an important and useful group for microbial control of lepidopteran pests including the Egyptian cotton leafworm *S. littoralis* (Boisd.), tobacco cutworm *S. litura* (F.), fall armyworm *S. frugiperda* Smith, beet armyworm *S. exigua* (Hübner) [18], corn borers *S. nonagrioides* (Lef.) and *Ostrinia nubilalis* (Hübner), cotton bollworm *Helicoverpa armigera* (Hübner), corn earworm *Helicoverpa zea* (Boddie) and soybean looper *Chrysodeixis includens* (Walker) [18-21]. The toxicity of Bt towards phytoparasitic nematodes such as potato cyst nematode (*Globodera pallida*) and root knot nematode (*Meloidogyne incognita, M. javanica*) [22-24], has also been demonstrated.

Baculoviridae is a family of viruses with circular, covalently closed, double-stranded DNA genomes that range in extremely tiny size from 100 to 180 kbp. The virion (an infective baculovirus particle) is occluded into large proteinaceous capsules or occlusion bodies that are rod-shaped polyhedrons. Two genera have been recognized, Nucleopolyhedrovirus (NPV) and Granulovirus (GV), known as Alphabaculoviridae and Betabaculoviridae, respectively [25]. Baculoviruses show a scanty host limit, generally infecting only closely related species. These baculoviruses are potentially useful as biological control agents and have been used effectively to manage different insect pests [26, 27]. NPVs have an impact on a massive range of insect pests and can cause lethal epizootics in susceptible species. The bacterial biopreparations demand around 74% of the market whilst the viral ones around 5% [28]. This study aims to evaluate the biological aspects of new biopreparation upon field application, and to illustrate their impact on Egyptian cotton leafworm and root knot nematodes.

2. Materials and Methods

2.1. Microbial agents

In the present study, *S. litura* nuclear polyhedrosis virus (*Slitura*NPV) and *B. thuringiensis* subsp. *mexicanensis* (Btm) were used. The Btm was isolated in 2003 from the citrus mealybug, *Planococcus citri* (Risso) on a sour orange tree, at the courtyard of Faculty of Agriculture, El-Shatby, Alexandria University, Alexandria, Egypt [29]. Serotyping of this local strain was kindly confirmed by Prof. Lecadet Marguerite, Pasteur Institute, Paris, France. The tested virus was originally isolated from moribund larvae of the cotton leafworm *S. littoralis*, collected in 2017, from fields of the Agricultural Research Station Farm, Nubaria, 47 km west of Alexandria City, Egypt. The NPV was propagated in *S. littoralis* larvae. NPV-murdered larvae were amalgamated in sterile water and then refined through four layers of cotton fabric [30] to obtain a suspension of 7.93×10^8 polyhedra g^{-1} of the dry preparation. The virus particles were then partially purified and subjected to a molecular identification using specific primers [31]. The formulation was produced by adding the suspensions of Btm and virus to a suitable proprietary adjuvant (Patent no. 473/2014). The biopreparation of Btm was produced by adding the adjuvant into other ingredients after sterilizing. The initial Btm preparation contained 1.23×10^9 CFU g^{-1} of the dry preparation.

2.2. Isolation and PCR amplification of the viral DNA

The viral DNA was obtained from the infected *S. littoralis* using DNA virus isolation kit (Qiagene, USA) according to the manufacturer's procedures. A 1 µl (100 ng) sample of the purified DNA was used for PCR amplification of the *Slitura*NPV-polyhedrin gene using specific primers. The PCR reaction mixture consisted of 1 µl DNA sample, 12.5 Master mix (Fermentas, USA), and 1 µl (20 pmol) of each primers of the polyhedrin gene; forward: 5' TA(CT) GTG TA(CT) GA(CT) AAC AA T 3' and reverse: 5' TTG TA(GA) AAG TT(CT) TCC CA(AG) AT 3' in a final volume of 25 µl. The cycling conditions for polymerase chain reaction were as follows: 1 cycle at 94°C for 5 min., followed by 35 cycles of; 94°C for 30 s; 49°C for 1 min and 72°C for 1 min, and an extension cycle at 72°C for 10 min. Amplification was accomplished using a DNA thermal cycler (Eppendorff, USA). The PCR amplified product was clarified by a PCR clean-up column kit (QIAGEN, Germany) and examined on agarose gel electrophoresis. After the sequencing process, the annotated nucleotide sequence was analyzed using NCBI-BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi), and compared with those of previously reported *Slitura*NPVs. The sequence was deposited in GenBank with the accession number MN649826 Spodoptera_LAST Spodopera.

2.3. Semi-field application test against *S. littoralis*

The experiment was carried out at Nubaria Agriculture Research Station, km. 47 Cairo-Alexandria road during 2017 and 2018. The experimental area was divided into plots of 3.5m x 3m (10.5 m^2), each arranged in a randomized complete block design with three replicates for each treatment. The common soybean variety Giza 111 was planted in mid-May each year and normal agriculture practice was followed. Treatments consisted of (1) Btm alone, (2) *Slitura*NPV alone, (3) a combination of the two entomopathogens and (4) an untreated control. The microbial agents, alone and in combination, were prepared with each of two concentrations, 20% and 30% (W/V), for a total of 7 treatments.

The preparations were sprayed at a rate of 494 l ha^{-1} using a manually pressurized 10 l backpack sprayer after observing the first egg mass in field, and after two weeks, untreated plants were sprayed by water only. Sprays were applied thoroughly to cover the plants. Some egg masses

of *S. littoralis* were taken to the laboratory for hatching and rearing. Everyday, soybean leaves were collected at random from each plot for feeding larvae in the laboratory. Each container contained ten middle-sized leaves, and one egg mass was placed on sprayed leaves after counting the eggs in the mass. Percent mortality of larvae was determined after five days; adjusted mortality rate (P) was calculated based on the following formula [32]:
where P = [P observed - P expected/control / (1- P expected/control] x 100.
At harvest, the seed weights per plot were recorded as proper indicator of yield.

2.4. Root-knot nematode preparation

The root-knot nematode *M. incognita* used in this study was supplied by the Plant Pathology Department, Faculty of Agriculture, Alexandria University. The nematodes were propagated on tomato plants, *Solanum lycopersicum* cv. Alisa, cultivated in sandy clay soil. Inocula of *M. incognita* were prepared by extracting nematode eggs from nine-week-old nematode-infected tomato roots. Active juveniles (J_2) of *M. incognita* were obtained by using Baermann plate technique [33, 34].

2.5. Nematicidal bioassay of Btm

2.5.1. *In vitro* effect

The microbial agent (Btm) was tested on second-stage juveniles (J_2) of *M. incognita* under laboratory conditions. Two rates (20 and 30%, w/v) of each treatment as described earlier were prepared in distilled water. Five replicates of each rate were used with about 150 *M. incognita* juveniles. The assays were conducted in 6-well plastic ELISA plates, with the plates incubated in the lab at room temperature ($27^{o}\pm 2^{o}C$). The control treatment was distilled water. The mortality of J_2s was determined after 24 h, 48 h and 72 h of exposure.

2.5.2. *In planta* effect

The nematicidal activity of Btm towards *M. incognita* was examined *in planta*, using tomato plants (*S. lycopersicum* cv. Alisa). The assay was performed in 15 plastic pots, 15 cm in diameter and 14 cm in depth, filled with 1 kg of an autoclaved mixture of a sandy loam soil (1:1, sand: loam). Three weeks after sowing, each pot was thinned to one plant. The pots were then each inoculated with close to 3000 second-stage juveniles (J_2) of *M. incognita* by running 10 ml of a nematode suspension into an aperture having a 3-5 cm depth around the plant stem. Each treatment consisted of 5 pots and an additional 5 pots were left to serve as controls. The pots were arranged in a completely randomized design, one plant per pot. Infested pots were kept on a bench in the greenhouse and maintained for two months at 25°C±5°C. After this incubation period, the root groups were harvested and assessed for galling (number of galls per root system), and egg masses per root using an aqueous solution of phloxine-B stain (0.15 g l^{-1} tap water) for 15-20 min, and then the roots were rinsed in tap water to remove residual stain [34]. The percent reduction was calculated using the Abbott correction [31].

2.6. Data analysis

Data were analyzed using IBM SPSS software package version 20.0 (Armonk, NY: IBM Corp). The Kolmogorov-Smirnov test was used to verify the normality of distribution of variables. Comparisons of categorical variables between groups were assessed using Chi-square test (Fisher or Monte Carlo). Smirnov, Shapiro, and D'agstino tests were used to verify the normality of distribution of

variables, Student t-test was used to compare two groups for normally distributed quantitative variables, while ANOVA was used to compare more than two groups for normally distributed quantitative variables. This was followed by Post Hoc test (Tukey) for pairwise comparison. Spearman coefficient was used to correlate between quantitative variables. Significance of the obtained results was judged at the 5% level.

3. Results and Discussion

Viral identification involved the use of PCR with specific polyhedrin gene primers, and an amplicon with a molecular size of ~1200 bp was detected (Figure 1). After PCR product purification and sequencing, the DNA sequence was delivered to the GenBank database under the accession number of MN649826. NCBI-BLAST alignment and phylogenetic analysis (Figure 2) indicated that our virus isolate was closely linked to the two isolates (*Spodoptera litura* NPV) of Chinese and Australian origin (AF037262, AF068189), respectively, with a similarity of 86% to viral isolates of *S. litura* from 4 countries. An Indian isolate of *Sl*NPV (Y10254) was considered an outlier for all the examined genes in the constructed tree. On the other hand, when the analysis performed, using the deduced amino acid sequences, it was observed that our isolate showed only 50% similarity with a French isolate of *Sl*NPV (X99073).

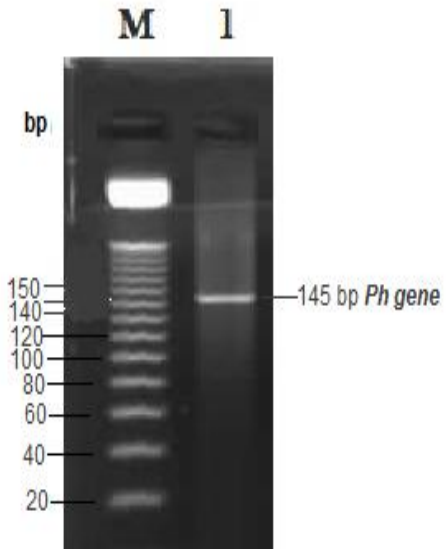

Figure 1. PCR amplification of the polyhedrin gene from the DNA extracted from the cotton leaf worm *S. littolaris* infected with the *Slitura*NPVs

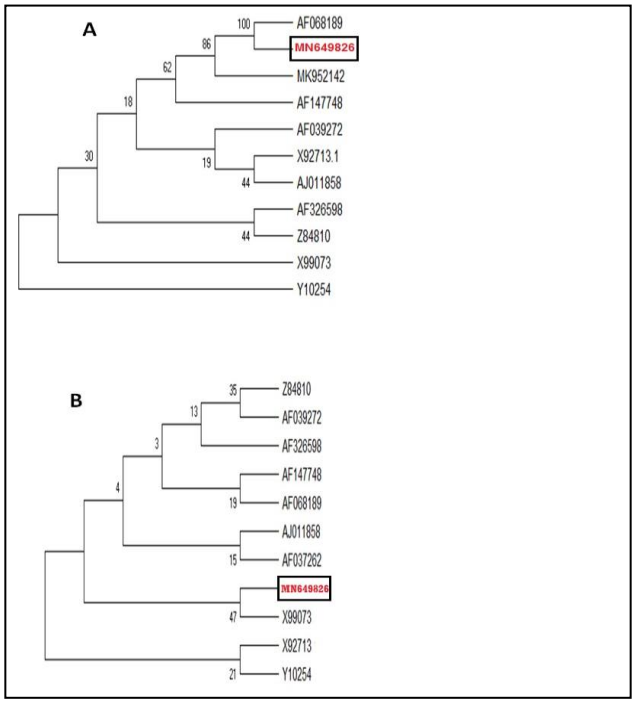

Figure 2. Phylogenetic tree of the isolated polyhedrin genes compared with the others published in the GenBank. The phylogenetic trees were constructed using the Mega 5 program using UPGMA type. A: phylogeny was constructed based on the nucleotide DNA sequence; while B: the phylogeny was constructed based on the deduced amino acid sequences

Data presented in Tables 1 and 2 illustrate the insect stage effect on responses of *S. littoralis* after foliar application by the treatments (Btm, *Slitura*NPV and both *Slitura*NPV and Btm) in 2017 and 2018. The percentage mortality of the treated *S. littoralis* larvae is presented in Table 1. Our results reveal that the impact of the combined *Slitura*NPV and Btm at 20% and 30% gave the highest activity of 87.9% and 94.0%, respectively, without any significant differences between these two concentrations. Mortality of *S. Littoralis* larvae treated with *Slitura*NPV alone, at 20% and 30%, was 73.6% and 77.3%, respectively, which was higher than the corresponding mortality of larvae treated with Btm alone, which was 60.2% and 63.8%, respectively, in 2017. In 2018, similar results were obtained (Table 2).

In both years, the results of all treatments showed a numerical but not statistically significant increase in the mortality percentage of *S. littoralis* larvae with highly significant differences between the type of treatments; but there were no significant differences between the concentrations of the same treatment used in 2017 and 2018 (Tables 1 and 2). According to the high percentage of mortality, the treatments of the combinations of Btm and SliturаNPV were highly effective. In 2017, after application of SliturаNPV or Btm (20 and 30%), the percentage of abnormal pupae of S. littoralis was 11.9 and 5.7%, respectively while it was 25.1% and 22.5% after the treatment with SliturаNPV (20 and 30%). However, the abnormal pupae percentage of the target insect, treated with Btm 20% was 37.1%, and with Btm 30% was 34.4% (Table 1). The pupal abnormality was the highest with Btm treatment, followed by SliturаNPV, and the lowest abnormal

Table 1. Biological parameters of *S. littoralis* after field foliar application in 2017

Biological parameters	**Btm**		***Slitura*NPV**		***Slitura*NPV and Btm**		**Untreated (control)**	χ^2	**p**
	20%	**30%**	**20%**	**30%**	**20%**	**30%**	**0%**		
Total number of eggs	1820	1914	1911	1857	1853	1912	1862		
Number of dead larvae	1095^{a} (60.2%)	1221^{a} (63.8%)	1406^{b} (73.6%)	1436^{b} (77.3%)	1629^{c} (87.9%)	1798^{d} (94.0%)	21^{e} (1.1%)	4714.052^{*}	$\leq 0.001^{*}$
Number of abnormal pupae	675^{a} (37.1%)	658^{a} (34.4%)	480^{b} (25.1%)	418^{b} (22.5%)	221^{c} (11.9%)	109^{d} (5.7%)	3^{e} (0.2%)	1419.329^{*}	$\leq 0.001^{*}$
Number of abnormal adult moths	41^{a} (2.3%)	27^{ab} (1.4%)	18^{bc} (0.9%)	3^{d} (0.2%)	3^{d} (0.2%)	5^{cd} (0.3%)	0^{d} (0.0%)	106.382^{*}	$\leq 0.001^{*}$
Number of normal adult moths	9^{a} (0.5%)	8^{ab} (0.4%)	7^{ab} (0.4%)	0^{b} (0.0%)	0^{b} (0.0%)	0^{b} (0.0%)	1838^{c} (98.7%)	12738.011^{*}	$\leq 0.001^{*}$

Different superscripts are statistically significant at $p<0.05$ in the same row.

Table 2. Biological parameters of *S. littoralis* after field foliar application in 2018

Biological parameters	Btm		*Slitura*NPV		*Slitura*NPV and Btm		Untreated (control)	χ^2	p
	20%	30%	20%	30%	20%	30%	0%		
Total number of eggs	1789	1852	1848	1798	1836	1817	1839		
Number of dead larvae	1228^{a} (68.6%)	1349^{a} (72.8%)	1431^{b} (77.4%)	1437^{b} (79.9%)	1695^{c} (92.3%)	1625^{c} (89.4%)	19^{d} (1.0%)	4944.390*	≤0.001*
Number of abnormal pupae	508^{a} (28.4%)	432^{b} (23.3%)	409^{b} (22.1%)	350^{b} (19.5%)	138^{c} (7.5%)	184^{c} (10.1%)	0^{d} (0.0%)	849.150*	≤0.001*
Number of abnormal adult moths	38^{a} (2.1%)	45^{a} (2.4%)	5^{b} (0.3%)	6^{b} (0.3%)	3^{b} (0.2%)	7^{b} (0.4%)	0^{b} (0.0%)	138.699*	≤0.001*
Number of normal adult moths	15^{ab} (0.8%)	26^{b} (1.4%)	3^{ac} (0.2%)	5^{ac} (0.3%)	0^{c} (0.0%)	1^{c} (0.1%)	1820^{d} (99.0%)	12232.302*	≤0.001*

χ^2: Chi square test
Different superscripts are statistically significant at $p<0.05$ in the same row.

pupae were in the combination treatment. There were no significant differences between different concentrations in the same treatment; with the except of the combination treatment where the result recorded at a high concentration (30%) was less significant than a lower one (20%). On the other hand, there was the difference between the two concentrations of Btm treatment in 2018 (Table 2), hence it was 28.4 and 23.3%, respectively, for Btm (20%) and (30%). Other treatments in 2017 revealed the same significance in pupa abnormality. There were significantly different percentages of abnormal moth after treatment, where the Btm (20 %) treatment gave the highest abnormal moth numbers, while the lowest was the combination (30 %) treatment.

In the two years of this study, significant differences were found in the number of normal and abnormal moths of *S. littoralis* after foliar application with the current treatments. Furthermore, all treatments used showed a clear effect on numbers of dead larvae, abnormal pupae, abnormal moths and normal moths of the target insect *S. littoralis* compared with the untreated control (Tables 1 and 2).

Some of the obvious symptoms in infected larvae, in addition to deformed pupae, are illustrated in Figure 3. These are the results after exposure to both bacteria and virus. The symptoms of viremias differed from those killed by the Btm bacteria. Infection with the virus led to build-up of fluid in the body of the larva and rupture of the cuticle upon touching with a probe; at the beginning of the injury, the bodies had a rubbery consistency. However, the action of the bacteria lead to harder cadavers and dark body colors due to paralysis and cessation of nutrition, in addition to septicemia.

Figure 3. Adverse effects of the tested biological control agents on *S. littolaris*-treated individuals

All biological parameters in both seasons showed that the treatment was very effective in the case of larval mortality, while it has had a weak effect on abnormal pupae or moths and vice versa. In addition to the presence of different significance between two seasons, the percentage of larval mortality was higher in the second season than in the first one, as shown in Table 3. There was no correlation between the number of mortality larvae and the number of abnormal pupae in the two seasons, where the Spearman coefficient was -0.250.

Table 3. Biological parameters of *S. littoralis* after field foliar application in 2017 and 2018 seasons

Biological parameters	2017	2018	χ^2	p
	No. (%)	No. (%)		
No. of dead larvae				
Btm at 20%	1095 (60.2%)	1228 (68.6%)	28.265*	≤0.001*
Btm at 30%	1221 (63.8%)	1349 (72.8%)	35.548*	≤0.001*
*Slitura*NPV at 20%	1406 (73.6%)	1431 (77.4%)	7.566*	0.006*
*Slitura*NPV at 30%	1436 (77.3%)	1437 (79.9%)	3.652	0.056
*Slitura*NPV and Btm at 20%	1629 (87.9%)	1695 (92.3%)	20.107*	≤0.001*
*Slitura*NPV and Btm at 30%	1798 (94.0%)	1625 (89.4%)	26.223*	≤0.001*
Untreated	21 (1.1%)	19 (1.0%)	0.078	0.781
No. of abnormal pupae				
Btm at 20%	675 (37.1%)	508 (28.4%)	30.935*	≤0.001*
Btm at 30%	658 (34.4%)	432 (23.3%)	55.904*	≤0.001*
*Slitura*NPV at 20%	480 (25.1%)	409 (22.1%)	4.638*	0.031*
*Slitura*NPV at 30%	418 (22.5%)	350 (19.5%)	5.098*	0.024*
*Slitura*NPV and Btm at 20%	221 (11.9%)	138 (7.5%)	20.420*	≤0.001*
*Slitura*NPV and Btm at 30%	109 (5.7%)	184 (10.1%)	25.205*	≤0.001*
Untreated	3 (0.2%)	0 (0.0%)	2.965	0.250
No. of abnormal moth				
Btm at 20%	41 (2.3%)	38 (2.1%)	0.070	0.792
Btm at 30%	27 (1.4%)	45 (2.4%)	5.213*	0.022*
*Slitura*NPV at 20%	18 (0.9%)	5 (0.3%)	6.963*	0.008*
*Slitura*NPV at 30%	3 (0.2%)	6 (0.3%)	1.102	0.336
*Slitura*NPV and Btm at 20%	3 (0.2%)	3 (0.2%)	0.000	1.000
*Slitura*NPV and Btm at 30%	5 (0.3%)	7 (0.4%)	0.445	0.505
Untreated	0 (0.0%)	0 (0.0%)	–	–
No. of normal moth				
Btm at 20%	9(0.5%)	15 (0.8%)	1.616	0.204
Btm at 30%	8(0.4%)	26 (1.4%)	10.226*	0.001*
*Slitura*NPV at 20%	7(0.4%)	3 (0.2%)	1.473	0.344
*Slitura*NPV at 30%	0(0.0%)	5 (0.3%)	5.171*	0.029*
*Slitura*NPV and Btm at 20%	0(0.0%)	0 (0.0%)	–	–
*Slitura*NPV and Btm at 30%	0(0.0%)	1 (0.1%)	1.053	0.487
Untreated	1838(98.7%)	1820 (99.0%)	0.527	0.468

χ^2 = Chi square test

* = Statistically significant at $p \leq 0.05$

Table 4 shows a comparison between the different treatment groups based on seed weight. In 2017, the mean weight ranged from 803.3±12.6 g/plot when treated with Btm at 20% to 847.7±4.0 g/plot when treated by the combination treatment at the 30% rate, compared with the untreated which was 545.7d±5.5 g/plot; and ranged from 807.0±4.4 g/plot when treated with Btm at 20% to 842.3±2.5 g/plot when treated with *Slitura*NPV and Btm at 30%, compared to the untreated which was 560±2.0 g/plot in 2018. Statistically, according to ANOVA test significant differences were showed in both years.

Treatments with Btm showed high efficacy against Juvenile (J_2) *M. incognita* after 24, 48 and 72 h of exposure in vivo, compared with the control (Table 5). This illustrated a highly significant difference in the percentage of mortality compared with the untreated control, which were the same for two concentrations (20 and 30%) after 24, 48 h at 87.5%, 90.6%, respectively and were 94%, 97% after 72 h for the two concentrations.

Table 4. Comparisons between the different studied treatments according to seed weight

Treatment	Weight of seed by g/plot				T	p
	2017		2018			
	Mean ± S.D.	% Increasing	Mean ± S.D.	% Increasing		
Btm at 20%	803.3^{c}±12.6	47.2	807.0^{d}±4.4	44.1	0.477	0.658
Btm at 30%	829.3^{b}±1.5	52.0	820.0^{c}±1.0	46.4	8.854*	≤0.001*
*Slitura*NPV at 20%	822.7^{b}±2.5	50.8	808.0^{d}±7.5	44.3	3.192*	0.033*
*Slitura*NPV at 30%	829.0^{b}±3.6	51.9	827.3^{bc}±1.5	47.7	0.737	0.502
*Slitura*NPV and Btm at 20%	846.0^{a}±3.6	55.0	836.0^{ab}±2.0	49.3	4.201*	0.014*
*Slitura*NPV and Btm at 30%	847.7^{a}±4.0	55.3	842.3^{a}±2.5	50.4	1.940	0.124
Untreated (control)	545.7^{d}±5.5		560.0^{e}±2.0		4.237*	0.013*
F	1029.080*		2262.120*			
P	<0.001*		<0.001*			

Data with the same letter(s) in each column, are not significantly different

Table 5. Evaluation of the nematicidal effects of *Bacillus thuringiensis* on mortality (M%) of juvenile (J2) *Meloidogyne incognita* for different exposure periods

Treatments	Juvenile (J2) mortality					
	24 h		48 h		72 h	
	L	M %	L	M %	L	M %
Control (distilled water)	6.4 ±1.08^{a}	0	6.4± 1.09^{a}	0	6.4±1.57^{a}	0
Btm at 20%	0.8 ±0.24^{b}	87.5	0.6±1.38 ^{b}	90.6	0.4±1.20^{b}	94
Btm at 30%	0.8± 0.44^{b}	87.5	0.6±0.47 ^{b}	90.6	0.2 ±1.43^{b}	97

Data of means with the same letter(s) in each column, are not significantly different at P ≤0.05.
M% (% Mortality) = [(Total number of J2 in control - No. of alive (L) J2 in treatment) ÷ No. of Total J2 in control] × 100

In Table 6, there were significant differences in the mean number of the root fresh weight, number of galls/ roots, number of egg masses/ root and number of egg /egg masses between the two Btm concentrations and the untreated control and among each other, the treatment with Btm 30%

showing the highest effectiveness in all parameters. The percentage reduction due to Btm at 20% and Btm at 30% ranged from 77%, 83% for galls/root; 67%, 80% for egg masses/root and 39%, 61% egg/egg masses.

Table 6. The effect of *Bacillus thuringiensis* on root-knot nematode *M. incognita* in tomato plants

Treatments	Nematode parameters and percent reduction (R)						
	Root fresh wt. (g)	Galls per Root	% Reduction	Egg masses per root	% Reduction	Eggs per egg mass	% Reduction
Untreated (control)	32.0[c]	129.7[a]	0	441.2[a]	0	510[a]	0
Btm at 20%	57.0[b]	30[b]	77	145[b]	67	312[b]	39
Btm at 30%	65.0[a]	21.5[c]	83	88[c]	80	200[c]	61

Data are means of 10 replicates, means with the same letter(s) in each column are not significantly different

The bacterium (Btm) and virus (*Slitura*NPV) gave the highest larval *S. litoralis* mortality when used in combination at 30%. The other treatments in order of decreasing efficacy were Btm/*Slitura*NPV combined at 20%, *Slitura*NPV alone at 30%, then 20%, and finally Btm alone at 30 and 20%. Çakici *et al*. [35] mentioned that *B. thuringiensis* subsp. *kurstaki* (MnD) and *B. thuringiensis* subsp. *kurstaki* (BnBt) were found to be the foremost successful of the strains tested, causing 100% mortality within 10 days of treatment, whereas in this study *Bacillus thuringiensis* serovar *mexicanensis* (Btm) had a mean efficacy of 68.3% efficacy, in both seasons, only 5 d after treatment. The efficacy appears to be due to the formula, which provided more protection to spores and crystals under conditions of continuous sun exposure [27, 36].

Nathan *et al*. [37] found that bacterial Cry toxin affected the lactate dehydrogenase (LHD) activity and this effect was more severe at low concentrations in *Cnaphalocrocis medinalis* (rice leaf folder). Similar results occurred in our study, hence the lower rate had better efficacy.

Sarker and Mahbub [38] reported that several *B. thuringiensis* strains were very effective for suppressing many insect pests. In addition, Konecka *et al*. [39] obtained results that are consistent with another effect of Bt toxicity in lepidopteran species.

Abad *et al*. [40] indicated the importance of NPV in controlling insect pests, especially lepidopteran pests. As the same in the studies of Eroglu *et al*. [41], when testing neonate, 3rd, and 5th instar larvae of *H. armigera*, the highest dose (8×10^6 PIBs ml^{-1}) of *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV-O1) caused 92, 88 and 57% mortality, respectively, within 14 days.

Raghunandan *et al*. [42] revealed that NPV infected *S. frugiperda* larvae on maize could cause high mortality ratio after 3 days; while Luna-Espino *et al*. [43] demonstrated that the biological efficacy of the local Mexican *Se*MNPV isolates was identical to the activity of the foreign isolate (SeUS2), and these native viruses are appropriate for the microbial control of *S. exigua* in Mexico. The native virus gave 92-100% mortality whereas the exotic virus gave only 78%.

Regarding nematode efficacy of Bt, Mohammed *et al.* [44] demonstrated that Bt7N reduced the number of egg masses by 78%, and the number of eggs by 84% compared to their control. These results were similar to those shown in Table 6, which had 80 and 67% from treatment with Btm at both 30% and 20% rates. Ashoub and Amara [45] confirmed that all their tested bacteria (*Bacillus thuringiensis, Pseudomonas fluorescens,* and *Rhizobium leguminosarum*) showed a significant efficacy in suppressing *M. incognita* in vivo and *in vitro* [46]. In evaluating three Egyptian-isolates

of *B. thuringiensis*, significant positive correlations between percentage reduction in the nematode populations and bacterial doses were found. *Bacillus thuringiensis* was also reported to suppress populations of *M. javanica* and *M. Incognita* [47-49]. Results in our study agree with the earlier data by Yamamoto and Powell [50] and Fernandes *et al*. [51]. However, their *Bacillus* spp. isolates had no potential as bionematicides for controlling of *M. incognita* in common bean.

4. Conclusions

Both NPV and Btm have considerable potential in managing *S. littoralis* populations. The combination provides a small incremental increase, but the economic implications remain to be better defined. According to the data in our study, Btm could also provide an excellent tool for management of root-knot nematodes. A serious problem for efficacy of NPV or bacteria against foliage feeding pests is maintaining sufficient persistence under the harsh, sunny conditions. This problem is alleviated by a new formulation modified for use in the present study. Synergism between Btm and NPV in our new formulation seemed to have good efficacy in the field. With lepidoptera only, the application of viral-insecticide is preferable because of the rapid effect of NPV on lepidopteran insect pests, especially Egyptian cotton leafworm, beet armyworm, and fall armyworm, which are new invasive pests in Africa and Asia.

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