El-Helaly et al. Egyptian Journal of Biological Pest Control (2020) 30:111
https://doi.org/10.1186/s41938-020-00314-0

Egyptian Journal of Biological Pest Control

RESEARCH Open Access

# Impact of emamectin benzoate on nucleopolyhedrosis virus infectivity of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae)

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## Abstract

**Background:** Attempts based on increasing the efficacy of Baculovirus and/or reducing the application concentration of synthetic insecticides through integrated lepidopteran management are appreciated role for conserving the environment. Impact of the multiple nucleopolyhedrosis virus (*Spli*MNPV) with emamectin benzoate (Em) against the cotton leaf worm, *Spodoptera littoralis*, was examined to identify the effective strategy for applying both agents in the control program successfully.

**Main body:** The $LC_{50}$ and $LC_{90}$ were drastically decreased from $1.9 \times 10^6$ and $1.0 \times 10^{10}$ PIB/ml in *Spli*MNPV treatment to reach $8.87 \times 10^1$ and $1 \times 10^4$ PIB/ml, respectively in the *Spli*MNPV concentrations + Em $LC_{25}$ treatment. This interaction was considered as potentiation. Larvicidal activity of Em was highly increased by Em concentrations + *Spli*MNPV $LC_{25}$ treatment than the separately Em treatment; however, this interaction was considered as additive. Moreover, the mixture treatment (*Spli*MNPV $LC_{99}$ + Em $LC_{50}$) provided almost full protection of viral pathogenicity up to 48 h at natural exposure periods. Furthermore, the mixture treatment had a negative impact on the insect survival and reproduction of treated individuals.

**Conclusion:** Results indicated that the virus infectivity was increased by a mixture treatment of *Spli*MNPV + Em in particular facing UV sunlight, which causes virus degradation as well as reduced the effective doses of Em. These findings suggest that this simultaneous treatment maybe an effective technique to be applied in *S. littoralis* control strategy.

**Keywords:** Baculovirus, Emamectin benzoate, *Spodoptera littoralis*, Larvicidal activity, Bio-pesticide interaction

## Background

The use of insecticides to control the cotton leaf worm, *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae), and other lepidopteran pests has an adverse impact on the ecosystem and its fauna, devastation of natural enemies, and development of insect resistance (Ahmad et al. 2009). Research into alternative strategies for limited use and/or reducing the dose application with that type of synthetic pesticide might be beneficial to save the environment as well as successfully suppress the insect population (Lalouette et al. 2016). Additionally, developing the efficacy of bio-pesticides such as natural enemies and pathogens, which are considered environmentally friendly methods to be more effective against insect pests, is appreciated. Baculoviruses is considered one of the bio-control methods against lepidopteran insects, which is commercially produced in some areas with a trade name (Sayed et al. 2020). The cotton leaf worm *S. littoralis* multiple nucleopolyhedrosis virus (*Spli*MNPV) belongs to Baculoviruses that may be used

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as a promising agent for its bio-control (Yang et al. 2012). Admittedly, sunlight UV radiation has adversely affected *Spli*MNPV persistence in the environment, causing pyrimidine dimers of the viral DNA chain, and rapid degradation of the virus (Yoon et al. 2000). Efforts have been made to protect the pathogenicity of *Spli*NPV against UV radiation, using various substances. Ignoffo and Batzer (1971) and Rabindra et al. (1989) proposed coax boric acid acted as UV protectants, respectively. Nevertheless, the researches into appropriate techniques to improve Baculovirus infectivity rather than UV protectants were also employed. Consequently, attempts have been conducted to boost NPV infectivity, such as juvenile hormones (Liao et al. 2016) and gamma irradiation (Sayed and El-Helaly 2018). Moreover, synthetic insecticides have been suggested as synergic agents when combined with Baculoviruses such as spinosad with both *Sf*NPV and *Pg*NPV on *Spodoptera frugiperda* (J.E. Smith) and *Pectinophora gossypiella* (Saund.), respectively (Méndez et al. 2002; Jackson et al. 2014); methoxyfenozide with *Spli*MNPV on *S. littoralis* (Pineda et al. 2009); and azadirachtin with SfMNPV on *S. frugiperda* (Nathan and Kalaivani 2006). Similarly, emamectin benzoate (Em) may interact with *Spli*NPV for improving *S. littoralis* control. Em is isolated from soil actinomycete, *Streptomyces avermitilis*, that is occurring in nature and is known to be an important natural chemistry insecticide against lepidopteran pests (Jansson et al. 1996). The key action of Em is to cause permanent paralysis on the nerve transmitter (Jansson and Dybas 1998). Given the high toxicity of Em on Lepidoptera, it is less harmful on most beneficial arthropods (e.g., honey bees, parasitoids, predators (Wolterink et al. 2012). However, its extensive use and lethal doses applied may have deleterious effect on biodiversity.

Thus, the aim of the study was to identify the Em-*Spli*NPV interaction in order to improve *S. littoralis* control. Besides, the larvicidal activity of the Em + *Spli*MNPV mixture to elucidate the pathogenicity of the virus and its persistence against UV sunlight was examined.

## Materials and methods

### Insect colony

A laboratory stock culture of the cotton leaf worm *S. littoralis* was initiated from eggs samples of infested tomato plant at Giza region, Egypt. Collected eggs were maintained under laboratory conditions of 25 ± 2 °C and 65 ± 5% RH inside a plastic cage until hatching. The newly hatched larvae were transferred to a larval rearing cage (40 × 40 × 10 cm) containing castor plant leaves, *Ricinus communis* as food for adaptation until pupation. The upcoming larval colony was maintained on a semi synthetic diet (Shorey and Hale 1965).

### Bioassay of emamectin benzoate toxicity and *Spli*MNPV pathogenicity

Local isolate of *Spli*MNPV was used in the bioassay experiments. The pathogenicity of *Spli*MNPV was evaluated on the 2nd instar larvae of *S. littoralis*. Eight different virus concentrations from $1 \times 10^2$ to $1 \times 10^9$ BIPs/ml (polyhedral inclusion bodies/ml) were prepared from the stock concentration ($2.3 \times 10^9$ PIB/ml); it was diluted in distilled water to adjust the experimental concentrations. Em (1.9% EC) was provided by Elhelb, Pesticides and Chemicals Company. Eight concentrations, 0.01, 0.1, 0.3, 0.5, 0.7, 1.0. 2.0, and 4.0 ppm, were prepared, using distilled water. The concentration-mortality response of both *SpliMNPV* and Em was calculated, following the diet surface contamination technique (Cisneros et al. 2002); 2 ml of each treatment was spread on the surface special plate divided into 50 cells containing 50 ml semi-artificial diet. Distilled water was used for untreated control experiments. Each treatment was repeated in 5 replicates with 50 larvae each. Mixture treatments (*Spli*NPV + Em) were conducted, using different *Spli*MNPV concentrations + $LC_{25}$ of Em and vice versa, following the methods mentioned above. Mortality responses of larvae were recorded daily in each treatment. Comparing evaluation of the mixture treatment to the single ones was carried out according to (Mansour et al. 1966) as follows:

Co-toxicity factor (%) = (% Observed mortality – % Expected mortality/% Expected mortality) 100, where the factor (– 20 to + 20) is additive, + 20 or more is potentiation, and – 20 or more is antagonism.

### *Spli*MNPV protection against UV irradiation

An experimental area (500 $m^2$) of tomato was set up in the spring season with the conditions of 24–27 °C, 61–65% RH, and daylight was approximately 14 h. At the time of field application, $LC_{50}$ concentration of Em and $LC_{99}$ of *Spli*MNPV were prepared and kept in the fridge till spraying. The virus and Em were thoroughly mixed (v/v) together, and the measured volume was used into a hand sprayer. Virus suspension and separate treatments were applied on tomato foliage, using hand sprayer (1 l). Leaves were randomly collected from treated and untreated plants at 0, 10, 24, 48, 96, and 168 days post application and kept individually. Every leaf was placed in a glass bottle that allowed 10 neonate larvae to feed for 48 h before being transferred to fresh leaves from the same treatment. Larval mortality was daily recorded as descripted by Shapiro et al. (2008). The experiments were repeated in 5 replicates.

### Latent effect of Em and/or *Spli*MNPV on *S. littoralis* biology

The impact of sub-lethal concentrations of *Spli*MNPV and Em either alone or in a mixture (Em 0.05 ppm +

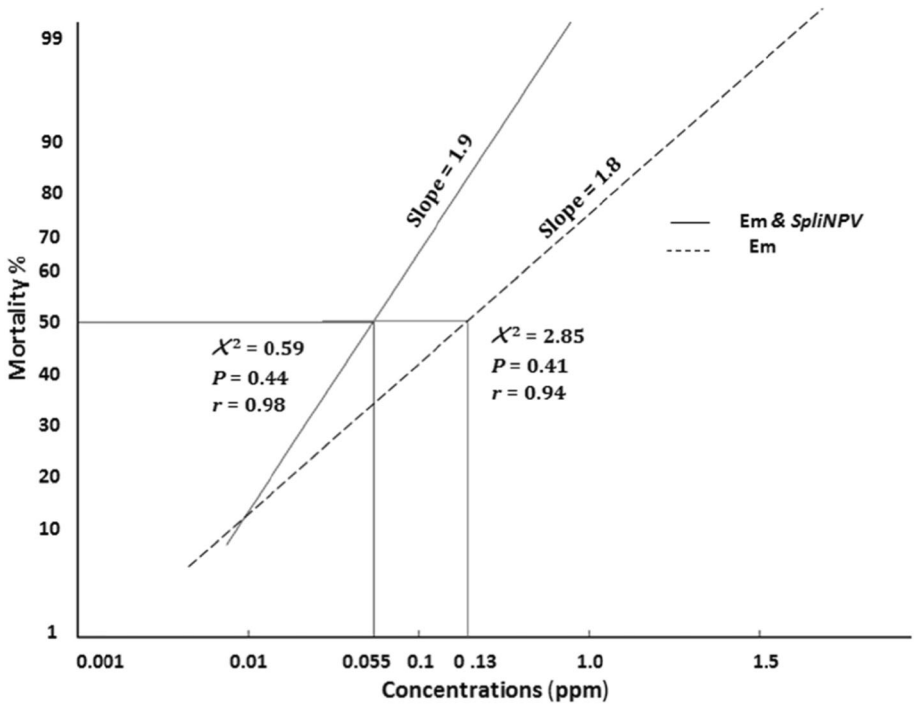


**Fig. 1** % mortality of *Spodoptera littoralis* 2nd instar larvae treated with different concentrations of Em (ppm) and (Em concentrations ppm) + *Spli*MNPV $1 \times 10^4$ PIB/ml

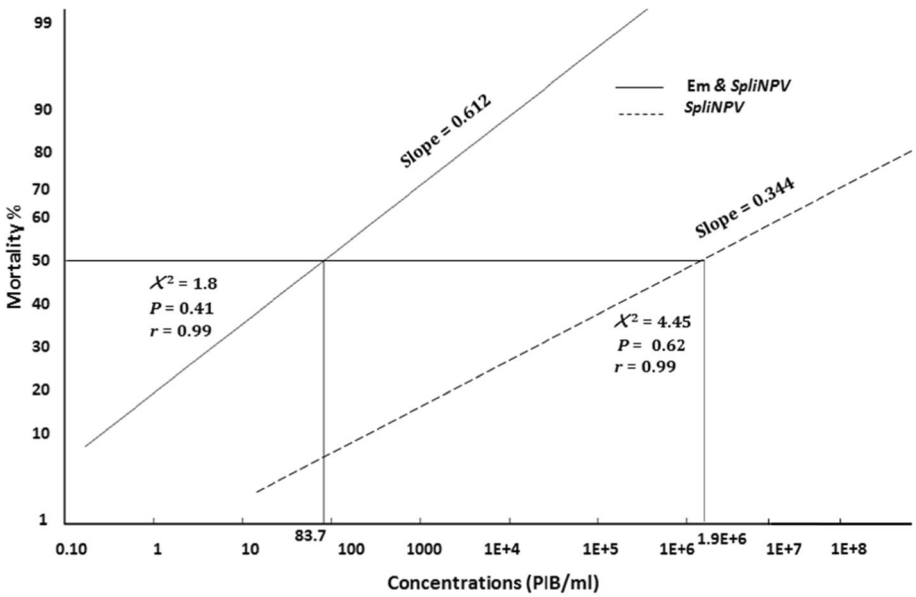


**Fig. 2** % mortality of *Spodoptera littoralis* treated as 2nd instar larvae with different concentrations of *Spli*MNPV (PIB/ml) and (*Spli*MNPV concentrations PIB/ml) + Em 0.05 ppm)

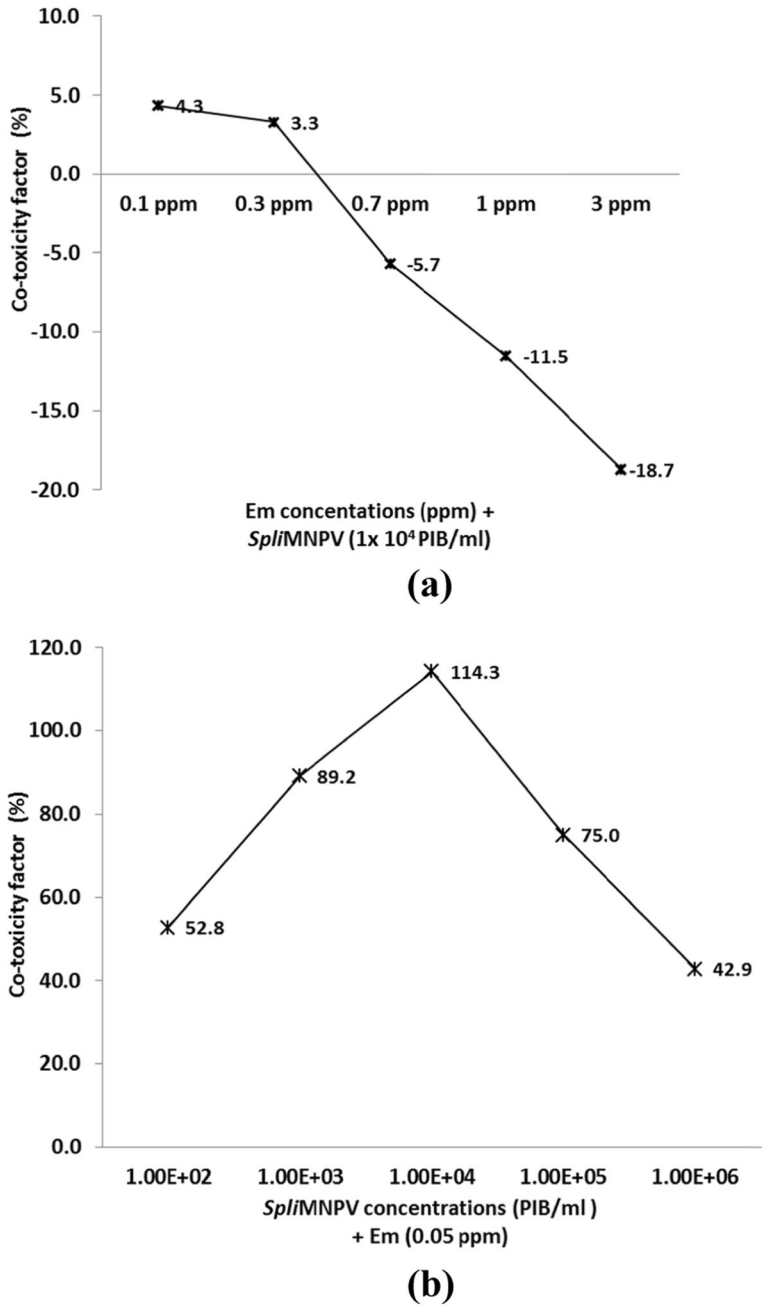


**Fig. 3** % Co-toxicity factors of *Spodoptera littoralis* 2nd instar larvae treated with the two different mixture treatments **a** *Spli*MNPV concentrations (PIB/ml) + Em (0.05 ppm) and **b** Em concentrations (ppm) + *spli*MNPV ($1 \times 10^4$ PIB/ml)

*Spli*MNPV $1 \times 10^4$ BIPs) treatment on the biological parameters of *S. littoralis* 2nd instar larvae was evaluated. Survival rate was estimated through the larvae survived at various treatments in comparison with the control; also, larval duration, pupal period, and adult longevity were included. Moreover, the daily eggs laid by emerged females and their hatchability were recorded. Each treatment was replicated 5 times, and every replicate contained 50 individuals.

### Statistical analysis

Mortality data of either *Spli*MNPV + Em or the separated treatments were analyzed, using Probit analysis, slope. $LC_{50}$ was calculated according to Finney (1971). Average rates of reduction in virus activity expressed in mortality percentages and the percentages of original activity remaining (% OAR) were conducted according to Muro and Paul (1985) and were calculated as the formula of Sun's model at each exposure time (Sun et al. 2004). The data of biological

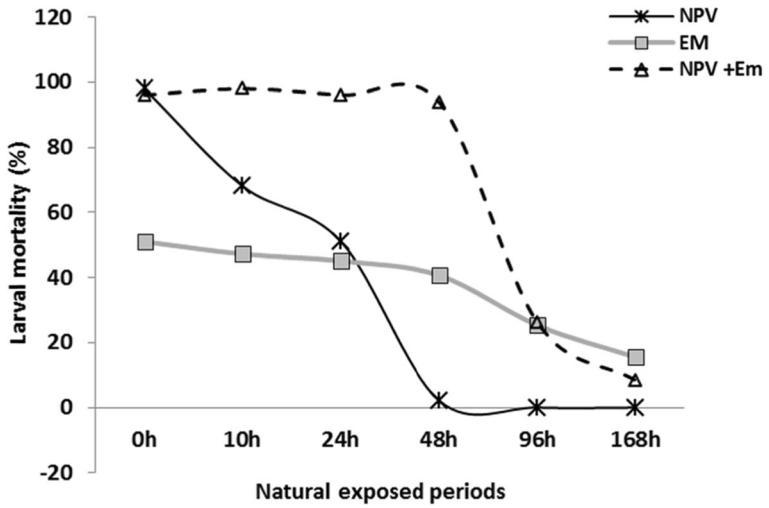


**Fig. 4** Average rates of reduction in the *spli*NPV activity expressed in mortality percentages of *Spodoptera littoralis* 2nd larvae treated with *Spli*MNPV, Em, and Em $LC_{50}$ + *Spli*MNPV $Lc_{99}$, all exposed to different natural sunlight periods

studies were analyzed by the analysis of variance (ANOVA) technique, and the means were analyzed, using Duncan' s multiple range test ($P$ = 0.05) (Steel and Torrie 1960). The potential of Em to prolong the virus persistence was analyzed as described by Muro and Paul (1985).

## Results and discussion

Toxicity effect of Em on the 2nd instar larvae of *S. littoralis* is shown in Fig. 1. The $LC_{50}$ and $LC_{90}$ were estimated at 0.13 and 0.68 ppm, respectively. The results indicated that the larvae of *S. littoralis* was highly sensitive to Em in comparison to those stated by Bengochea et al. (2014) on *S. exigua* and Ahmad et al. (2003) on *Helicoverpa armigera*. Furthermore, the data of *Spli*NPV pathogenicity is illustrated in Fig. 2; the concentrations of $1.9 \times 10^6$ and $1.0 \times 10^{10}$ PIB/ml of SpliNPV were reported in $LC_{50}$ and $LC_{90}$, respectively. The *Spli*NPV pathogenicity was significantly improved by adding 0.1 Em concentration, where the $LC_{50}$ and $LC_{90}$ concentrations of the mixture decreased to $8.87 \times 10^1$ and $1 \times 10^4$ PIB/ml, respectively (Fig. 2). The co-toxicity factors measured in this experiment indicated that they were more than + 20 at all tested concentrations and could be considered as potentiation. This positive interaction resulted from the overall mortality rate of Em and

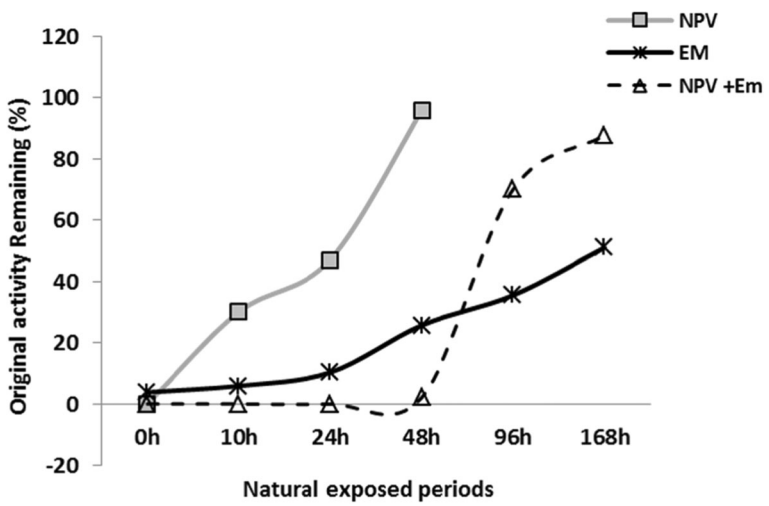


**Fig. 5** Percentage reduction in the *spli*NPV activity expressed in original activity remaining (OAR) of *Spodoptera littoralis* 2nd instar larvae treated with *Spli*MNPV, Em, and Em $LC_{50}$ + *Spli*MNPV $Lc_{99}$ all exposed to different natural sunlight periods

*Spli*MNPV separately was lower than that for their mixture (Fig. 3a). The synergistic effect of the mixture treatment can be referred to a high toxic effect of Em against insect mid gut cells (Aljabr et al. 2014) that may increase the penetration of viral bodies into the nucleus and/or an immunity degradation in the treated larvae by Em (Birah et al. 2008; Zamora-Avilés et al. 2013) and may support the pathogen infection. Meanwhile, the data of other (*Spli*MNPV 1 × $10^4$PIB/ml + Em concentrations) treatment revealed that the toxicity of Em increased in the mixture than in separate ones, where $LC_{50}$ and $LC_{90}$ decreased to 0.055 and 0.026 ppm, respectively (Fig. 2). The co-toxicity factors of this experiment ranged from − 20 to + 10 at the tested concentrations and could be considered as additive (Fig. 3b). This interaction was based on the overall mortality rate of Em and *Spli*MNPV separately that was approximately equal with the mortality rate of their mixture. The present findings contradict with those reported an antagonistic effect when chemical pesticides combined with NPV, for instance cartap hydrochloride with *S. littura* granulovirus *Splt*GV (Baculovirus) on *S. littura* (Subramanian et al. 2005) and carbamate methomyl with *Autographa californica* nucleopolyhedrovirus *Ac*NPV on *Heliothis virescens* (McCutchen et al. 1997). Recently, Dader et al. (2020) identified a synergy of emamectin with *Ac*MNPV and *Spli*NPV on *S. exigua* and *S. littoralis* when they were sequentially feeding of NPV where the $LC_{50}$ of Em was followed by the $LC_{50}$ of NPV.

Field test presented higher rates of virus protection against natural sunlight in the (*Spli*MNPV + Em) treatment than the separate ones (Figs. 4 and 5). Percentages

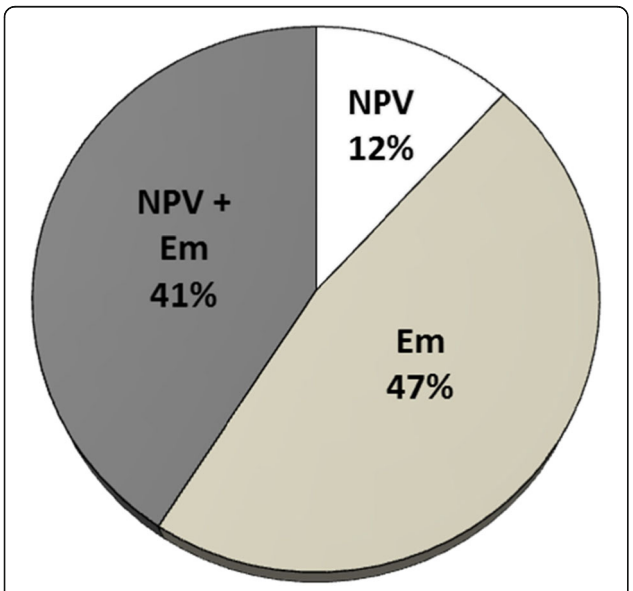


**Fig. 6** Median lethal inactivation time ($LIT_{50}$) of *Spodoptera littoralis* 2nd instar larvae treated with *Spli*MNPV, Em, and Em $Lc_{50}$ + *Spli*MNPV $Lc_{99}$ all exposed to different natural sunlight periods

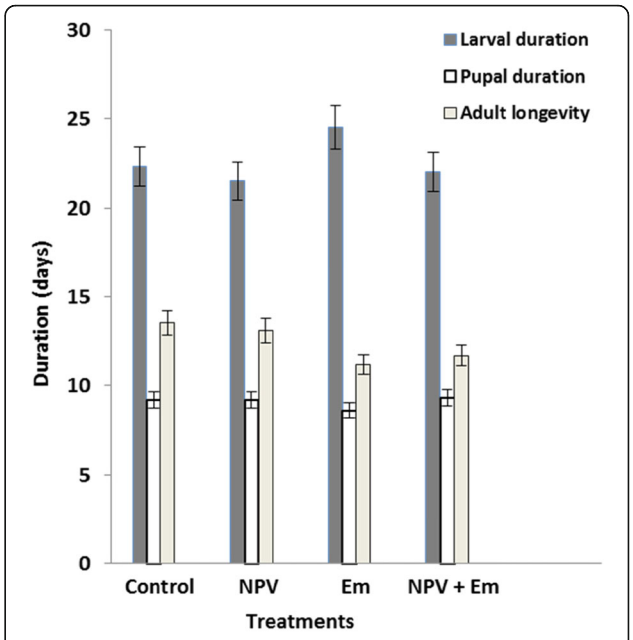


**Fig. 7** Average larval duration, pupal duration, and adult longevity of *Spodoptera littoralis* stages previously treated as 2nd instar larvae with *Spli*MNPV Em 0.05 ppm, Em 1 × $10^4$ BIPs and Em 0.05 ppm + SpliMNPV 1 × $10^4$ BIPs

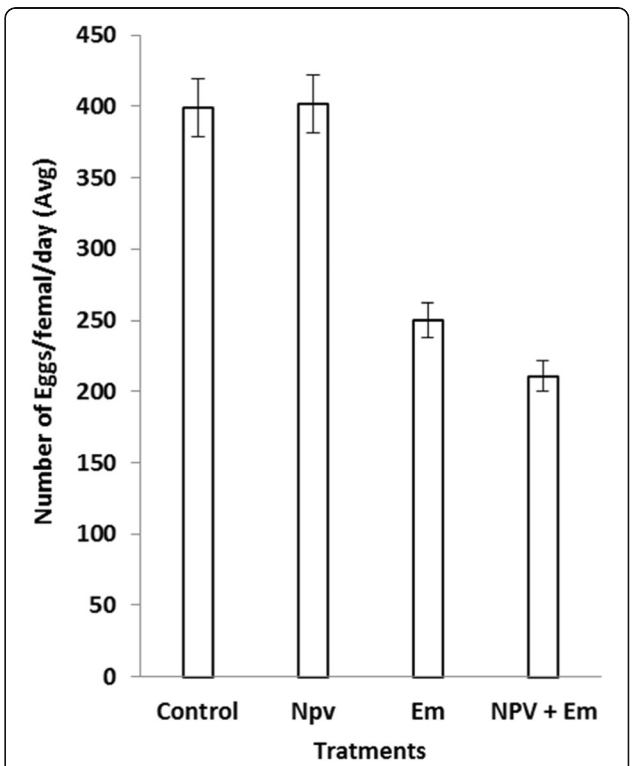


**Fig. 8** Average number/eggs/day of *Spodoptera littoralis* female previously treated as 2nd instar larvae with *Spli*MNPV Em 0.05 ppm, Em 1 × $10^4$ BIPs, and Em 0.05 ppm + SpliMNPV 1 × $10^4$ BIPs

of larval mortality were significantly high at the exposure periods from 10 to 48 h than *Spli*MNPV separately. The original activity remaining (OAR) revealed that Em could extent the viral persistence since the OAR was similar until 48 h of natural sunlight exposure time as compared to the *Spli*MNPV separately. The percentages of larval mortality in the mixture treatment were significantly reduced at 69 and 168 h. These results could refer to those reported in a degradation degree of Em under UV light, where its photodegradation was relatively high (Zhu et al. 2011). The median lethal inactivation time ($LIT_{50}$) showed slightly higher in the mixture treatment than *Spli*MNPV separately (Fig. 6). It gave in ascending potency 3.5-folds that means a high preservation to the virus. In the obtained results on the interaction of Em + *Spli*MNPV in mixture, synergistic effect identified may lead to many benefits, enhancing the *S. littoralis* control in short-term application, delaying the development of insect resistance, and replacing the conventional pesticides with that environmentally friendly product.

The impact of mixture treatment on insect survival is shown in Fig. 7. The data showed that the reduction in larval period in Em was significantly shorter than in *Spli*MNPV and in mixture treatment; the reduction in the pupal duration was non-significant among various treatments. Additionally, the periods of adult longevity in Em and mixture treatments were significantly shorter than those observed in *spli*MNPV and control treatments. Such variability in insect survival via Em treatment may be attributed to the direct action of Em on insect cell physiological functions (Rothman and Myers 1996). Moreover, the average numbers of daily eggs laid/female that treated as larvae were significantly lower in Em and mixture tratments than in *spli*MNPV and control treatments (Fig. 8), while the hatched egg reductions were significantly higher in all tested treatments than the control (Fig. 9). These findings are consistent with Lalouette et al. (2016) who reported hormonal changes in the insect pests when sub lethal concentrations were applied.

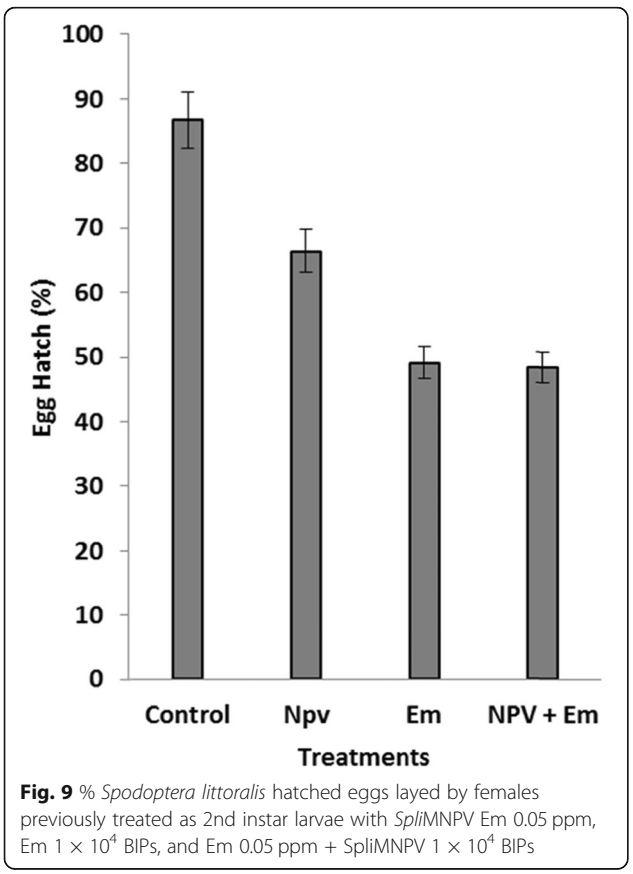


**Fig. 9** % *Spodoptera littoralis* hatched eggs layed by females previously treated as 2nd instar larvae with *Spli*MNPV Em 0.05 ppm, Em 1 × $10^4$ BIPs, and Em 0.05 ppm + SpliMNPV 1 × $10^4$ BIPs

## Conclusion

Baculovirus and Em are safe promising tools against *S. littoralis.* The larvicidal activity of Em + *Spli*MNPV $LC_{25}$ increased than the single Em treatment. Evidently, the treatment of SpliMNPV $LC_{99}$ + Em $LC_{50}$ effectively protected the *Spli*MNPV against natural sunlight. The synergistic effect of the mixture treatment improved the pathogenicity of *Spli*MNPV.

### Abbreviations

*Spli*MNPV: *Spodoptera littoralis* multiple nucleopolyhedrosis virus; PIB: Polyhedral inclusion bodies; Em: Emamectin benzoate; $LC_{50}$: Median lethal concentration or lethal concentration 50; $LC_{99}$: Lethal concentration 99; UV: Ultraviolet radiation

### Acknowledgements

The authors wish to express their gratitude to Prof. Dr. Adel Hatem and Dr. Gamal Hassan from Plant Protection Institute Research, Ministry of Agriculture, Giza, Egypt, for helpful discussion and technical support of the manuscript.

### Authors' contributions

AE and WS carried out the bioassay and biological studies. AE and HE conducted the isolation and propagation of the virus. WS carried out the filed treatment and analyzed the data. AE, WS, and HE contributed in the experimental design and writing the manuscript. All authors read and approved the final manuscript.

### Funding

No specific fund was indicated in this study.

### Availability of data and materials

The authors declare that they have no objection to the availability of data and materials.

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

All authors agreed to publish this manuscript.

### Competing interests

The authors declare that they have no competing interests.

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Received: 22 June 2020 Accepted: 31 August 2020
Published online: 10 September 2020

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