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EFFICACY AND VIRULENCE OF *Spodoptera littoralis* NUCLEOPOLYHEDROVIRUS on *S. littoralis* LARVAL FEEDING AND SUSCEPTIBILITY

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ABSTRACT: Entomopathogenic baculoviruses have long been recognized as vital environmentally safe alternatives to chemical pesticides. A study for investigating the virulence and susceptibility of *Spodoptera littoralis* larvae to *S. littoralis* nucleopolyhedrovirus (SpliNPV) isolate was carried out. Bioassay experiments on the 1st and 2nd larval instars using droplet feeding method revealed that polyhedral for 50% or 90% mortality of the 2nd larval instar of *S. littoralis* was significantly lower compared with the 1st instar. While, the 1st instar was killed faster than the 2nd one especially with the higher concentration. The susceptibility of the infected 2nd instar when feeding on different hosts showed that larvae were more susceptible when feeding on eggplant leaves followed by lettuce leaves, artificial diet and tomato leaves as shown by larval weight and reduction in feeding. The reduction in both larval weight and feeding were significantly higher with $1x10^7$ PIB/ml than $1x10^4$ PIB/ml in the majority of cases. *S. littoralis* larvae showed more than 2 times of tolerant to the 3rd passage of SpliNPV on the level of LC_{50} and more than 18 times tolerant on the level of LC_{90}. The tolerant levels of *S. littoralis* larvae for the 3rd passage of virus was associated with more than 20% increase in time taken for kill with no changes in DNA patterns regarding subsequent passages in *S. littoralis* larvae using a restriction enzyme *Eco*RI.

Key words: *S. littoralis*, SpliNPV, susceptibility, plant hosts, DNA profile.

INTRODUCTION

Baculoviruses are insect specific entomopathogens belonging to family Baculoviridae. Their occlusion bodies (OBs) contain rod-shaped virions which have circular double stranded DNA. Baculovirus genomes are ranging from 90 to 160 kbp (Blissard and Rohrman, 1990). All baculoviruses composed of the genera nucleopolyhedrovirus (NPV) and granulovirus (GV) (Volkman *et al*., 1995; Theilmann *et al*. 2005) that infect lepidopteran species. Jehle *et al.* (2006) classified baculoviruses into four genera on the basis of hosts. Lepidopteran-specific NPV and GV named Alphabaculovirus and Betabaculovirus, respectively. Hymenopteran-specific NPV named Gammabaculovirus and dipteran-specific baculovirus named Deltabaculovirus. *Spodoptera littoralis* nucleopolyhedrovirus (SpliNPV) is an alphabaculovirus that has been isolated from populations of *S. littoralis* in Egypt, Morocco, France, the Azores islands, Tunisia, and Turkey (Abul Nasr, 1956; Cherry and Summers, 1985; Croizier *et al.*, 1989; Toprak and Gurkan, 2004; Martins *et al.*, 2005; Laarif *et al.*, 2011). Baculoviruses occur naturally and are relatively host-specific. They showed safe to non-target organisms and the mammals (Ashour *et al.*, 2007; Cheng and Lynn, 2009).

The successful application of baculoviruses was initiated from the application of *Anticarsia gemmatalis* (Hübner) nucleopolyhedrovirus (AgNPV) for controlling *A. gemmatalis* in soybean (Moscardi, 1999 and 2007). The initial field experiments indicated that AgNPV has a potential as a biopesticide in soybean IPM programs. One reason for AgNPV success in controlling velvetbean caterpillar in Brazil is the collaborative work of researchers and extension

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workers to get farmers familiar and convinced with the benefits of using AgMNPV as a major part in the biological control of the pest in field (Moscardi, 2007 ; Moscardi *et al.,* 2011).

Field and vegetable crops in Egypt are infested by the Egyptian cotton leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). It is an important and widespread pest not only in Egypt but also in tropical and subtropical regions (Brown and Dewhurst, 1975). According to its many plant hosts, *S. littoralis* named as a polyphgous insect that primarily causes economical yield losses on several crops (Carter, 1984). Examples of economically important crops attacked by *S. littoralis* are cotton, spinach, alfalfa, pepper, eggplant, tomato, lettuce, bean, strawberry, and some ornamental crops throughout the year in Africa, Asia and Europe (Bayoumi *et al.*, 1998; Pineda *et al.*, 2007). Accordingly, more than 40 insecticide formulations belonging to different groups have been registered and used for its control in Egypt (El-Sheikh, 2015a). Because this insect developed high levels of resistance against different groups of conventional insecticides (Smagghe *et al.*, 1999; Aydin and Gürkan, 2006), application of specific programs such as biopesticides would be useful. To focus on the probability of change in the susceptibility, this study aims to test the effects of SpliNPV on reducing growth of *S. littoralis* larvae and accordingly reduce plant feeding damage. Differ in *S. littoralis* susceptibility to SpliNPV was also studied against the first and third viral passes in *S. littoralis*. For confirming the stability of viral genome during infection cycles, DNA analysis using *Eco*RI was carried out.

MATERIALS AND METHODS

Insect and Rearing

A laboratory colony of the cotton leafworm, *S. littoralis*, was obtained from Agricultural Research Center (ARC), Dokki, Giza, Egypt. *S. littoralis* larvae were reared on a modified version of artificial diet (Gelernter *et al.*, 1986) in the laboratory at 27 ± 2ºC and 65 ± 5% RH, with a photoperiod of 16:8 hr., (light:dark). The composition of artificial diet used for preparation was 220 g white beans, 14 g agar powder, 4.8 g ascorbic acid, 0.9 g sorbic acid, 2.4 g methyl-p-hydroxybenzoate, 40 g brewer's yeast, and 700 ml distilled water. Egg masses were obtained by rearing larvae on the diet and allowing emerged adults to feed on 10% (*W/V*) of honey solution in wide glass jars provided with clean white papers for oviposition. Egg masses were collected daily and transferred into clean plastic containers provided with artificial diet or plant leaves for continuous rearing or experiments.

Virus and Propagation

An isolate of *S. littoralis* nucleopolyhedrovirus (SpliNPV) from infected *S. littoralis* larvae was used. Surface contamination method of artificial diet was used for virus propagation in the 3rd and 4th larval instars of *S. littoralis*. The diet was prepared as mentioned above and purified viral particles were spread on the diet surface in a rate of 1×10^4 polyhedral inclusion bodies (PIBs)/ mm^2. Thirty 3rd and 4th instar larvae were transferred into each plastic container (15 × 10 × 5 cm) containing the contaminated diet. Dead larvae were collected and virus was isolated and purified as mentioned in Breitenbach *et al.* (2013).

Droplet Feeding Bioassay

Droplet feeding method (Hughes *et al.*, 1986) was used for testing the virulence of SpliNPV on both 1st and 2nd larval instars of *S. littoralis*. Five concentrations of SpliNPV ranging from 1×10^4 to 1×10^8 PIBs/ml were prepared by mixing viral suspension with 10% sucrose and 5% blue food dye (El-Sheikh *et al.*, 2011) to give the required concentrations. New 1st and 2nd instar larvae (within 12 hr.) of *S. littoralis* were exposed to concentrations of SpliNPV in the form of droplets for ~10 min. Thirty larvae showed drinking from virus concentrations were individually transferred for each concentration into 1 OZ cups (Frontier Agricultural Services, Newark, DE) supplied with fresh clean diet and kept on the above mentioned conditions. Treatments were observed daily and the number of dead larvae was recorded for 10 days post infection (d.p.i.). Larvae dead in the initial 24 hr., were excluded from final larval mortality and considered injured during transfer. Control

experiments were done by exposing larvae (1st or 2nd) to droplets without virus. All treatment or control experiments were repeated three times.

Surface Contamination Bioassay

The propagated and purified virus in *S. littoralis* larvae at the beginning of experiments was considered the 1st passage. The 1st passed polyhedra was used for second infection of larvae to get the purified particles of the 2nd passage. The 2nd passed polyhedra was used for third infection of larvae to get the purified particles of the 3rd passage. The 1st and 3rd viral passes of SpliNPV were used in surface contamination bioassay on the 2nd larval instar. Six concentrations ranging from 1.6 to 5000 PIB/ mm^2 were prepared in dH_2O and used. Concentrations were spread on the surface of artificial diet prepared in plastic containers (15 × 10 × 5 cm). Containers were left for 15 min at room temperature for air dry. Thirty larvae of the 2nd instar larvae were transferred into each contaminated diet. Larvae were allowed to feed on the contaminated diet for 24 hr., before transferring individually into clean diet in 1 OZ cups. Times of transferring larvae on clean diet were considered as time zero for calculating mortality percentage. Larval mortality was recorded daily for 10 d.p.i. on the previous conditions. Control was used for comparison and done by feeding larvae on clean diet only. Experiments of the treatments and control were repeated three times as reported in El-Sheikh (2006).

Time-Mortality Response Determination

Time required for 50% (LT_{50}) or 90% (LT_{90}) mortality was calculated for droplet feeding experiments on the 1st and 2nd instar larvae of *S. littoralis* using concentrations of $1x10^6$ and 1×10^8 PIB/ml. For surface contamination experiments on the 2nd instar, LT_{50} was calculated using concentrations of 200 and 5000 PIB/mm^2. The same procedures mentioned above were used except that mortality was recorded 12 hr., intervals. All experiments were repeated three times.

Effect of Infection on Larval Weight and Feeding Damage

The effect of SpliNPV on larval weight and feeding damage was examined by infecting the 2nd larval instar of *S. littoralis* using two concentrations of 1×10^4 and 1×10^7 PIB/ml through droplet feeding method. Preparation of virus concentrations and infection were done as mentioned above. After infection, 30 larvae were individually transferred into 1 OZ cups containing enough amounts of diet, tomato leaves, lettuce leaves or eggplant leaves for each concentration. Larval weight was recorded in the beginning of experiments (day 0) and at days 3, 6 and 9 post infection (p.i.). Diet and plant leaves were replaced each day with fresh ones and their weights were recoded before feeding and after 24 hr., of feeding. Decrease in weight of diet or plant leaves due to feeding of infected or un-infected control larvae were recorded at days 1, 4, 7 and 10. A piece of diet or plant leaves were used as control without any feed for subtracting the percentage of normal decrease from those recorded due to larval feeding.

DNA Extraction and Analysis

Infected larvae from the 1st and 3rd passages were collected separately in 50 ml conical tubes. Occlusion bodies (OBs) were released from infected larvae, suspended in 30 ml of PBS, and filtered twice through cheesecloth. OBs were incubated in 15 ml of 0.1 M sodium bicarbonate for 30 min at 37°C for releasing virions. DNA was obtained from virus particles by incubating virions overnight at 37°C in the presence of proteinase K (200 µg/ml). Viral DNA was subsequently phenol:chloroform extracted and re-suspended in dH_2O as described in Breitenbach *et al.* (2013). For analyses, DNA was digested for 1 hr., at 37°C with a restriction enzyme *Eco*RI (Invitrogen Corp., CA, USA). Digested DNA was electrophoresed on 1% agarose gel and stained with ethidium bromide solution according to standard techniques of Sambrook *et al.* (1989).

Statistical Analysis

Probit analysis program version 3.1 (Finney, 1971) was used to calculate lethal concentrations (LCs) and their corresponding 95% confidence limits (CLs). ViStat 2.1 program (Hughes, 1990) was used for calculating the lethal time (LT) values. Values of LC_{50} and LC_{90} were considered significantly different when their respective CLs did not overlap. Larval and leaf/diet weights were analyzed using SPSS 14 for Windows by least significant difference (LSD) of one-way ANOVA.

RESULTS AND DISCUSSION

Susceptibility of the 1st and 2nd larval instars of *S. littoralis* to SpliNPV was tested using different concentrations by droplet feeding method (Tables 1 and 2). Data presented in Table 1 show lethal concentrations of 50 and 90% of SpliNPV against 1st and 2nd instars of *S. littoralis* which indicate that number of polyhedra used to kill 50% or 90% of the 2nd instar is less than that used to kill the same percentage of the 1st instar (Table 1). This difference may be attributed to the difference in consumed amounts of viral solution by each instar that is higher with older instar larvae compared with early instar larvae. The high amounts consumed by the 2nd instar compared with the 1st instar might reflect high numbers of viral polyhedra consumed and causing high mortality.

To show the effect of viral concentration in reducing time taken for killing *S. littoralis* larvae, two concentrations of 1×10^6 and 1×10^8 PIB/ml were used (Table 2). When 1×10^6 PIB/ml was tested, the time taken for killing 50 and 90% of the 1st larval instar was longer than that of the 2nd instar, while it was opposite when 1×10^8 PIB/ml was the infection concentration through droplet feeding method. Percentage of decreasing time by increasing concentration was higher in the 1st instar comparing with the 2nd instar larvae (Table 2).

Several characteristics should exist in baculoviruses to make them ideal candidates as pest control agents. Efficacy and biosafety are major parameters in their use. Wild type and genetically modified baculoviruses tested on non-target organisms showed no direct effects on beneficial insects and rats (McNitt *et al.*, 1995; Ashour *et al.*, 2007). Also, they showed high virulent effects on lepidopteran insects (El-Sheikh *et al.*, 2011; El-Sheikh, 2014). Efficacy of SpliNPV was studied on *S. littoralis* under field (Topper *et al.*, 1984; Jones *et al.*, 1994) and laboratory (Grzywacz *et al.*, 1998; Ashour *et al.*, 2007) conditions as well as other spodopteran species (El-Sheikh, 2015b). This virus showed successful control of *S. littoralis* in the field as reported by Topper *et al.* (1984) and showed high effectiveness against other species of *S. frugiperda* and *S. exigua* (El-Sheikh, 2015b) in a rate similar to what reported in the current study. Time taken for mortality was higher in late instars compared with early instars especially with higher concentrations as showed in the current study. In agreement, Abbas and Young (1991) found that LT_{50} values were higher in late instars of the soybean looper, *Pseudoplus includes* than in early instars.

The effect of infection on the weight of the 2nd instar larvae was investigated using different feeding hosts to show the effect of host on larval growth of infected larvae compared with control (Fig. 1). Also, it was showed that larval growth was depending on the type of the host. Control larvae of *S. littoralis* showed the highest increase in weight when fed on artificial diet followed by feeding on lettuce, tomato and eggplant leaves. Larval weight of infected larvae, with 1×10^4 and $1x10^7$ PIB/ml, was affected started from day three when fed on diet, lettuce or eggplant leaves. Significant decrease in weight of larvae fed continuously for 9 days p.i on diet, tomato and lettuce leaves compared with control. All larvae infected with both concentrations and fed on eggplant leaves dead before day 6 p.i. probably because eggplant is un-favorite host for *S. littoralis* comparing with other plant hosts tested in this study. Larvae infected with 1×10^7 PIB/ml showed significant decrease in weight compared with 1×10^4 PIB/ml and control in different time points in all hosts except with tomato leaves (Fig. 1).

Significant reduction in leaf weight started in the 1st day with non-infected control larvae especially with lettuce and eggplant. Higher concentration of 1×10^7 PIB/ml was more effective in reducing leaves and artificial diet weight. The lower concentration of 1×10^4 PIB/ml was effective in reducing leaves damage before day 10 as it showed significant reduction in diet/leaf weights as much as in control experiments (Fig. 2).

The reduction in larval weight and growth is a critical parameter for minimizing plant damage by insect hosts. In this study, higher concentration of SpliNPV was effective in reducing *S. littoralis* larval weight and subsequently reduced larval feeding damage comparing with the lower viral concentration and un-infected control larvae.

Table 1. Lethal concentrations of *Spodoptera littorais* nucleopolyhedrovirus (SpliNPV) on the 1st and 2nd instars of *S. littoralis* after exposure into virus concentrations by droplet feeding method

Instar*	No.**	Lethal concentrations (PIB/ml) (95% CL)		Slope ± SE	X^{2***}
		LC_{50}	LC_{90}		
1st	120	2.5×10^{5A} (1.3×10^{5}-4.5×10^{5})	1.2×10^{8A} (4.5×10^{7}-4.9×10^{8})	0.5±0.04	5.3
2nd	120	$1.4x10^{5B}$ (8.7×10^{4}-2.3×10^{5})	$1.2x10^{7B}$ (6.2×10^{6}-2.8×10^{7})	0.6±0.05	4.8

- Values of lethal concentrations (LC_{50} or LC_{90}) between 1st and 2nd instars are considered significantly different when their confidence limits (CL) did not overlap and showed with different uppercase letters.

* New emergence 1st and 2nd instars were used in experiments which repeated 3 times.

** No. is the total number of larvae used for each experiment.

*** X^2 means chi-square heterogeneity.

Table 2. Lethal-time (LT) of the 1st and 2nd instars of *Spodptera littoralis* due to feeding on *S. littorais* nucleopolyhedrovirus (SpliNPV) droplets containing viral particles at concentrations of 1×10^6 or 1×10^8 PIB/ml

Instar*	No.**	Lethal times (hr.)				Decrease in time (%)	
		1×10^6 PIB/ml		1×10^8 PIB/ml			
		LT_{50}	LT_{90}	LT_{50}	LT_{90}	LT_{50}	LT_{90}
1st	120	168.0	357.6	55.2	132.0	67.1	63.1
2nd	120	151.2	328.8	84.0	141.6	44.4	56.9

*New emergence 1st and 2nd instars were used in experiments which repeated 3 times.

**No. is the total number of larvae used for each experiment.

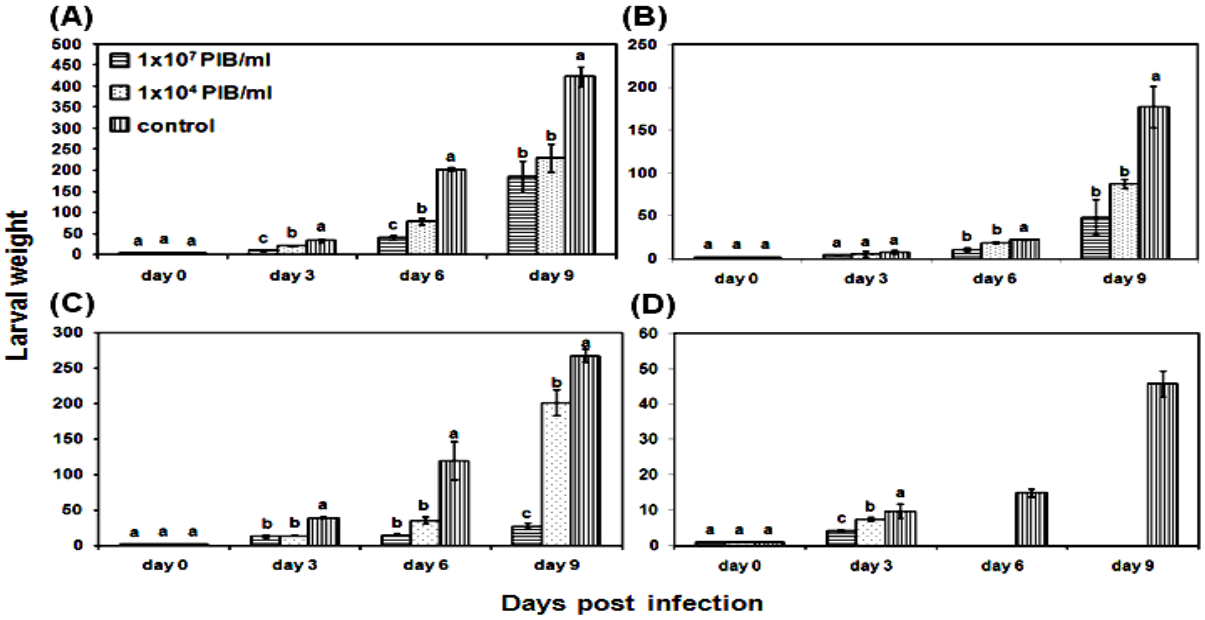

Fig. 1. Increase in individual larval weight of the 2nd instar of *Spodoptera littoralis* after infection with 1 × 10^4 or 1 × 10^7 PIB/ml then transferred on artificial diet (A), tomato leaves (B), lettuce leaves (C), or eggplant leaves (D) for 10 days. Mean weight of alive larvae was recorded immediately before infection (day 0) and after 3, 6, and 9 days of infection. Differences between treatments and control were analyzed by SPSS 14 using LSD of one-way ANOVA for each time point. Bars holding different letters are statistically different at P<0.05

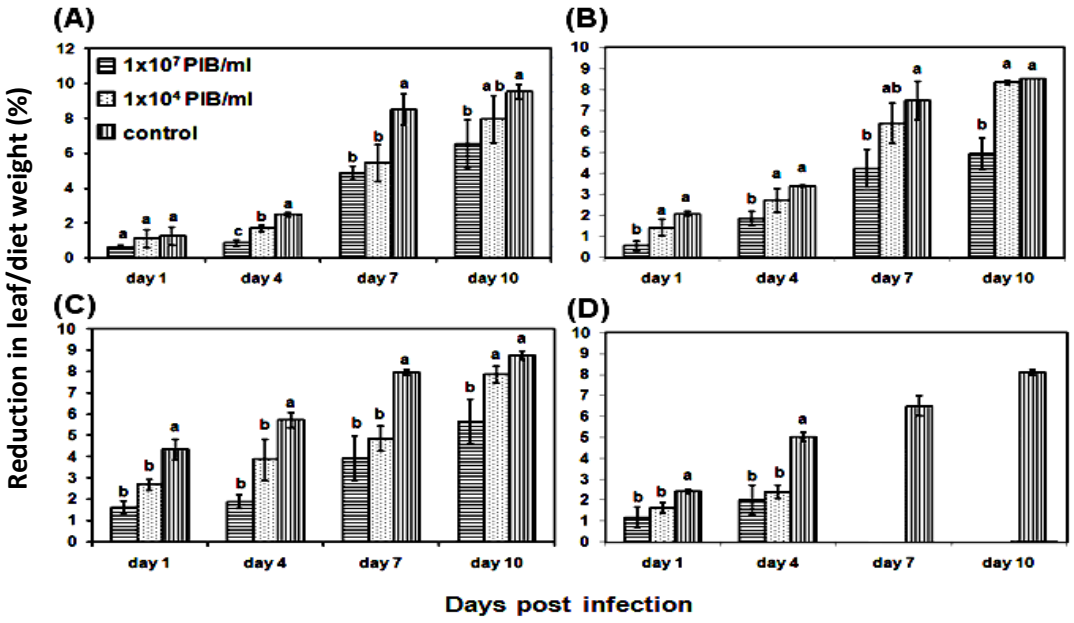

Fig. 2. Mean percentage of reduction in artificial diet (A), tomato leaves (B), lettuce leaves (C), and eggplant leaves (D) due to feeding of *Spodoptera littoralis* 2nd instar larvae for 10 days after exposure to droplets containing 1 × 10^4 or 1 × 10^7 PIB of SpliNPV/ml (treated) or free virus droplets (control). Differences between treatments and control were analyzed by SPSS 14 using LSD of one-way ANOVA for each time point. Bars holding different letters are statistically different at P<0.05

The effects of continuous exposure of *S. littoralis* larvae to SpliNPV on the susceptibility of the 2nd instar of *S. littoralis* to different virus passages are presented in Tables 3 and 4. First and 3rd passages were examined on the 2nd larval instar of *S. littoralis* to show the virulent effect of the virus (Table 3) and time for 50% mortality (Table 4). Obtained data showed that concentrations of the tested virus caused 50% and 90% mortality of the 1st passage are 0.5×10^2 PIB/mm^2 and 1.7×10^4 PIB/mm^2, respectively. While, mortality data of the 3rd passage of SpliNPV showed that concentrations caused 50% and 90% mortality increased comparing with 1st viral pass and recorded 1.4×10^2 and 3.2×10^5 PIB/mm^2, respectively. Increase in the number of viral polyhedra used to kill 50% and 90% of *S. littoralis* larvae in the 3rd passage comparing to 1st passage are 2.8 and 18.8 times, respectively (Table 3).

When the 2nd instar larvae of *S. littoralis* was exposed to the concentration of 200 PIB/mm^2, time to kill 50% was increased from 206.4 hr., in the 1st viral pass to 254.4 hr., in the 3rd viral pass with 23.3% increase. While, time increased by 30% in the 3rd passage comparing with the 1st passage when larvae exposed to 5000 PIB/mm^2. Times to kill 50% were decreased by increasing concentration which recorded 206.4 and 120 hr., in the 1st viral pass for 200 and 5000 PIB/mm^2, respectively with 14.9% decrease in time. Although that isolate of the 3rd viral pass takes more time for killing 50%, using high concentrations (5000 PIB/mm^2) decreases time taken for 50% mortality by 38.7% comparing to 14.9% in the 1st pass (Table 4).

Continuous exposure of *S. littoralis* larvae to a strain of SpliNPV has resulted in low mortality as indicated by higher number of polyhedra required for killing 50 or 90% in the 3rd passage comparing to the 1st passage. Also, time for 50% mortality increased by increasing number of virus passages inside *S. littoralis* larvae. The decrease in larval susceptibility due to continuous exposure to NPV was reported in *S. frugiperda* as indicated in the 1st larval instar that showed high levels of tolerance to a selected *S. frugiperda* nucleopolyhedrovirus (SfNPV) strain comparing to a wild type strain (Fuxa and Richter, 1992). In an experiment for measuring relative susceptibilities to NPV, Briese *et al.* (1980) showed that a strain of light-brown apple moth, *Epiphyas postvittana*, exposed several times to NPV are more than 50 times resistant to NPV than a laboratory strain and even more than 100 times resistant compared with a field strain.

Analysis of SpliNPV genome using a restriction endonuclease *Eco*RI for the 1st and 3rd passages (Fig. 3) revealed no differences in the patterns of DNA fragments. The similarity in DNA patterns indicates that SpliNPV genome did not change during subsequent infection of the insect host in the laboratory, which its stability previously reported in the semi field experiments (Ashour *et al.*, 2007).

Table 3. Susceptibility of the 2nd instar larvae of *Spodoptera littoralis* when exposed to subsequent passes/ replications of *S. littoralis* nucleopolyhedrovirus (SpliNPV)

Replications/passes*	No.**	Lethal concentrations (PIB/mm^2) (95% CL)		Slope±SE	X^2***
		LC_{50}	LC_{90}		
1st replication/pass	40	0.5×10^{2A} (0.07×10^2-3×10^2)	1.7×10^{4A} (1.6×10^4-2.6×10^6)	0.5±0.1	1.2
3rd replication/pass	60	1.4×10^{2A} (0.7×10^2-3×10^2)	3.2×10^{5A} (4.9×10^4-7.6×10^6)	0.8±0.1	6.9
DT (times)****		2.8	18.8		

- Values of lethal concentrations (LC_{50} or LC_{90}) between 1st and 2nd instars are considered significantly different when their confidence limits (CL) did not overlap and showed with different uppercase letters.

* 1st pass obtained from infected larvae with SpliNPV, and then the 1st pass was used for insect infection and obtaining the 2nd pass. While, the 3rd pass obtained from infected larvae with the 2nd pass.

** No. is the total number of larvae used for each experiment.

*** X^2 means chi-square heterogeneity.

**** DT= degree of tolerant which calculated by dividing LC_{50} or LC_{90} of the 3rd pass/LC_{50} or LC_{90} of the 1st pass.

Table 4. The effect of increase in *Spodoptera littoralis* nucleopolyhedrovirus (SpliNPV) polyhedra and increase in viral passes on the time taken for 50% mortality (LT_{50}) in the 2nd larval instar of *S. littoralis*

Replications/Passes*	No.**	Median lethal time (hr.)		Decrease in time**** (%)
		200 (PIB/mm^2)	5000 (PIB/mm^2)	
1st replication/pass	120	206.4	120.0	14.9
3rd replication/pass	120	254.4	156.0	38.7
Increase in time (hr.) ***		1.23	1.3	
Increase in time (%)		23.3	30	

* 1st pass obtained from infected larvae with SpliNPV, and then the 1st pass was used for insect infection and obtaining the 2nd pass. While, the 3rd pass obtained from infected larvae with the 2nd pass.

** No. is the total number of larvae used for each experiment.

*** Increase in time was calculated by subtracting LT_{50} value of the 1st pass from 3rd pass for 200 or 5000 PIB/mm^2.

**** (%) decrease in time was calculated between viral concentrations used for 1st or 3rd pass by [(LT_{50} value of 200 PIB ml^{-1} – LT_{50} value of 5000 PIB ml^{-1}) × 100/LT_{50} value of 200 PIB ml^{-1}].

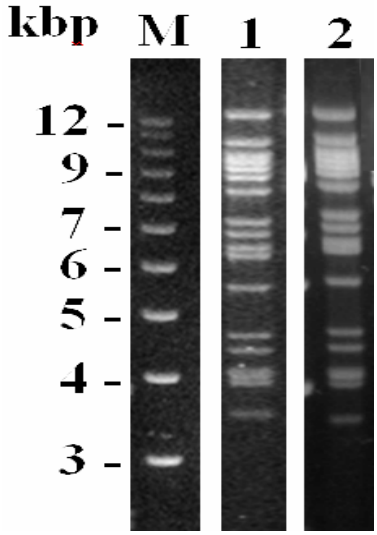

Fig. 3. *Spodoptera littoralis* nucleopolyhedrovirus genome for 1st pass (lane 1) and 3rd pass (lane 2) cut with *Eco*RI restriction enzyme and run on 1% Agarose gel. Lane M represents the 1 kbp plus DNA ladder with standard fragments visable in this figure from 3000 bp to 12000 bp

In conclusion, SpliNPV is a valuable bio-pesticide against the Egyptian cotton leaf worm. It is effective against early instars in killing and reducing both larval weight and feeding damage when a reasonable concentration is used. In the opposite way, continuous exposure of *S. littoralis* larvae to virus particles might change insect susceptibility toward resistance in a very few generations. This phenomenon requires using SpliNPV within integrated pest management programs.

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الفعالية والقدرة الإبادية للفيروس البوليهيدري النووي SpliNPV على تغذية وحساسية يرقات دودة ورق القطن الكبرى

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عُرفت الفيروسات العصوية الممرضة للحشرات بأنها بدائل آمنة بيئيا للمبيدات الكيميائية، تم دراسة القدرة الإبادية وحساسية يرقات دودة ورق القطن الكبرى للفيروس البوليهيدري النووي (SpliNPV) لهذه الحشرة، أظهرت تجارب التقييم الحيوي على العمرين اليرقيين الأول والثاني باستخدام طريقة droplet feeding أن عدد البوليهيدرا التي أدت إلى قتل ٥٠% أو ٩٠% من يرقات العمر الثاني لدودة ورق القطن الكبرى كانت أقل معنوياً مقارنة بيرقات العمر الأول، في حين أن يرقات العمر الأول ماتت أسرع من يرقات العمر الثاني خاصة مع التركيز الأعلى، وعند دراسة حساسية يرقات العمر الثانى المعداة بالفيروس نتيجة للتغذية علي عوائل مختلفة، أظهرت النتائج أن اليرقات كانت أكثر حساسية عند التغذية علي أوراق الباذنجان، يليه التغذية على أوراق الخس، البيئة الغذائية وأوراق الطماطم، وذلك من خلال التأثير على أوزان اليرقات والخفض في معدل التغذية، كما وجد أن تأثير التركيز الفيروسي ١ × ١٠^{٧} بوليهيدرا/مل على الخفض في الوزن اليرقى والتغذية كان أعلي معنوياً مقارنة بالتركيز ١×١٠^{٤} بوليهيدرا/مل، أظهرت يرقات دودة ورق القطن تحمل للفيروس SpliNPV بأكثر من مرتين علي مستوي LC_{50}، وأكثر من ١٨ مرة علي مستوى LC_{90} وذلك للعزلة الفيروسية الثالثة (3rd passage)، كما وجد أن مستويات تحمل يرقات دودة ورق القطن الكبرى للعزلة الفيروسية الثالثة كانت مرتبطة بأكثر من ٢٠% زيادة في الوقت المستغرق لقتل اليرقات مع عدم وجود تغير في نماذج تفريد DNA للعزلات الفيروسية المختبرة من يرقات دودة ورق القطن الكبرى باستخدام إنزيم القصر *Eco*RI.

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