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Nuclear polyhedrosis virus DNA identification and first evaluation against Strawberry pest, *Pentodon algerinum* (Coleoptera: Scarabaeidae)

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Abstract

In Egypt, The white grub, *Pentodon algerinum* (Coleoptera: Scarabaeidae) damages various economic important plants recently strawberry. This study aimed to DNA identification and first evaluation of the *Spodopteda littoralis Nuclear polyhedrosis virus* (*SpliNPV)* and *Pentodon algerinum Nuclear polyhedrosis virus* (*PNPV*) against *Pentodon algerinum* third instar larvae in the laboratory to find successful safe alternative control method against this pest. Bioassay was made by two experiments, one used five *SpliNPV* concentrations, $6.7X10^6$, $3.11X10^7$, $1.1X10^8$, $3.64X10^9$ and $3.64X10^{10}$ Polyhedral inclusion bodies (PIB/ml) achieved 80, 100, 100, 80 & 90% mortality respectively during thirteen days. Another experiment used four *PNPV* concentrations, $8.2x10^7$, $8.2x\ 10^8$, $2.6x10^9$ and $8.2x10^9$ PIB/ml caused 100, 70, 67 & 72.7% mortality respectively during ten days. *SpliNPV* Lethal concentrations LC_{50} $6.2x10^{14}$ & LC_{90} $8.9x10^9$ PIB/ml were more than *PNPV* LC_{50} $2.8X10^9$ & LC_{90} $1.8X10^8$ PIB/ml. Thus *PNPV* and *SpliNPV were effective against Pentodon* larvae but *PNPV* was effective and specific more than *SpliNPV*. DNA of *SpliNPV* and *PNPV* was identified by RAPD PCR using four RAPD primers, Operon A9, A20, B17 & B7 (Operon Technologies, Inc., Alameda, CA, USA) using automated (Bio Rad, USA) thermal cycler. Total 33 bands were produced including 24 *PNPV* bands with molecular weights between 152-1204 base pairs (bp) included between resulted 9 *SpliNPV* bands having molecular weights between 138 - 1767 bp. Therefore *PNPV* is similar to *SpliNPV* in almost its DNA sites and also is a polymorphic copy from *SpliNPV*. Thus *PNPV* and *SpliNPV* can be recommended against this pest.

***Keywords*:** the white grubs; *Pentodon algerinum*; larval stage ; strawberry plants; RAPD PCR; *SpliNPV*; *PNPV*

Introduction

Strawberry yield (*Fragaria x ananassa* Duch.) is a major economic vegetable crop for local consumption and exportation in Egypt. Recently in Egypt, the white grub *Pentodon algerinum* (Coleoptera: Scarabaeidae) extensively destroys strawberry. The larvae are known as the waste organic manure larvae (White grubs) and the adult stage is named as the hard black beetle. [1, 2]. The larvae live in the soil and feed on organic matters and plant roots [3]. The larvae go near to the soil surface, looking for any roots or tubers to feed on. These larvae cluster around the roots when soil is removed. The infection causes plant wilt and death. The larvae prefers living in the sandy soil (newly reclaimed area).White grubs are basic pests of different agricultural crops and the most damaging group for the turf grass, nurseries and ornamentals in the worldwide [4,5, 2]. The white grubs cause extensive damage to the roots of grasses, legumes, small fruit plants, shrubs and trees in many parts of the world [6, 7]. Strawberries producers faced problems with declining the efficacy of the chemical insecticides and developing resistance against these compounds, [8]. Pathogenic virus for controlling of insect pests has the advantage of tolerance to extreme climatic conditions of heat& humidity and that one time use is enough to reduce the number of insect pests. Viruses are tiny obligate intracellular parasites being either RNA or DNA genome involved by a protective, virus coded protein envelope. They are of cellular origin and distinguished by a long co-evolution of virus and host. Infection by viruses depends on specialized host cells providing the complex metabolic and biosynthetic machinery of eukaryotic or prokaryotic cells. [9, 10]. The entomopathogenic Baculovirus cannot be transmitted to human because this virus requires an alkaline based cell structure to replicate itself while Human is acidic based. Intracellular

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pathogenic viruses need to complement with its specific receptor sequence on the target cell surface for their entry to initiate infection. Virus receptor binding is too specific, and this specificity marks both the species and the cell type that can be infected by a given virus. Baculovirus receptor on insect host cells is not found in human cells. Entomopathogenic baculoviruses are considered as safe alternative method for insect pest control such as the Nuclear Polyhedrosis Virus (NPV) being a double stranded DNA baculovirus and highly specific to their host insects. [9,10]. Today Nuclear polyhedrosis virus (NPV) (Baculoviridae) is an ideal tool in the pest management programs, as it is highly specific to its host insects, safe to the environment, humans, other plants and beneficial natural enemies[11,12,13].

The NPV has large polyhedron shaped structures called polyhedral containing many virions [14]. The occlusion of the virus inside a protein coat is important to protect the infective particles in the transmission of the virus from insect to insect [15, 16]. The Baculoviridae family contains the *Nucleopolyhedrovirus* such as the multiple and single Nuclear polyhedrosis viruses (MNPV, SNPV) and the *Granulovirus* such as granulosis viruses [17]. During larvae infection, occlusion bodies (OBs) consisting of enveloped virions enclosed in polyhedrin protein are dissolved in the alkaline insect host midgut releasing virions making a primary infection. [18,19]. They replicate when ingested by the insect host larvae by biphasic cycle containing budded virus produced in the primary infection, and later, when viral particles are produced, they become occluded virus (polyhedral inclusion bodies). The budded virus is responsible for virus infection within the host while the occlusion bodies are responsible for spreading of virus in the environment between susceptible larvae. This is achieved through cell lysis of infected larvae resulting in contamination of the feeding plant parts such as leaf surfaces eaten by healthy larvae becoming infected by virus. Other important advantages of baculovirus for pest control are a lack of toxic residues, allowing farmers to treat their crops even shortly before harvest, with low possibility to develop stable resistance [20]. The identification of the DNA of cells can be carried out by Random Amplification of Polymorphic DNA **(**RAPD **PCR) which** is a type of Polymerase chain reaction (PCR). The segments of DNA are randomly amplified by using short primers (8–12 nucleotides) to make DNA fingerprint. This method is now one of the most commonly used assays for obtaining a particular segment of DNA or RNA. It is rapid and extremely sensitive. This method does not require knowledge of the nucleotide sequence at the ends of the region needed to be amplified. Once that is known, one can make large quantities of that region starting with tiny amounts of material, such as the DNA within a single human hair. With the availability of almost complete or complete sequences of genomes from many species, the range of genes to which it can be applied is enormous. [21].

This study aimed to DNA identification and first evaluation of the *Spodopteda littoralis Nuclear polyhedrosis virus* (*SpliNPV)* and *Pentodon algerinum Nuclear polyhedrosis virus* (*PNPV*) against *Pentodon algerinum* third instar larvae in the laboratory to find successful safe alternative control method against this pest.

Experimental:

1- Rearing of *Pentodon algerinum*

The first and second instars of *Pentodon algerinum* larvae were collected from infested organic strawberry fields at Tokh town; Qalubia Governorate. Infestation was firstly recorded at September and Novamber 2018. These fields were not treated with insecticides nor entomopathogenic agents during the previous year. Reared collected *P. algerinum* larvae fed on a mixture of sandy soil, organic manure, and potato pieces with roots of grass plant under laboratory conditions (Temp. of 22.0±3.0ºC & RH of 70.0±5.0) according to **[2]**. These larvae have three larval instars. Third instar larvae were used for laboratory experiments.

2. Production and isolation of Nuclear Polyhedrosis virus

The original Nuclear Polyhedrosis virus (NPV) types were produced and isolated from infected *Spodoptera littoralis* larvae as *(SpliNPV)* and from infected *Pentodon algerinum* larvae as (*PNPV*) by (Samah M.M. Abd EL-Aziz) according to **[9,10]** at the laboratory of Pests & Plant Protection Department, National Research Centre and stored at -20^{o} C till use.

3-Preparation of *SpliNPV* and *PNPV* viral concentration

The virus stocks of *SpliNPV* and *PNPV* were used to prepare different dilutions for each of them.

The number of polyhedral inclusion bodies (PIB /ml) was counted under light microscope by haemocytometer according to **[22]**.

4- Bioassay tests:

Bioassay was made by two experiments, one used five concentrations of *SpliNPV*, $6.7X10^{6}$, $3.11X10^{7}$, $1.1X\ 10^{8}$, $3.64X10^{9}$ and $3.64X10^{10}$ PIB/ml. Another

experiment was done by preparing four concentrations of *PNPV*, $8.2x10^7$, $8.2x\ 10^8$, $2.6x10^9$ and $8.2x10^9$ PIB/ml for Bioassay tests.
Ten larvae of *Pentodon algerinum* for each virus concentration were put individually in 10 plastic cups (350 ml) containing *NPV* contaminated food. Every day each larva was investigated until death. Also there were 10 larvae for control were put under the same conditions without any NPV contamination and were followed up daily. The dead larvae were recorded daily. Each treatment and control had ten replicates. Lethal concentrations $LC_{50\&90}$ and lethal times $LT_{50\&90}$ were calculated according to **[23]**, using Probit analysis of mortality data from bioassays**.**

5- Molecular Study:

5.1- Isolation of DNA from *PNPV* & *SpliNPV:* DNA of *PNPV* & *SpliNPV* was isolated according to **[24]**.

5.2-Identification of *PNPV* DNA & *SpliNPV* DNA by RAPD PCR

The quantities used in the RAPD PCR reaction for amplification of all the genes of *PNPV* and *SNPV* DNA had been carried out with different reaction mixture compositions in a final volume of 25.0 µl and 20 µl at different RAPD PCR programs in the automated (Bio Rad, USA) thermal cycler. Four Operon (A9, A20 & B7, and B17) were used with RAPD PCR.

5.2.1- For Operon A9 (5′ GGG TAA CGC C 3′):13µl;12.5; 10 µl master mix (2x), 2µl;1; 2µl Operon A9 (10 pmol) , 1µl ;2; 5 µl template DNA and 9µl ;9.5; 3 µl PCR grade water (nuclease free) at total volume of 25 µl; 25 µl and 20 µl, respectively (the initial denaturation at 95ºC for 3 minutes (one cycle only), denaturation at 95ºC for1 minute, , primer annealing at 40ºC for1 mins., extension at 72 ºC for1 mins. for 40 cycles and final extension at 72 ºC for10 mins); Also at (initial denaturation at 98ºC for 2 mins for one cycle only, denaturation at 98ºC for 30 sec , primer annealing at 42 °C 30 sec, extension at 72 °C 1 min.for40 cycles and final extension at 72 °C for 10 minutes for one cycle only). And at (initial denaturation at 95ºC for 3 mins for one cycle only, denaturation at 95ºC for 1 mins, , primer annealing at 41ºC for 1 mins., extension at 72 ºC for 1 mins.,for 42 cycles and final extension at 72 ºC for 10 mins. for one cycle only) for each reaction mixture respectively.

5.2.2- While in a final volume of 20 µl. with different reaction mixture compositions for Operon A20 (5′ GTT GCG ATC C 3′) and for Operon B17(5′ AGG GAA CGA G 3′) , 10µl master mix(2x), 0.3µl; 2µl Operon A20 (10 pmol) or Operon B17(10 pmol) , 2µl; 5µl template DNA and 7.7µl; 3µl PCR grade water(nuclease free).at (initial denaturation at 95ºC/4mins for one cycle only., denaturation at 94ºC for45 sec., , primer annealing at 41 ºC for45 sec., extension at 72 ºC for45 sec., for 40 cycles and final extension at 72 ºC for10 mins., for one cycle only).Also at (initial denaturation at 95ºC/ 3 mins for one cycle ,denaturation at 95ºC for 1 mins, , primer annealing at 41ºC for 1 mins, extension at 72 ºC for 1 mins., for 42 cycles and final extension at 72 ºC for 10 mins. for one cycle only) for each reaction mixture respectively.

5.2.3- While in a final volume of 20 µl. with different reaction mixture compositions for Operon B7 (5′ GGT GAC GCA G 3′), 12.5 µl ;10µl master mix(2x), 1 µl ; 2µl Operon B17(10 pmol) , 2 µl ;5µl template DNA and 9.5 µl; 3µl PCR grade water(nuclease free) at total volume of 25 µl and at 20 µl respectively. at (initial denaturation at 98 °C/ 2 min for one cycle only, denaturation at 98 °C 30 sec , primer annealing 42 °C 30 sec , extension at 72 °C 1 mins ., for 40 cycles and final extension at 72 °C for 10 min for one cycle only.). Also at (initial denaturation at 95ºC/ 3 mins for one cycle ,denaturation at 95ºC for 1 mins, , primer annealing at 41ºC for 1 mins, extension at 72 ºC for 1 mins., for 42 cycles and final extension at 72 ºC for 10 mins. for one cycle only) for each reaction mixture respectively.

The amplification cycles were carried out in the automated (Bio Rad, USA) thermal cycler. The used thermal cycling programs depend on the primer, length of amplified fragment, 'GC' content and the structure of fragment.

5.3-PCR products were loaded into1.7- 2 % agarose gel after mixing it with 6x loading dye in a ratio of 1:5. The DNA ladder was added as a standard in a separate well. The voltage was adjusted at 5.0 volt/1.0 cm² of the tray gel area till the loaded samples reach to the half of the gel.
The gel containing PCR products was then carefully transferred to the UV trans- eliminator, visualized under UV and photographed directly by using a gel documentation system (BIO RAD, USA).Gel pictures were analyzed by using (Gel –Pro Analyzer Version:3). Illustrative graph was made by SPSS (Version 19.0)

Results & Discussion

***SpliNPV* virus evaluation**

The results in table (1) & fig (1) showed that five *SpliNPV* concentrations, $6.7X10^6$, $3.11X10^7$, $1.1X10^8$, $3.64X10^9$ and $3.64X10^{10}$ PIB/ml used

against *P. algerinum* third instar larvae in the laboratory achieved 80, 100, 100, 80 & %90 mortality respectively during thirteen days. Also these concentrations had Lethal Concentrations (LC_{50}8.9x10^9 & LC_{90} 6.2x10^{14} PIB/ml).

From these results it can be observed that as the *SpliNPV* virus concentrations decreased, the mortality percentage of *P. algerinum larvae* increased especially at the concentrations 3.64X10^9 and 3.64X10^{10} PIB/ml. It is realized that LC_{50}value was higher than the LC_{90}value which is a unique result needing great investigations. But these results may be due to that *SpliNPV* was evaluated for the first time against this insect pest so the virus considered this pest as the foreign host for it so it was looking for its receptor on the mid gut to enter the cell and initiate infection inside the cell nucleus. It probably took two roots to enter into this pest larvae, one is by entering through cuticle then fat bodies, trachea, until find its receptor on the mid gut but until it reaches that there were many immunological defenses from insect against it, also there was competition between these virus entering particles to reach this receptor, so not all virus particles can succeed to reach their receptors. So that as the virus concentrations decreased ,the success of it to reach its receptor and initiate infection increased. While another root is its ingestion by insect during feeding on its contaminated food.

While the results in table (2) and figs. (2) Showed that the concentration , 1.1x10^8 PIB/ml recorded the lowest LT_{50} (at 5.348 days) and LT_{90} equal to 10.43 days. Viral concentrations 6.7x10^6, 3.11x10^{7}PIB/ ml had approximate values of LT_{50} and LT_{90} while high concentrations (3.64x10^9, 3.64x10^{10}PIB/ ml) need 29.76, 18.36 days to reach LC_{90}.

Table (1) Lethal Concentrations of *SpliNPV* against *P. algerinum* larvae

Concentration (PIB/ml)	%mortality	LC_{50}	LC_{90}	Slope (±SE)	Probability	Chi-square (X^2)
6.7x10^6	80	6.2x10^{14}	8.9x10^9	-0.26± 0.16	6.0	24.0628
3.11x10^7	100					
1.1x10^8	100					
3.64x10^9	80					
3.64x10^{10}	90					

Units LC = PIB/ml, applied for 13 days.

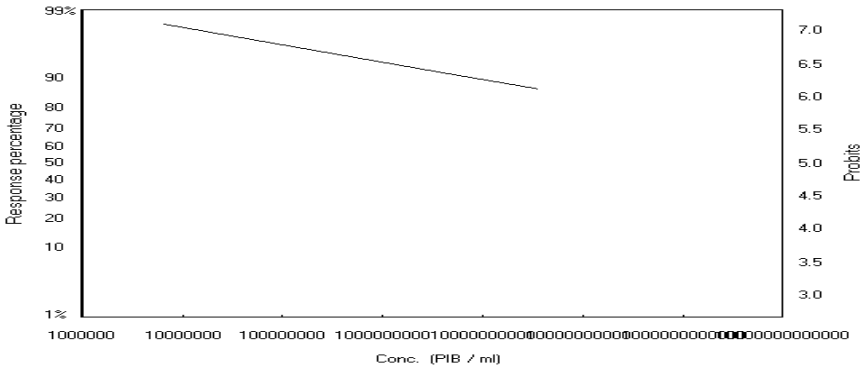
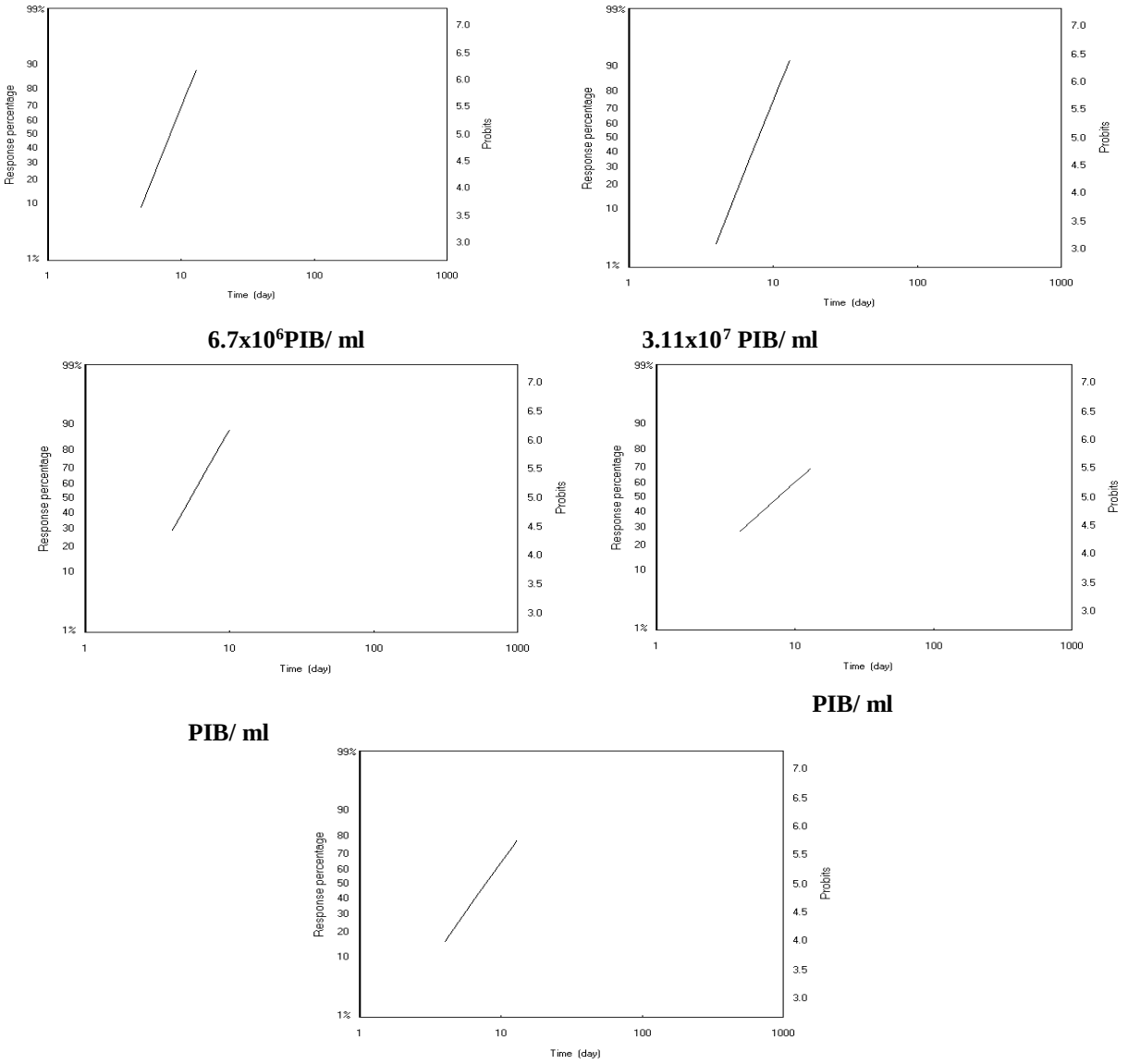


Fig.(1) Probit analysis of toxicity line *SpliNPV* against *P. algerinum* larvae in the laboratory

Table (2) Lethal Times of SpliNPV against P. algerinum larvae

Conc. (PIB/ml)	LT_{50} (day)	LT_{90} (day)	Slope (±)	Probability	Chi-square
6.7x10^6	8.288	13.42	6.12±1.42	9.5	0.21
3.11x10^7	7.888	12.46	6.44±0.38	11.1	54.01
1.1x10^8	5.348	10.43	4.41±0.48	6.0	38.19
3.64x10^9	7.536	29.76	2.14±0.84	11.1	1.43
3.64x10^{10}	7.798	18.36	3.44±0.91	11.1	2.31



Figs. (2) Probit analysis Lethal times (LT) of each *SpliNPV* concentration against *P. algerinum* larvae in the laboratory

***PNPV* virus evaluation**

The results in table (3) and fig. (3) showed that four *PNPV* concentrations, $8.2x10^7$, $8.2x\ 10^8$, $2.6x10^9$ and $8.2x10^9$ PIB/ml used against *P. algerinum* larvae in the laboratory caused 100, 70, 67 & %72.7 mortality respectively during ten days. Also these concentrations had Lethal Concentrations ($LC_{50}2.8X10^9$ & $LC_{90}1.8X10^8$ PIB/ml). These results indicated that as the *PNPV* virus concentrations decreased, the mortality percentage of *P. algerinum larvae* increased, leading to that $LC_{50}$was more than LC_{90} being a unique result needing great studies. But these results may be due to that *PNPV* was evaluated for the first time against this insect pest. This virus probably took two roots to enter into this pest larvae, one is by entering through cuticle then fat bodies, trachea, until find its receptor on the mid gut but until it reaches that there were many immunological defenses from insect against it, also there was competition between these virus entering particles to reach this receptor, so not all virus particles can succeed to reach their receptors. So that as the virus concentrations decreased, the success of it to reach its receptor and initiate infection increased. While another root is its ingestion by insect during feeding on its virus contaminated food.

While the results in table (4) and figs. (4) showed that at concentration $2.6x10^9$ recorded the lowest

LT_{50} and LT_{90} (4.61&8.17) while the concentration $8.2x10^9$ PIB/ ml recorded LT_{50} at 6.80 days and had LT_{90} equal to 19.29 days. While viral concentrations $8.2x10^7$, $8.2x10^8$ PIB/ ml had LT_{50} and LT_{90} (6.95, 25.24 and 7.96 , 17.24 days) for each concentration respectively.

From all these results, it can be noticed that $LC_{50}6.2x10^{14}$ & LC_{90} $8.9x10^9$ PIB/ml for *SpliNPV* virus were were more than $LC_{50}2.8X10^9$& LC_{90} $1.8X10^8$ PIB/ml for *PNPV* virus. The results confirmed that *PNPV* and *SpliNPV* were effective against *Pentodon* larvae but *PNPV* was effective and specific more than *SpliNPV*. **[25, 26]** confirmed that viruses had high virulence when passed in the original insect host, the virulence differs greatly when infected other hosts. The viruses isolated from other species have a lower virulence than those isolated from the homologous insect species. However, The current study indicated that *PNPV* was more active than *SpliNPV* against *P. algerinum larvae*.

Pathogenicity and virulence of the baculovirus are the most frequently evaluated parameters. The pathogenicity can be defined as that the ability of an organism to cause disease and virulence refers to the degree of pathogenicity caused by this organism. The screening of highly-virulent isolates is a first step in the biopesticide development, but other factors need to be taken in consideration, including persistence, host range and impact on non-target insects**[27,28]**. The most common used biological control of white grubs is such as: 1-Milky spore disease caused by a soil inhabiting bacterium (*Paenibacillus popilliae*) ingested by the grub during normal feeding. 2-Beneficial nematodes, such as *Heterorhabditis bacteriophora* entering through natural openings in the early larval stage body, then releases toxic bacteria that kill the insect. 3-Chemical Control: There are many effective insecticides for grub control by either preventative or curative, such as contact insecticides giving curative control of grubs. Contact insecticide applications are more effective on smaller, younger grubs, which are present during the early to mid-summer **[29]**.

White grubs are naturally infected by various entomopathogens including fungi, bacteria and nematodes. Entomopathogenic fungi genera *Beauveria* and *Metarhizium* are widely used against white grubs **[7]**.

Also **[2]** evaluated the efficacy of Imidacloprid , *Metarhizium anisopliae* and nimbecidine against Strawberry White grubs (*Pentodon algerinum*) in Egypt. The results showed that Imidacloprid was the highest effective followed by bio-catch while nimbecidine was the lowest one .The first application revealed that the effective formulations were not significant after the 1st week from application. While, the 2nd week from application, Imidacloprid, *Metarhizium anisopliae* formulation and Nimbecidine achieved 78.0, 75.0 and 67.2 % reduction in alive white grubs, respectively. The second application clearly confirmed the efficiency of these compounds.

While there were few studies made from other authors on the experimenting of virus on coleopteran insects such as **[30]** who studied the effect of other type of virus known as The *Oryctes virus* on *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) and found after concentrated laboratory studies, the virus was isolated and identified as the first non-occluded, rod shaped insect virus, morphologically resembling the baculoviruses. Infection experiments clarified the pathology, histopathology, and virulence of the virus and demonstrated that the virus was extremely virulent to larvae after oral application. Also **[31]** studied *Oryctes rhinoceros nudivirus* causing severe disease in *Allomyrina dichotoma* in Korea and found that the healthy larvae were infected after taking 30 μl orally of diseased cadaver hemolymph, and after 6 wk, about 62% of larvae died with virus symptom.

These previous used control methods mentioned above including fungi, bacteria, nematodes and chemical insecticides are not positively effective enough for controlling of these grubs because of that some of them are limited in their effect on all larval instars of these grubs, also there are many harms caused by some of these compounds. So that there is strong need for finding an effective safe alternative control method against these grubs such as baculoviridae Nucleopolyhedrosis viruses (NPVs). NPV belongs to family baculoviridae which is specific against a variety of insect pests of forests and economically important crops **[10, 32, 33].**

Table (3) Lethal Concentrations of PNPV against P. algerinum larvae

Conc. (PIB/ml)	%mortality	LC_{50}	LC_{90}	Slope ±SE	Probability	Chi-square (X^2)
$8.2x10^7$	100%	**$2.8X10^9$**	**$1.8X10^8$**	**-0.5829± 0.1094**	**6**	20.4152
$8.2x10^8$	70%					
$2.6x10^9$	%67					
$8.2x10^9$	72.7%					

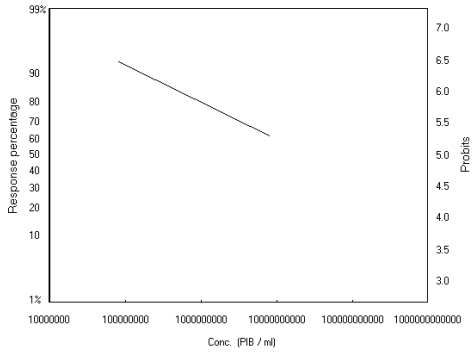
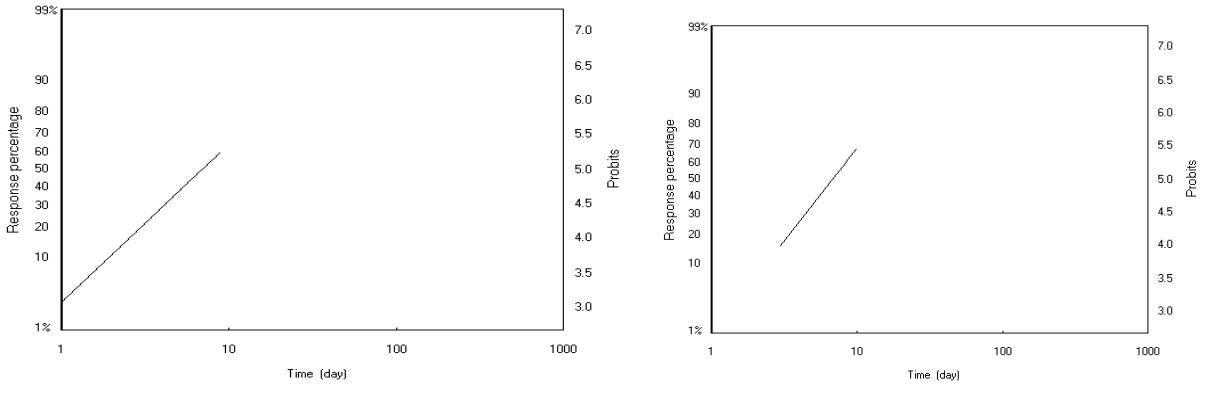
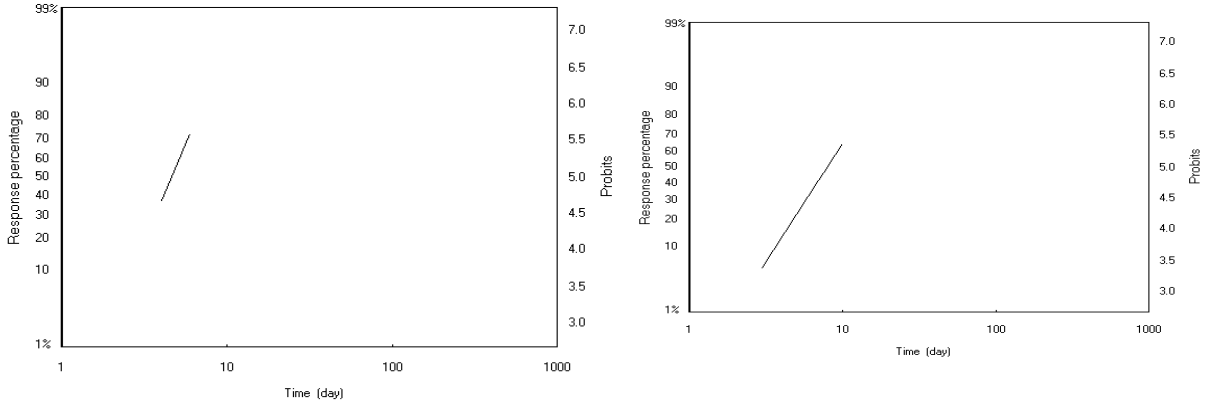


Fig. (3) Probit analysis of toxicity line of PNPV against *P. algerinum* larvae in the laboratory



$8.2x10^7$ PIB/ml **$8.2x10^8$ PIB/ml**



PIB/ml **PIB/ml**

Figs.(4) Probit analysis Lethal times (LT) of each *PNPV* concentration against *P. algerinum* larvae in the

Table (4) Lethal Times of PNPV against P. algerinum larvae

Conc (PIB/ml)	$LT_{50\ (day)}$	$LT_{90\ (day)}$	Slope (±)	Probability	Chi-Square
$8.2x10^7$	6.95	25.24	2.28±0.77	9.5	9.30
$8.2x10^8$	7.96	17.24	3.82±1.33	9.5	2.25
$2.6x10^9$	4.61	8.17	5.17± 1.05	3.8	6.1624
$8.2x10^9$	6.80	19.29	2.83±1.03	9.5	0.49

Identification of *PNPV* DNA & *SpliNPV* DNA by RAPD PCR:

The results in Tables (5a-5b) & Figs.(5-8) indicated that DNA of all samples (*SpliNPV* and *PNPV*) was amplified using four RAPD primers, Operon A9, A20 , B17 & B7 (Operon Technologies, Inc., Alameda, CA, USA) and generated total 33 bands as 24 bands for *PNPV* having molecular weights between 152 - 1204 bp and 9 bands for *SpliNPV* having molecular weights between 138 - 1767 bp, as shown in figures (5-8) and tables (5a-5b) . These results confirmed that produced *PNPV* bands molecular weights were included between resulted *SpliNPV* bands indicating that *PNPV* is similar to *SpliNPV* in almost sites of its DNA and also is a polymorphic copy from *SpliNPV*. Four arbitrary oligonucleotide primers generated different magnified segments of DNA, (figs 5-8). The primers did not behave the same when attached to *SpliNPV* and *PNPV* (figs 5-9).

The differences between the templates primer reactions in RAPD-PCR proved that the virus has species variability when infected *Pentodon* larvae. Viruses are intracellular pathogens that need binding factors to specify receptor molecules on the target host cell surface for its entry to initiate infection. Virus receptor binding molecules are greatly specific and this specificity is determined by both insect species and the cell type being infected by a given virus **[35]**.

Budded *PNPV* virus particles took part of the infected cell wall to form their polyhedral envelopes (occlusion bodies) and then became occluded virus particles. So that there were some genetic variability between *PNPV* and *SpliNPV* due to genetic difference between the cell wall of insect which they were isolated from, in addition the random compatible binding between RAPD primers used with *PNPV* DNA and *SpliNPV* DNA.

Comparative analyses of baculovirus genome sequences have revealed a high degree of diversity in genome size, organization, and gene content **[36-38]**. Phenotypic diversity is obviously observed among NPV viruses **[39, 40]**. This reflects the host-dependent evolution of NPVs **[41]**.

The detected genetic variability in the current study results was previously observed in NPV species including *Spodoptera litura NPV*, *Helicoverpa armigera NPV*, and *Spodoptera rugiperda NPV* **[42-44]**.

RAPD, markers generated by Polymerase Chain Reaction (PCR) is widely used since 1990's to assess the genetic variation at nucleic acids level (21). RAPD PCR gives a quick and efficient screen for DNA sequence based polymorphism at many loci. This method was used as initial identification method for amplified DNA **[45]**.

RAPD analysis has been previously used for various purposes such as identification and classification, **[46]**. It utilizes short synthetic oligonucleotides (10 bases long) of random sequences as primers to amplify nanogram amounts of total genomic DNA using PCR gave reproducible results to standardize the conditions used **[47-50]** used a group of RAPD primers included A9, A20, B17&B7 which we used and studied the genetic variations and diversity of *Helicoverpa armigera* NPV isolates from India , using RAPD PCR . The resulted polymorphic bands had molecular weight ranged from 1873 to 141 bp., which includes produced bands in the current study for *PNPV* and *SpliNPV*.

The full length of polyhedrin gene from lepidopteran NPVs was determined by **[51]**, and found that it ranged from 483 bp to 747 bp. Polyhedrin gene from *Spodoptera sp*. NPVs had full length ranging from 510 bp to 747 bp . In case of polyhedrin gene from *Spodoptera sp*. NPV full length ranged between 510 - 747 bp **[52]** . Therefore, it could be said that Polh-cr represented about 65% of the full length of polyhedrin gene. On comparing nucleotide sequence of Polh-cr to all available sequences in the GenBank, it created a significant homology with 100 NPV and 11 GV genes. It appeared 99% identity with *S. littoralis* polyhedrin gene (Acc# D01017), 95% with *S. litura* polyhedrin gene (Acc# AY552474) and 93% with *S. litura* polyhedrin genes (Acc# AY549963, AF325155, AF037262 and AF068189) **[53]**. From these previous studies it was shown that resulted bands of *PNPV* and *SpliNPV* included these molecular weights of the polyhedron gene.

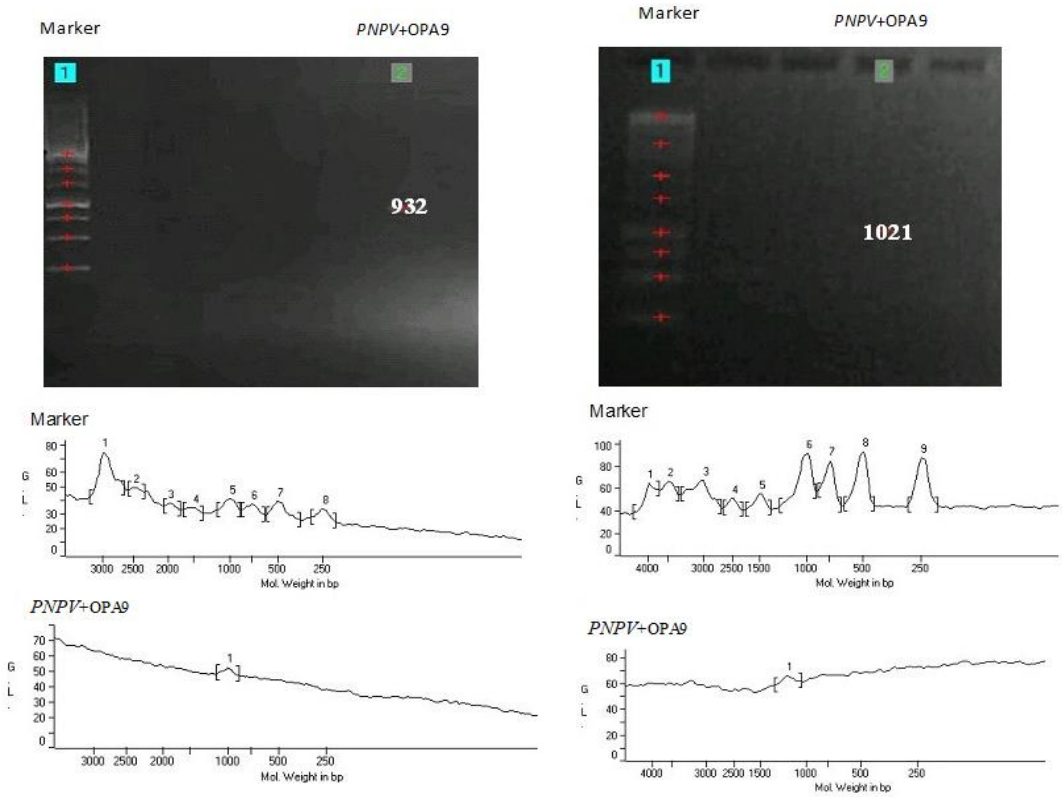
Table(5). RAPD PCR produced bands for *PNPV*DNA & *SpliNPV* DNA using four RAPD primers, Operon A9, A20& B17&B7 (Operon Technologies, Inc., Alameda, CA, USA)

RAPD PCR Primers	Total numbers of produced bands with		Molecular weights of produced bands with	
	PNPV	*SpliNPV*	*PNPV* DNA	*SpliNPV* DNA
Operon A9	7 bands	3 bands	between 185 -1204 bp	between 188 – 355 bp
Operon A20	14 bands	2 bands	between 227 -900 bp	between 208– 755 bp
Operon B17	3 bands	one band	between 152-724 bp	138 bp
Operon B7	-	3 bands	-	between 294 - 1767 bp
Total bands	24 bands	9 bands	between 152 - 1204 bp	between 138 - 1767 bp

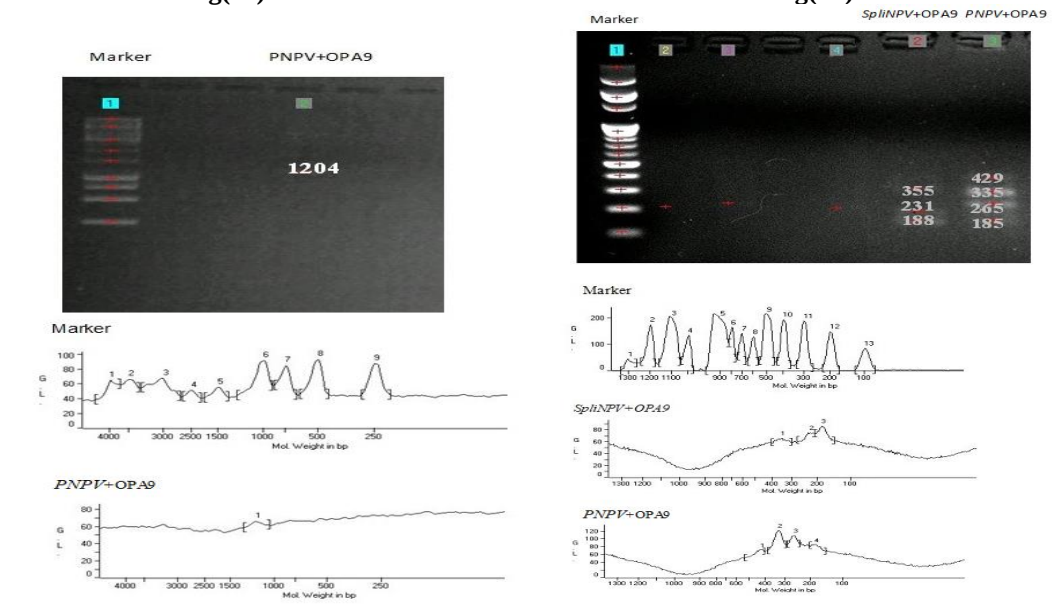
Table (5a)

Table (5b)

Marker1	Marker2	OPA9 m.wt		OPA20 m.wt		OPB7 m.wt		OPB17 m.wt	
		PNPV	*SpliNPV*	*PNPV*	*SpliNPV*	*PNPV*	*SpliNPV*	*PNPV*	*SpliNPV*
4000									
3500									
3000									
2500									
							1767		
1500	1500								
	1400								
	1300								
	1200	1204							
	1100								
	1000	1021							
	900	932		900					
	800								
750	700			733	755			724	
	600								
500	500			520					
	400			476					
				457					
		429		422					
				400					
	300			368					
			355	343					
		335		333					
				313					
	200	265		264					
250			231	242					
				227	208			200	
	100	185	188					152	138



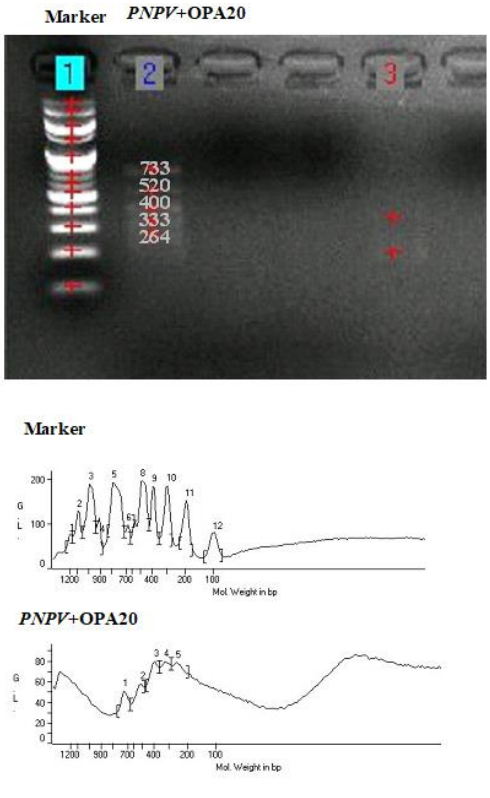
Fig(5a) **Fig(5b)**



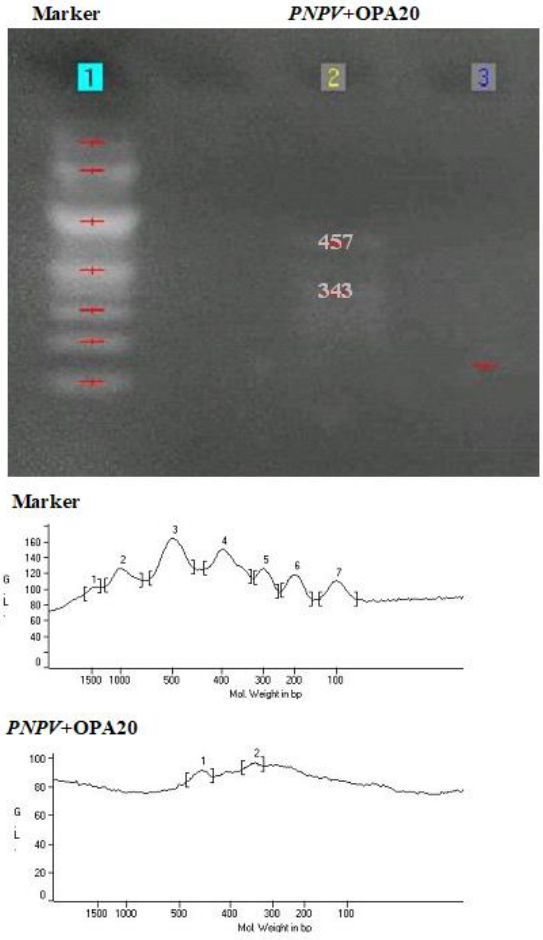
Fig(5c) **Fig(5d)**

Figs. (5a,b,c,d): Agarose gel electrophoresis& Denistometric scanning for RAPD PCR product of *PNPV* and *SpliNPV* using Operon A9 giving 7 bands with *PNPV* having molecular weights of (185,265,335,429,932, 1021&1204 bp) while with *SpliNPV* producing 3 bands with molecular weights of (188, 231&355 bp)

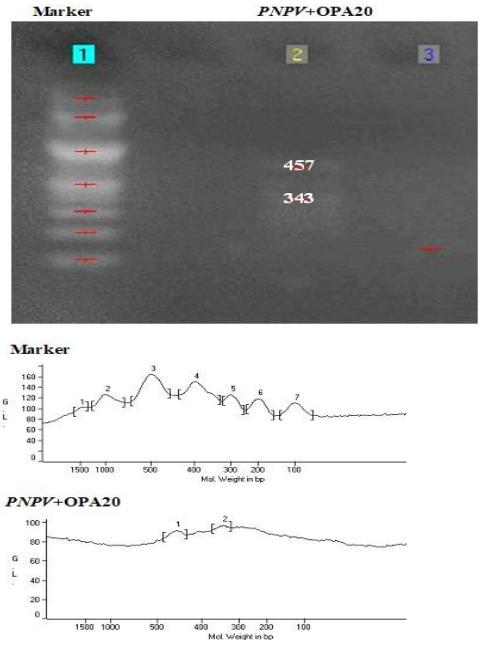
Marker 1 Kb : DNA marker ranged from 10000 - 250bp
Marker : DNA marker ranged from 1400 - 100bp
***PNPV* : *Pentodon* nuclear polyhedrosis virus DNA**
***SpliNPV* : *Spodoptera littoralis* nuclear polyhedrosis virus DNA**



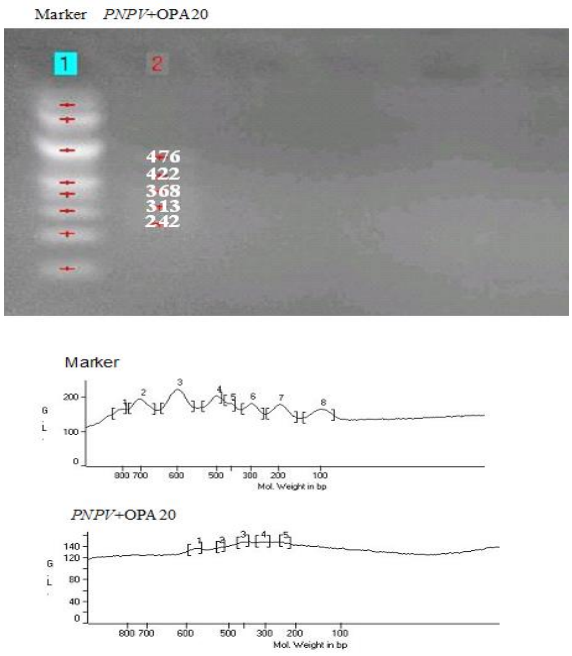
Fig(6a)



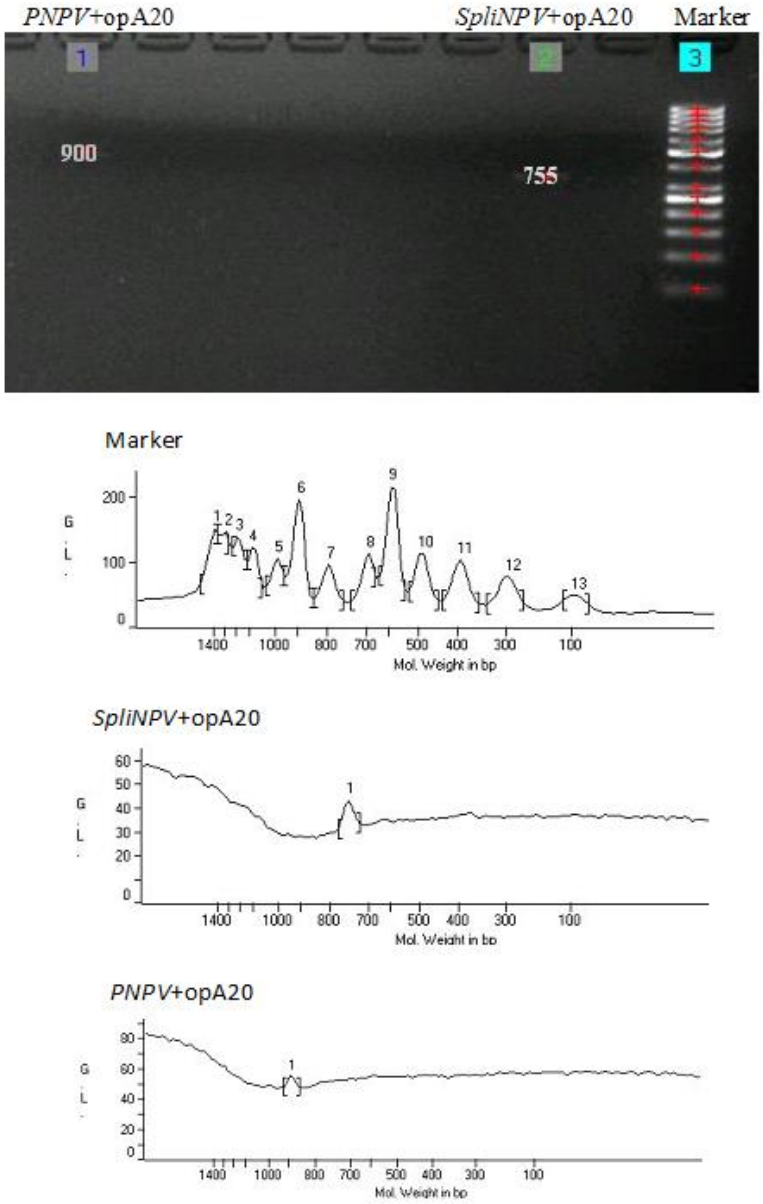
Fig(6b)



Fig(6c)



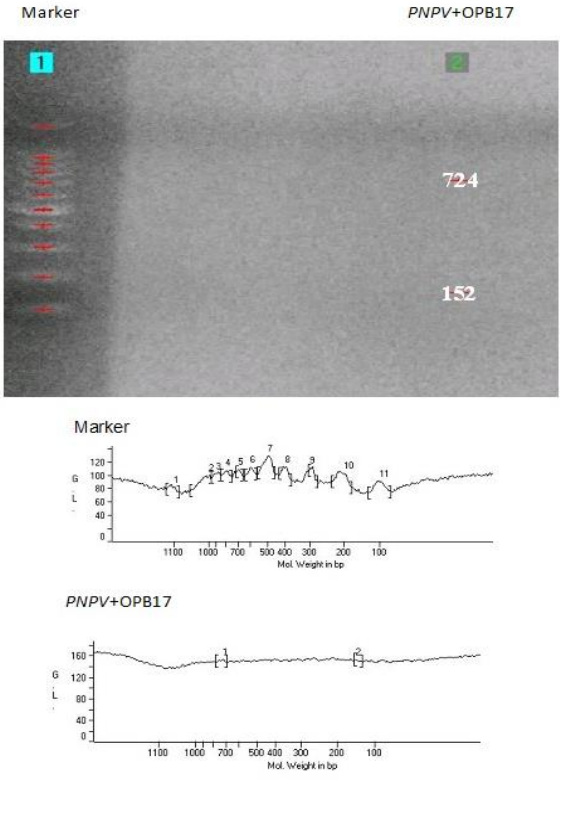
Fig(6d)



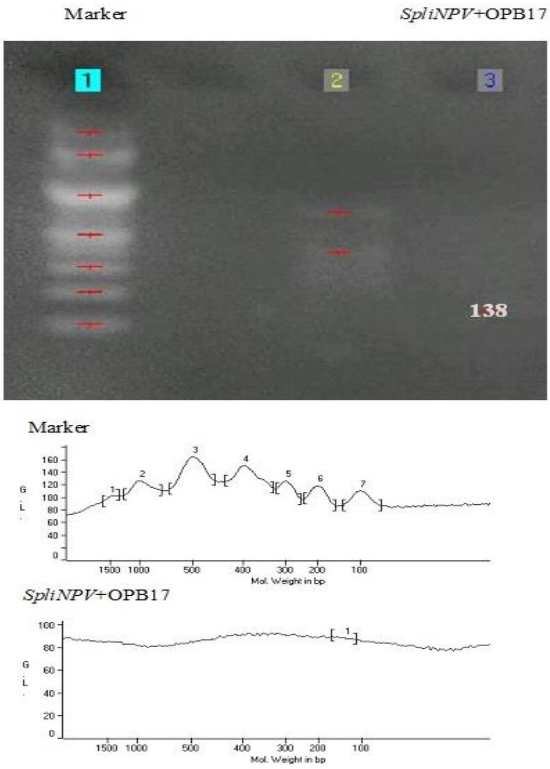
Fig(6e)

Figs. (6a,b,c,d,e): Agarose gel electrophoresis& Denistometric scanning for RAPD PCR product of *PNPV* and *SpliNPV* using Operon A20 giving 14 bands with *PNPV* having molecular weights of (227,242, 264,300, 313, 343, 368, 400, 422, 476, 457,520, 733 & 900 bp) while with *SpliNPV* producing 2 bands with molecular weights of (208 & 755 bp)

Marker : DNA marker ranged from 1400 - 100bp
***PNPV* : *Pentodon* nuclear polyhedrosis virus DNA**
***SpliNPV* : *Spodoptera littoralis* nuclear polyhedrosis virus DNA**



Fig(7a)



Fig(7b)

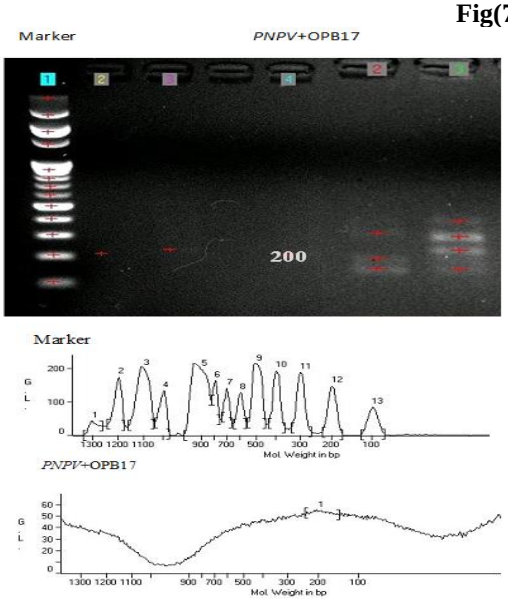
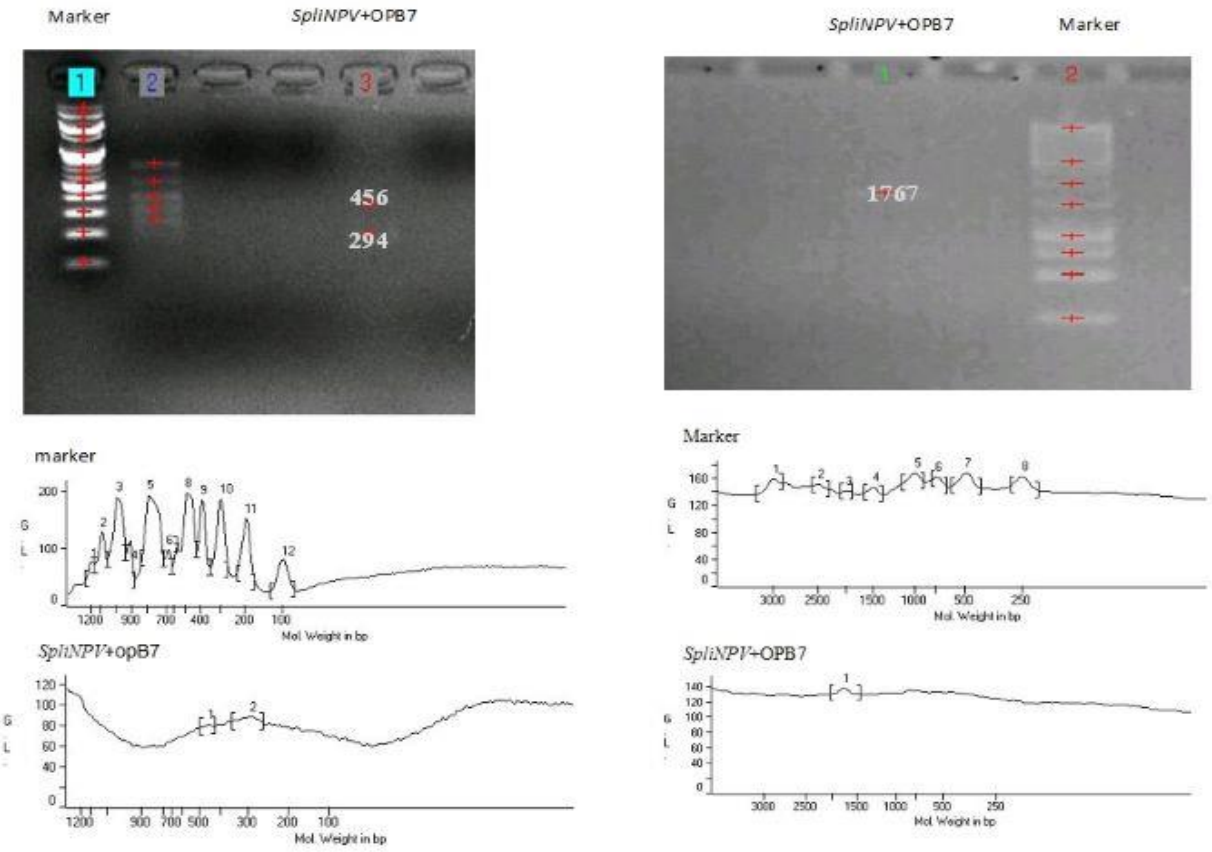


Fig (7c)

Figs. (7a,b,c): Agarose gel electrophoresis& Denistometric scanning for RAPD PCR product of *PNPV* and *SpliNPV* using Operon B17 giving 3 bands with *PNPV* having molecular weights of (152,200 &724 bp) while with *SpliNPV* producing one band with molecular weights of (138 bp)

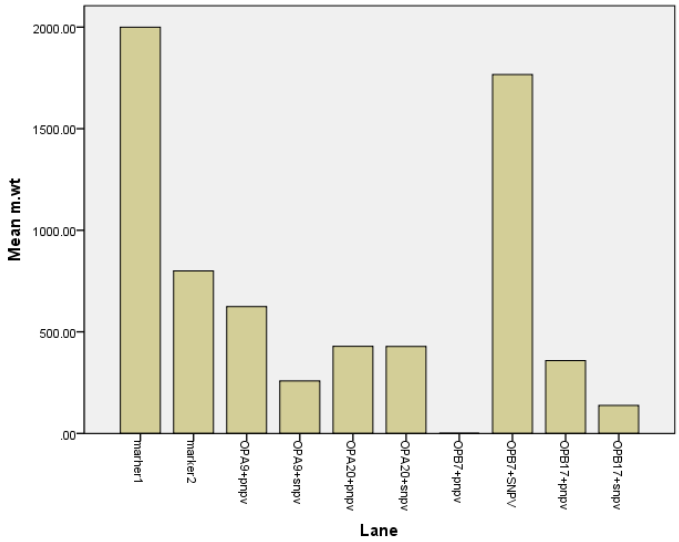
Marker : DNA marker ranged from 1400 - 100bp
***PNPV* : *Pentodon* nuclear polyhedrosis virus DNA**
***SpliNPV* : *Spodoptera littoralis* nuclear polyhedrosis virus DNA**



Fig(8a) **Fig(8b)**

Figs. (8a,b): Agarose gel electrophoresis& Denistometric scanning for RAPD PCR product of *PNPV* and *SpliNPV* using Operon B7 giving no shown accurate band with *PNPV* while with *SpliNPV* producing 3 bands with molecular weights of (294,456 &1767 **bp)**

Marker : DNA marker ranged from 1400 - 100bp
Marker 1 Kb : DNA marker ranged from 10000 - 250bp
***PNPV* : *Pentodon* nuclear polyhedrosis virus DNA**
***SpliNPV* : *Spodoptera littoralis* nuclear polyhedrosis virus DNA**



Fig(9) accumulative comparison between complementation between the used RAPD primers and *PNPV* DNA & *SpliNPV* DNA

Conclusion:

In Egypt, The white grub, *Pentodon algerinum* (Coleoptera: Scarabaeidae) damages various economic important plants recently strawberry.So that this current study aimed to DNA identification and first evaluation of the *Spodopteda littoralis Nuclear polyhedrosis virus* (*SpliNPV)* and *Pentodon algerinum Nuclear polyhedrosis virus* (*PNPV*) against *Pentodon algerinum* third instar larvae in the laboratory to find successful safe alternative control method against this pest. Bioassay was made by two experiments; one used five *SpliNPV* concentrations against pentodon larvae during thirteen days. Another experiment used four *PNPV* concentrations against this pest during ten days. The results indicated that as *PNPV and SpliNPV* concentrations decreased, the success of them to reach their receptor and initiate infection increased , that may be due to the faced insect immunity and competition between virus particles to reach the specific receptor. *SpliNPV* Lethal concentrations LC_{50} & LC_{90} were more than *PNPV* LC_{50} & LC_{90} . Thus *PNPV* and *SpliNPV were effective against Pentodon* larvae but *PNPV* was effective and specific more than *SpliNPV*. DNA of *SpliNPV* and *PNPV* was identified by RAPD PCR using four RAPD primers, Operon A9, A20, B17 & B7 (Operon Technologies, Inc., Alameda, CA, USA) using automated (Bio Rad, USA) thermal cycler. Total 33 bands were produced including 24 *PNPV* bands with molecular weights included between resulted 9 *SpliNPV* bands molecular weights. Therefore *PNPV* is similar to *SpliNPV* in almost its DNA sites and also is a polymorphic copy from *SpliNPV*. Budded *PNPV* virus particles took part of the infected cell wall to form their polyhedral envelopes (occlusion bodies) and then became occluded virus particles. So that there were some genetic variability between *PNPV* and *SpliNPV* due to genetic difference between the cell wall of insect which they were isolated from, in addition the random compatible binding between RAPD primers used with *PNPV* DNA and *SpliNPV* DNA. Thus *PNPV* and *SpliNPV* can be recommended against this pest.

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