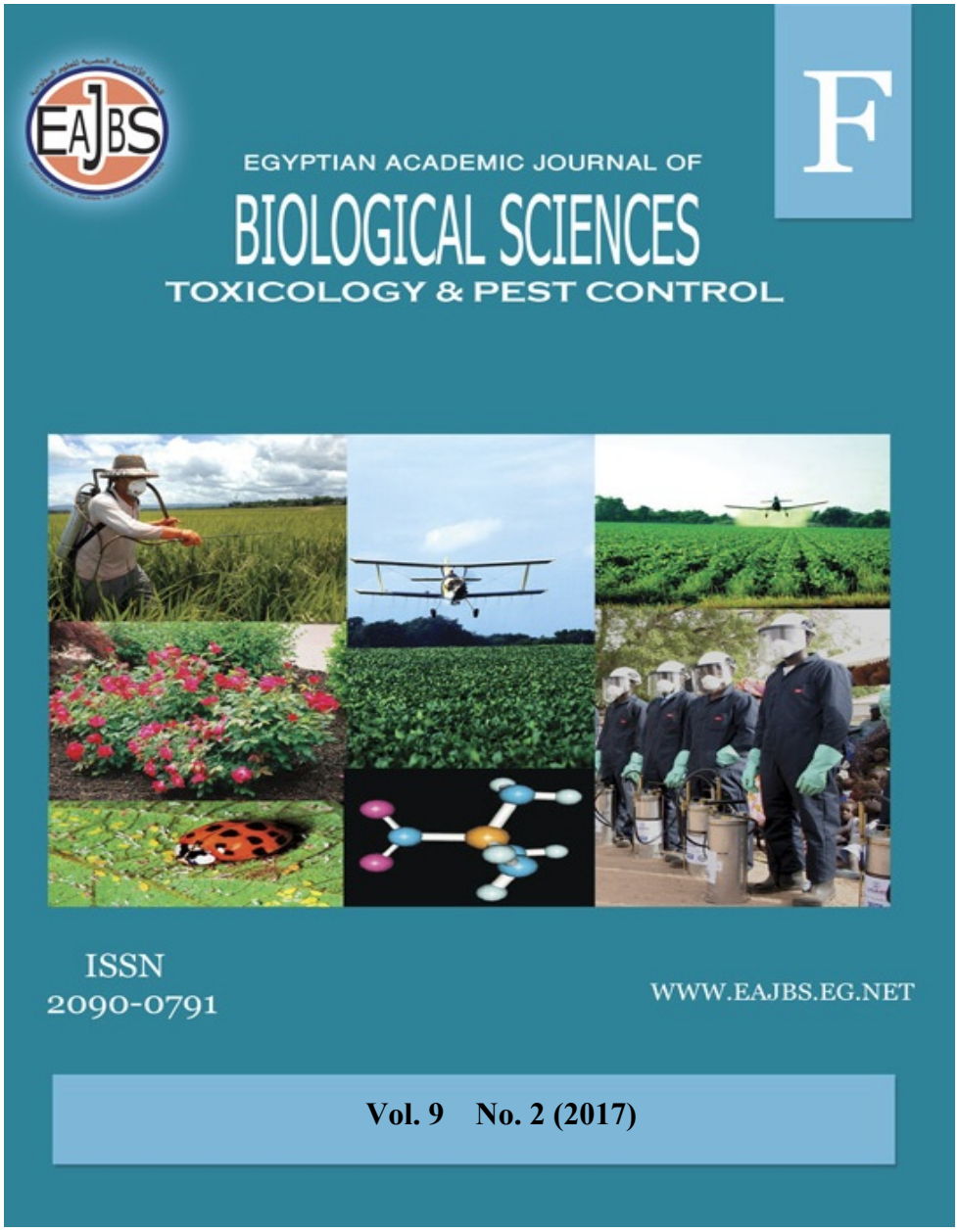

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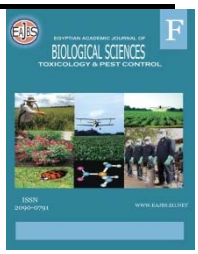

Comparative in Vitro Evaluation of Three Geographically Different Isolates of Nucleopolyhedrovirus of *Spodoptera littoralis* in Egypt

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ABSTRACT

The present study aimed to evaluate the effect of three isolates of Egyptian Nuclearpolyhedrovirus geographically different, namely NPV $_{\text{El-Qalioubia}}$, NPV $_{\text{Al-Fayoum}}$ and NPV $_{\text{El-Beheira}}$ which isolated in Bio-insectcide Production Unit Plant Protection Research Institute, were evaluated against *Spodoptera littoralis* larvae under laboratory conditions. Five concentrations of Occlusion Bodies (OBs) from each of the three NPVs isolates (1×10^6, 1×10^7, 1×10^8, 1×10^9 and 1×10^{10} PIB/ml) were used against 2nd insters larvae of *S. littoralis*. The results showed that the percentage mortality of the larvae increased with increasing concentrations of tested pathogens. Also, the isolate NPV $_{\text{Al-Fayoum}}$ was the most effective against *S. littoralis* larvae at different tested concentrations. In biological Studies all tested NPVs isolates decreased the mean larval duration than untreated larvae and decreased of pupation. NPV $_{\text{Al-Fayoum}}$ recorded the least Pupation percentage 45.7%. All tested NPVs isolates showed significantly shortening in the mean adult longevity for both males and females. All tested NPVs isolates significantly decreased the mean number of eggs laid and hatched /female.

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Biosd.) (Lepidoptera: Noctuidae), is a serious polyphagous insect pest in Egypt. It attacks numerous economically important crops throughout the year. Conventional insecticides were successful in controlling insect pests. However, use of these chemical pesticides has led to several problems, including environmental pollution and endangered human health, such as cancer and several immune system disorders (Devine and Furlong, 2007).

Due to extensive using of insecticide groups, many populations of *S. littoralis* have acquired resistance towards most of them (Alford, 2000). The problems and hazards that have arisen as a result of using conventional insecticides were incentives for the search of alternative control agents. Microbial control agents are a primary means of biological control for insect pests. The use of microbial control agents is targeted for a particular pest species. The entomopathogens that have most been used in biological control include representatives of bacteria, fungi, viruses, nematodes,

protozoa and insect growth regulator (Dent, 2000, Abdel-Aziz, 2012, Bakr *et al.,* 2013 and El-Sheikh, 2017)

Baculoviruses are considered to be the largest and most broadly studied insect viruses. They are infectious for arthropods, particularly insects of the order Lepidoptera. Baculovirus infections have been reported in over 600 insect species of the orders Hymenoptera, Diptera, Coleoptera, Neuroptera, Trichoptera, and Thysanura, as well as in the Crustaceae order Decapoda (Murphy *et al*., 1995). Baculoviridae includes nuclearpolyhedrovirus (NPV) which has polyhedron-shaped occlusion bodies. The baculovirus isolates have a limited host range, and infect only closely related species as for insects mostly of order Lepidoptera (Moscardi, 1999). Aim of the present investigation is to evaluate the effect of three Egyptian isolates of baculovirus against *S. littoralis* (Boisd.), to finding for bio-insectsid nuclearpolyhedrovirus isolate (s) with better insecticidal characteristics. Fergani, (2015) and El Sayed, *et al.,* (2016).

MATERIALS AND METHODS

Virus isolate, virus propagation and viral occlusion bodies (VOBs) purification:-

The original virus isolate was obtained from diseased *S. littoralis* larvae collected manually from cotton, tomato, and maize fields in three Governorates, El-Qalioubia, Al-Fayoum and El-Beheira. The larvae showed baculovirus infection symptoms were brought to our laboratory and examined to confirm the presence of virus by light microscope with Giemsa staining according to (Mustafa, *et al*, 2001), in which a thin smear of infected worm tissue was prepared on glass slide and dried in air. The smear was immersed for 1-2 min in Giemsa, rinsed under running tap water for 5-10 sec then the smear was stained for two hours in 10% Giemsa stain (10g of Giemsa dissolved in 100 ml. distilled water), the dye was rinsed off in running tap water for 5-10 sec and allowed to dry in air then examined under light microscope to detect the Occlusion Bodies (OBs). After the examination the diseases larvae kept at -80^{o} C until the purification of OBs (polyhedra).

The propagation of the virus isolate was performed by inoculation of the 3rd instar larvae of *S. littoralis* with *SlNPV* isolate which collected from the field and tested by light microscopy by surface contamination of the artificial diet. The inoculated larvae were observed daily to identify the *NPV* infected ones based on the sign and symptoms of disease. The tissues of dead larvae were examined as soon as possible with the naked eye and tissue smears under light microscopy as mentioned above.

The method of OBs purification was done as (Sudhakar *et al.,* 1997) with some modification. The individually dead larvae showing symptoms of *NPV* were transferred to a micro centrifuge tube and homogenized in 300 µl. of distilled water. The homogenates were filtered through a cheese cloth. The filtrate were subjected to sucrose layer (60% wt/vol) and centrifuged for 30 min at 10.000 rpm. The band was formed on top of the layer containing OBs was collected and again subjected to sucrose layer (40% wt/vol) and centrifuged at 10.000 rpm for 30 min. The band at the bottom of the gradient containing the OBs was collected and washed with distilled water. All the above steps were carried out at 4^{o}C. Pure OBs were suspended in distilled water and stored at –80^{o}C. For evaluation of OBs purification method, slide of OBs was stained with Giemsa stain as mentioned above and examined under light microscopy. The number of OBs was

counted by using Neubaur Hemocytometer to determine the concentration of PIB /ml. Five concentrations were prepared from the viral OBs mother suspension by serial dilution to be used in bioassay experiment.

Rearing of the *S. littoralis* (Boisd):

Egg masses of a sensitive strain of the cotton leaf worm, *S. littoralis* (Boisd.) were incubated under laboratory condition at 27±2^{O}C, 60 ± 5% RH and 8:16 LD photoperiod (Smits, 1987). The original insect culture was obtained from the Research Division of the Cotton Leaf worm, Plant Protection Research Institute. Newly hatched larvae were transferred to clean glass jars covered with muslin cloth held in position with rubber bands. Larvae fed on artificial diet described by (Shorey and Hale, 1965).

The toxicity experiment was carried out using diet surface treatment procedure (Addy, 1969). 2nd instars larvae of *S. littoralis* were starved for 8-10 h at 30^{o}C (Smits and Vlak, 1988) then transfer to cups with contaminated artificial diet with 20µl of ($1x10^{10}$, $1x10^{9}$, $1x10^{8}$, $1x10^{7}$, and $1x10^{6}$ PIB/ml) concentrations individually. After 2 days of feeding, the diet had become unpalatable, and the larvae were transferred to clean cups of diet and observed daily. Bioassays with 30 larvae per virus concentration plus 30 larvae as control were replicated 3 times. The experiment was conducted at a constant temperature 30^{o}C. Larval mortality was recorded at 2 day intervals during 10 days. The mortality percentages were corrected according to Abbott's formula (Abbott, 1925).

Toxicity was presented graphically as log/probit regression lines, and LC_{25},LC_{50}, and LC_{90} values as well as the slope of the probit lines were calculated (Finney, 1971).

Biological studies:

Newly hatched larvae from the maintained insect colony were collected and offered daily with artificial diet with 20µl of ($1x10^{10}$, $1x10^{9}$, $1x10^{8}$, $1x10^{7}$, and $1x10^{6}$ PIB/ml) Treated instars larvae were examined daily in order to study the following parameters: larval and pupal duration of each instar and percentage of pupation. Pupae were sexed and then placed in 3 pairs in the glass jars of the following combinations: treated male x treated female and for a control untreated male x untreated female. Subsequently, percentage of adult emergence, longevity of moths and the fecundity and fertility of eggs/female, were determined.

RESULTS AND DISCUSSION

Efficacy of the nuclearpolyhedrovirus isolates against 2nd instars larvae of *S. littoralis* larvae was performed by testing five concentrations of occlusion bodies from each of the three nuclearpolyhedrovirus isolates (1×10^{6}, 1×10^{7}, 1×10^{8}, 1×10^{9} and 1×10^{10} PIB/ml). The data in Table (1 and 2) and fig. (1) indicate that the percentage mortality of the larvae was increased with increasing concentrations of tested isolates. Furthermore, NPV $_{\text{Al-Fayoum}}$ exhibited higher toxicity to 2nd inster larvae followed by NPV $_{\text{El-Qalioubia}}$, and NPV $_{\text{El-Beheira}}$. The results of our study showed that, high concentrations of NPVs caused a high mortality rate; this conclusion is parallel to that found by (Duan and Otvos 2001) who reported that mortality was higher when younger larvae of *Choristonura fumiferana* were used. Similar findings were recorded with Abd-El Wahed *et al*. (2011) who mentioned that viruset was more effective on 2nd instar larvae than profect. LC_{90} and LC_{50} for viruset were $1x10^{6}$ and $1x10^{3}$ PIB/ml, respectively, corresponding values were $5x10^{8}$ + $1.6x10^{7}$ and $5x10^{4}$ + $1.6x10^{3}$ PIB/ml + IU/ml when profect was tested. The bioassay test revealed that the *S. littoralis* larvae showed symptoms during the first three days post-inoculation, these observations agree with (Federici 1997)

who confirmed that in typical NPV infections, such as the disease caused by the NPV of *Autographa californica* (Speyer.) (Lepidoptera: Noctuidae) (AcMNPV), there are few signs of disease during the first 3 days of infection. El Sayed, *et al.,* (2016), who found that the mortality percentage in the two tested instar larvae increased with increasing the concentrations and time elapsed post treatment. The NPV concentration $1x10^{10}$ PIB/ml ingested by larvae produces approximately 96% mortality percentage at 10 days post inoculation for *S. littoralis* 2nd instar larvae and 83% of the same concentration for 4th instar larvae. The remaining concentrations produce mortality percentages ranged from 90 to 48% and 75 to 45% of the two tested instars larvae used, respectively. That was in agreement with Abdel-Aziz, (2007), El Sayed, (2015) and El-Sheikh, (2017).

Table (1): Efficacy of three of nuclearpolyhedrovirus isolates against 2nd instar larvae of *S. littoralis* after 10 days post treatment

Concentrations PIB/ml*	**Mortality %**		
	NPV El-Qalioubia	**NPV Al-Fayoum**	**NPV El-Beheira**
$1x10^{10}$	89	90	88
$1x10^{9}$	81	85	80
$1x10^{8}$	70	72	68
$1x10^{7}$	55	65	50
$1x10^{6}$	48	49	42

*PIB: Polyhedral inclusion body

Table (2): Susceptibility of three of nuclearpolyhedrovirus isolates against 2nd instar larvae of *S. littoralis* after 10 days post treatment**.**

Virus isolates	**LC_{90} (PIB/ml)***	**LC_{50} (PIB/ml)***	**LC_{25} (PIB/ml)***	**Slope±SE**
NPV El-Qalioubia	1.89E+11	2.28E+07	1.97E+05	0.327 ± 0.045
NPV Al-Fayoum	8.84E+10	9.09E+06	72404.08	0.321 ± 0.046
NPV El-Beheira	2.95E+11	5.28E+07	5.64E+05	0.334 ± 0.044

*PIB: Polyhedral inclusion body

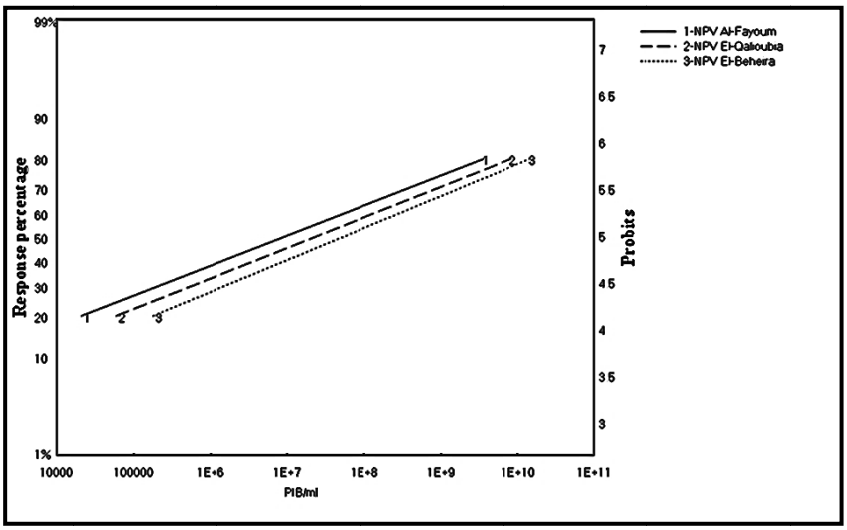

Fig (1): Toxicity of three of nuclearpolyhedrovirus isolates against 2nd instar larvae of *S. littoralis* after 10 days post treatment

The obtained results in Table (3) clarified the effect of the tested isolates virus on the mean larval duration, pupation%, and pupal duration. The treatment of the 2nd larval instar with LC_{50} of the three isolates decreased the mean larval duration about 0.6-1.6 day than untreated larvae (Table 2) this was in agreement with El Sayed, (2015) who found that the treatment of 2nd instars larvae of *S. littoralis* with Littovir (NPV) has reduced the mean larval duration about 72 hr. than untreated larvae. The three isolates affect the percentages of larvae entering pupation which was decreased in 2nd instar larvae treated than untreated larvae (47.6, 45.6 and 47%) for NPV $_{\text{El-Qalioubia}}$, NPV $_{\text{Al-Fayoum}}$ and NPV $_{\text{El-Beheira}}$ respectively. Meanwhile, the pupal stage were decrease in all isolates of 2nd instars larvae treated with NPV $_{\text{El-Qalioubia}}$, NPV $_{\text{Al- Fayoum}}$ and NPV $_{\text{El-Beheira}}$ were decreased (14, 13, and 14.3 days) respectively, than untreated larvae (15.3 days). These results were agreed with those of Abd El-Kareem, (2012) who treated *S. littoralis* with Protecto, Viruset, and Profect has shorted the pupal duration of the treated instars larvae.

(Table 4) showed that the three isolates affect the percentage of adult emergence was only slightly decreased than the control to 91, 90.3 and 94.6% for the three isolates NPV $_{\text{El-Qalioubia}}$, NPV $_{\text{Al-Fayoum}}$ and NPV $_{\text{El-Beheira}}$ respectively. Also, all tested isolates showed significantly shortening in the mean adult longevity for both males and females (Table 4). That was in coincides with Abd El-Kareem (2012) and El Sayed, (2015) who noticed decrease in the percentage of adult emergence and mean adult longevity of treated larvae of *S. littoralis* with Viruset.

Table (5) showed the latent effect of the treated *S. littoralis* with LC_{50} of tested isolates NPV on the mean number of laid and hatched eggs/female. All tested isolates NPV significantly decreased the mean number of eggs laid/female. NPV $_{\text{Al-Fayoum}}$ was the most effective virous, followed by NPV $_{\text{El-Qalioubia}}$, and NPV $_{\text{El-Beheira}}$. On the other hand, significantly reduction in the mean number of hatched eggs/female was observed when treated instar larvae with recommended dose of all tested (Table 5). That was in agreement with Abdel-Aziz, (2007), Abd El-Kareem, (2012) and El Sayed, (2015).

The results of this study showed that the three isolates could be used in the pests control. The best isolates were the isolation of El-Fayoum Governorate (NPV $_{\text{Al-Fayoum}}$) which gave the highest percentage of mortality from other isolates as well as the largest reduction rate in all biological experiments, Followed by El-Qalioubia Governorate NPV $_{\text{El-Qalioubia}}$ and then the El-Beheira Governorate NPV $_{\text{El-Beheira}}$.

Table (3): Effect of three of nuclearpolyhedrovirus isolates on larval duration, pupation rate and pupal duration of 2nd instars larvae of *S. littoralis*.

Virus isolates	Mean larval duration (days) ± S. E.	%Pupation	Mean pupal duration (days) ± S. E.
NPV $_{\text{El-Qalioubia}}$	15 ± 0.34^{ab}	47.6^{b}	14 ± 0.59^{ab}
NPV $_{\text{Al-Fayoum}}$	14.3 ± 0.33^{b}	45.6^{c}	13 ± 0.58^{b}
NPV $_{\text{El-Beheira}}$	15 ± 0.59^{ab}	47^{bc}	14.3 ± 0.33^{ab}
Control	15.6 ± 0.34^{a}	100^{a}	15.3 ± 0.34^{a}
F values	2.44^{ns}	2319.8^{***}	4.166^{*}
L.S.D.	1.3313	1.8026	1.537

-Means with the same letter are not significantly different ($p<0.05$).

Table (4): Effect of three of nuclearpolyhedrovirus isolates on adult emergence percentage and adult longevity of 2nd instars larvae of *S. littoralis*.

Virus isolates	Adult emergence %	Mean adult longevity (days) ± S. E.	
		♂	♀
NPV El-Qalioubia	91[c]	10 ± 0.58[b]	9.6 ± 0.33[b]
NPV Al-Fayoum	90.3[c]	9.6 ± 0.33[b]	10 ± 0.58[b]
NPV El-Beheira	94.6[b]	11.6 ± 0.39[ab]	10.33 ± 0.67[ab]
Control	100[a]	12 ± 0.59[a]	11.3 ± 0.34[a]
F values	141.33[***]	3.15[ns]	3.77[ns]
L.S.D.	1.215	1.802	1.33

-Means with the same letter are not significantly different ($p<0.05$).

Table (5): Effect of three of nuclearpolyhedrovirus isolates on fecundity of *S. littoralis* treated as new hatch instars.

Virus isolates	Mean no. of eggs/female ± S.E.	Mean no. hatched eggs/female ± S.E.
NPV El-Qalioubia	741.67 ± 59.9[b]	692±57.04[b]
NPV Al-Fayoum	586 ±13.5[c]	502±51.3[c]
NPV El-Beheira	750.3 ± 52.09[b]	685±56.40[b]
Control	2068 ± 46.5[a]	1956±53.09[a]
F values	229.78[***]	156.95[***]
L.S.D.	148.8	174.6

-Means with the same letter are not significantly different ($p<0.05$).

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ARABIC SUMMERY

مقارنة معملية لثلاث عزلات مختلفة جغرافياً من الفيروس النووي لدودة ورق القطن في مصر

مروة محمد محمود عبد العزيز الصباغ

معهد بحوث وقاية النباتات-مركز البحوث الزراعية- دقي- جيزة- مصر

هدفت الدراسة الحالية إلى تقييم تأثير ثلاث عزلات من الفيروس النووي لدودة ورق القطن الكبري من ثلاث محافظات مختلفة وهي $NPV_{El\text{-}Qalioubia}$, $NPV_{Al\text{-}Fayoum}$ and $NPV_{El\text{-}Beheira}$ والتي تم عزلها في معهد بحوث وقاية النبات بوحدة انتاج المبيدات الحيوية ضد يرقات دودة ورق القطن الكبري تحت الظروف المعملية .تم استخدام خمسة تركيزات من البولي هيدرا لجميع العزلات الثلاثة (1×10^6, 1×10^7, 1×10^8, 1×10^9 and 1×10^{10} PIB/ml) وذلك ضد يرقات العمر الثاني لدودة ورق القطن وأظهرت النتائج أن نسبة موت اليرقات زادت مع زيادة تركيزات الفيروسات المختبرة . وقد كانت عزلة $NPV_{Al\text{-}Fayoum}$ الأكثر فعالية ضد يرقات دودة ورق القطن لجميع التركيزات المختلفة وبدراسة التغيرات البيولوجية للحشرة والتي سبق معاملتها بالتركيز النصف مميت في بداية الطور اليرقي الثاني ومتابعة التطور البيولوجي له بالمقارنة بالحشرات غير المعاملة بالفيروس وجد حدوث انخفاض في طول الطور اليرقي للعزلات الثلاثة وانخفاض في نسبة التعذير والتي بلغت ٤٥.٦ % من نسبة التعذير للحشرات المتبقية من $NPV_{Al\text{-}Fayoum}$ وكذلك انخفضت فترة الطور العذري في جميع المعاملات بالمقارنة بالحشرات غير المعاملة ثم انخفضت نسبة ظهور الفراشات في جميع المعاملات وقصرت فترة بقاء الفراشات الاناث والذكور علي حد سواء في جميع المعاملات واخيرا حدث خفض كبير في اعداد البيض التي وضعتها الفراشات في جميع المعاملات وكذلك خفض كبير في متوسط اعداد الفقس .

من نتائج هذه الدراسة اتضح انة يمكن استخدام العزلات الثلاثة في المكافحة وان افضل العزلات هي عزلة محافظة الفيوم $NPV_{Al\text{-}Fayoum}$ حيث أعطت اكثر نسبة موت من العزلات الأخرى وكذلك اكبر نسبة خفض في جميع المعاملات البيولوجية المختبرة يليها محافظة القليوبية$NPV_{El\text{-}Qalioubia}$ ثم محافظة البحيرة $NPV_{El\text{-}Beheira}$.