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# Biological Control of Two Date Fruit Insect Pests Using Entomopathogenic Viruses

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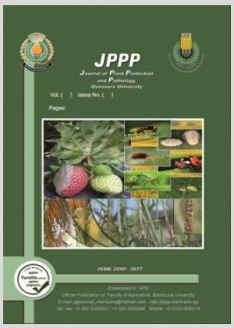


## ABSTRACT

The date palm is one of most important fruits in Middle East. Date fruits are exposed to infestation by many insect pests like *Ephestia cautella* (Walker) and *Plodia interpunctella* (Hübner) which decrease their quantity and quality. This study aimed to evaluate efficiency of two viruses (NPV and GV) on mentioned these insects. Seven concentrations of each virus were tested ($1x10^1$ to $1x10^7$) against these two insects at various durations: 5, 7, and 10 days. Results showed that corrected larval mortality% of *E. cautella* and *P. interpunctella* increased by increasing viruses concentrations and exposed durations with highest percentages were 54.44 and 73.33% for *E. cautella* and *P. interpunctella* larvae respectively compared with control after treating with NPV virus $1x10^7$ for 10 days. While these were 74.33 and 100% for *E. cautella* and *P. interpunctella* respectively in case of GV virus compared with control. Data obtained revealed that *P. interpunctella* larvae were more susceptible to tested viruses than *E. cautella* larvae. Further, the two insects were more susceptible to GV concentration than NPV concentrations. The corrected mortality percentages of *E. cautella* and *P. interpunctella* larvae were increased to 95.56 and 98.89% respectively after using mixture of $LC_{50}$s of two viruses compared to $LC_{50}$ of each virus separately. In addition, both viruses exhibited no-effect on tested chemical contents of date's fruits. As a conclusion, combination of both viruses increased their efficacy against *E. cautella* and *P. interpunctella* and this has to be considered in biocontrol programs of both insect pests.

***Keywords:*** Date palm, *Ephestia cautella*, *Plodia interpunctella*, NPV, GV.

## INTRODUCTION

The date palm (*Phoenix dactylifera*) is one of the most important and oldest fruit trees. It is found and cultivated mainly in North Africa and the Middle East (Haldhar *et al.*, 2017) Date palm considers the source of carbohydrate in many regions), shelter, wood products and the whole palm is also used. Egypt produces about 1.8 million tons of dates annually which produced from 15 Million fruitful palm trees; this is made it occupy the first production place in the world with 21% of the total world production (Egyptian Ministry of Agriculture, 2018).

Date fruits are affected by many insect pests that reduce their production quantitatively and qualitatively. Whereas, the insect pests that infest date fruits, whether in the field, and which extend with it to the storeroom, sometimes cause a decrease of 20-27% of production Ali, *et al.*, (2002). Date fruit pests cause a decrease in production of up to 50% after storing them for a period of 6-7 months. Mewtally, *et al.*, (2007). Palm cultivation therefore faces some problems due to insect and disease infestations (Erskine, *et al.*, 2004).

The Amri date worm (*Ephestia cautella*) is considered one of the most important insect pests that infest the date fruits in Egypt. Infestation begins in the field and moves with the date fruits to the storeroom, where it can reproduces for many generations with a great loss (Howard *et al.*, 2001; El-Shafei, 2018; El-Shafei, *et al.* 2020). *Plodia interpunctella* is one of the most dangerous insects that infests a wide range of dried fruits, vegetables and stored materials such as stored date fruits (Perez-Mendoza and Aguilera-Pena, 2004; Mohandass, *et al.*, 2006). Larvae of this pest decreases both quality and quantity of stored products through feeding, webbing, and fecal matter (Johnson *et al.*, 1997; Hansen and Jensen, 2002; Johnson, *et al.*, 1997). *Plodia interpunctella* attacks the date fruits and causes severe damage during storage (El-Shafei, *et al.* 2018; Zinhoum, *et al.*2019).

The control of these pests in storage systems mainly depends on fumigants such as methyl bromide, which has been banned in many countries since 2004 because of its ozone depleting properties (Hansen and Jensen, 2002). Fumigation by Phosphine is used as an insecticide fumigates grains, storage food, date fruits and animal feed. The toxicity of phosphine by inhalation exposure cause dizziness, headaches, vomiting, cough nausea, fatigue, drowsiness and burning substernal pain Sciuto, *et al.* (2016). Furthermore, the use of chemical pesticides has led to many other problems, including environmental pollution and human health hazards (Bravoa, *et al.*, 2011). Thus there is an urgent need to develop safe alternatives that have the potential to replace the toxic fumigants, with effective, economical and convenient uses (Ayvaz and Karaborklu, 2008). Many alternatives have been tested to replace these fumigants for stored product and quarantine uses such as controlled atmospheres (Hallman and Denlinger, 1999).

Biological control is considered one of the most important methods to protect the economic plants and

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husbandry from pests, plant diseases, weeds and mites by using living organisms like bacteria, fungi and viruses (Eilenberg, *et al.* 2001). Viral biocontrol used as bio-agent for resistance insect pests. Family *Baculoviridae* is an important group to control lepidopteran species pests. It's known an insect specific viruses. *Baculoviridae* include many viruses like Nucleopolyhedroviruses (NPVs) and Granuloviruses (GVs) (Kroemer, *et al.* 2015). The NPVs has occlusion bodies (OBs) formed by viral polyhedrin protein which contains multiple nucleocapsids packed singly or in groups while GVs has it's OBs with single nucleocapsid formed by the viral granulin proteins (King, *et al.* 2011). Bio-insecticides are becoming increasingly attractive as alternative pesticides. In particular, *Baculoviridae* has long been recognized as an environmentally safe potential alternative to chemical pesticides since viruses are highly host-specific, non-pathogenic to beneficial insects and other non-target organisms, including mammals, which making them effective candidates for integrated pest management IPM (Miller, 1997). Insect viruses can be classified into a number of groups using structural features alone. Of these groups the occluded viruses in which the virus particles are occluded in protein crystal that having a variety of forms are most different from viruses pathogenic to man, plants and animals. Nuclear polyhedrosis viruses are a group of Baculoviruses in which the majority are highly host specific and that have received more attention than other groups of insect viruses. Although viruses differ in the exact shape or size of inclusion body, identification by shape and size is generally unreliable, as many viruses with quite different host ranges are identical in appearance. Granulo-virus form small bodies called granules containing a single virion. Thus, the objective of this work aims to evaluate the effect of two viruses, NPV and GV, against *E. cautella* and *P. interpunctella* larvae either in a separate or mixed way and to evaluate their effect on date fruits characteristics, as well.

## MATERIALS AND METHODS

Experiments were conducted at date pests and diseases Dept., Central Laboratory of Date Palm, Agricultural Research Center, Giza, Egypt, and the laboratory of virology, Microbiology Department, Faculty of Agriculture, Ain shams University.

**Rearing of Insects Culture:**

The two tested insect species were collected from infested date fruits and reared on their standard food diets. The adults were reared on semi dry date fruits, Siwi-cultivar. The date fruits were sterilized before using in the experiments using freezing method by continuous freezing (-10°C) for at least two months, then kept under laboratory condition for 12 h, before use (Hussain, 2008). Insects culture was kept in an incubator at 27±2°C and 65±5% Rh. The larvae of *E. cautella* and *P. interpunctell*a were separately evaluated.

**Source of Viruses:**

**NPV:**

The viral isolate was obtained from laboratory of virology, Microbiology Department, Faculty of Agriculture, Ain shams University, Cairo, Egypt.

**GV:**

The viral isolate was obtained kindelly collected, from biological control unit, plant protection research institute, Agricultural Research Center, Dokki, Giza, Egypt.

**Preparing the insects samples for bioassay tests of biological control:**

Number of 30 second instar larvae of each insect species *E. cautella* and *P. interpunctella* were kept into plastic gar 1000 ml volume, each gar contained 250 g. Date fruits sprayed with seven concentrations of each virus (serial from $1x10^1$ to $1x10^7$ PIB/ml) and covered with muslin clothe and closed with rubber bands. Three of untreated gars were kept as control for each species which sprayed only with water. Each virus was applied individually and mixed against the date fruit pests *E. cautella* and *P. interpunctella* larvae in stored date fruit under laboratory conditions of; 27±2°C and 65±5% R.H. The treatments were replicated three times. The number of dead larvae in each jar was counted on specific dates after 5, 7 and 10 days from the treatments and the percentages of mortality were recorded. Mortality of the insects was corrected according to Abbott 1925 as following:

$$\text{Corrected Mortality \%} = \frac{\text{\% Mortaity in treatment - \% Mortality in control}}{\text{100 - \% Mortality in control}} \times 100$$

**Combination of the two viruses:**

After the calculation of the $LC_{50}$ value of each virus another experiment was conducted to evaluate the effect of mixing the two viruses on the mortality of the two insect species compared to the mortality percentages those treated separately with each of NPV and GV $LC_{50}$s.

**Cytotoxicity of NPV and GV assay:**

Cytotoxicity of NPV and GV ($1x10^8$ PIB for each virus) was estimated based on normal mammalian cells (normal lung cells wi-38) according to (Repetto, *et al.* 2008). Cytotoxicity was calculated depending on the percentage of viability of cells that treated with different concentration of NPV and GV (10, 15 and 20% for each virus from the initial concentration) for 24h/20ºC/ 20% RH. Cytotoxicity test was assessed in the cell culture Laboratory, Faculty of Agriculture Research Park (FARP), Cairo University.

**Biochemical Studies:**

**Determination of reducing sugar and soluble sugars:**

The alkaline potassium ferricyanides colorimetric method of Schales and Schales (1945) was used determine reducing sugars and total soluble sugars.

**Estimation of total amino acid:**

The total amino acid was estimated using ninhydrin as described by McGrath (1972)

**Determination of total phenol contents:**

Estimation of total phenol contents was done by Folin Ciocalteu's method according to Elizabeth and Kelly (2007) and Patel *et al.* (2010).

**Determination of total indols:**

The total indols were determined according to Selim, *et al.* (1978).

**Statistical Analysis:**

Mortality rates of insects were corrected using the Abbott formula (Abbott, 1925) compared with the control (untreated) and $LC_{50}$ and $LC_{90}$ were calculated through the probit analysis as described by Finney (1971). Data of quality analysis of date fruits were analyzed using Proc., ANOVA in SAS (SAS Institute 2006).

## RESULTS AND DISCUSSION

**Results**

**1. Efficacy of using virus NPV on controlling *E. cautella* and *P. interpunctella* larvae on stored date fruits.**

The efficacy of seven concentrations of NPV virus (serial from $1x10^1$ to $1x10^7$ PIB/ml) against the larvae of *E. cautella* and *P. interpunctella* at tested temperatures (27°C ±1 and 65 ± 5% R.H. and different exposure periods,5,7and 10 days is illustrated in Fig.(1).The results revealed that the corrected mortality percentages of *E. cautella* larvae after five days of exposer to NPV virus started by 1.11 %for the lowest concentration ($1x10^1$) and increased gradually by increasing of concentration to reach 41.11 % at the highest concentration ($1x10^7$).While the mortality percentages of *P. interpunctella* larvae after five days of exposer to NPV virus started by 2.22 % for the lowest concentration ($1x10^1$) and increased gradually by increasing of concentration to reach 42.22 % at the highest concentration ($1x10^7$).After seven days of exposer to NPV virus the mortality percentages of *E. cautella* and *P. interpunctella* started by (10.00and12.22%) respectively for the lowest concentration ($1x10^1$) and reached (52.22and52.22%) respectively at the highest concentration ($1x10^7$).Finally after ten days of exposer to NPV virus the mortality percentages of *E. cautella* and *P. interpunctella* larvae ranged from (11.11 and31.11%) to (54.44and73.33 %) for the lowest and highest ($1x10^1$ and $1x10^7$) concentration respectively .

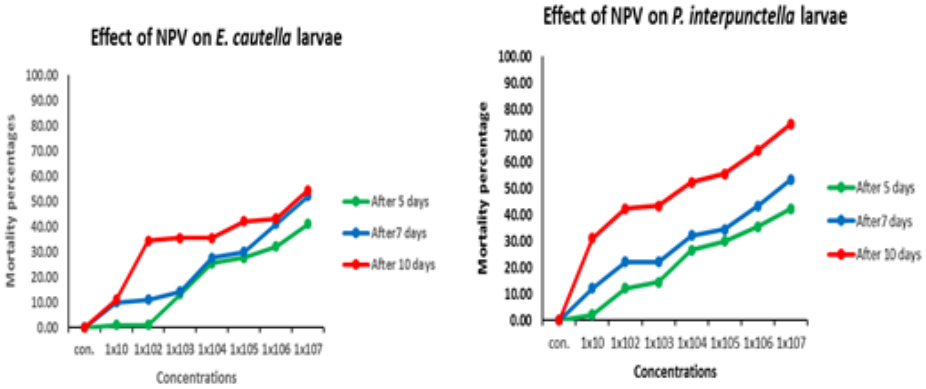


**Fig. 1. Efficacy of using virus NPV against *E. cautella* and *P. interpunctella* larvae on stored date fruits. a) Effect of NPV on *E. cautella* larvae, b) Effect of NPV on *P. interpunctella* larvae.**

**2. Efficacy of using virus GV on controlling *E. cautella* and *P. interpunctella* larvae on stored date fruits.**

The efficacy of seven concentrations of GV virus ($1x10^1$, $1x10^2$, $1x10^3$, $1x10^4$, $1x10^5$, $1x10^6$ and $1x10^7$ PIB/ml) against the larvae of *E. cautella* and *P. interpunctella* at tested temperatures (27°C ±1 and 65 ± 5% R.H. and different exposure periods,5,7and 10 days is illustrated in Fig.(2). The results indicated that the corrected mortality percentages of *E. cautella* and *P. interpunctella* larvae after five days of exposer to GV virus started by (3.33 and 5.55%) respectively for the lowest concentration$1x10^1$and increased gradually by increasing of concentration to reach (42.22 and 43.33%) respectively at the highest concentration $1x10^7$. After seven days of exposer to GV virus the corrected mortality percentages of *E. cautella* and *P. interpunctella* larvae started by (14.44 and15.55 %) respectively for the lowest concentration$1x10^1$and reached (53.33 and 82.22 %) respectively at the highest concentration $1x10^7$. Finally after ten days of exposer to GV virus the mortality percentages of *E. cautella* and *P. interpunctella* larvae ranged from (33.33 and 38.89 %) to (74.44 and 100 %) for the lowest and highest ($1x10^1$ and $1x10^7$) concentration respectively.

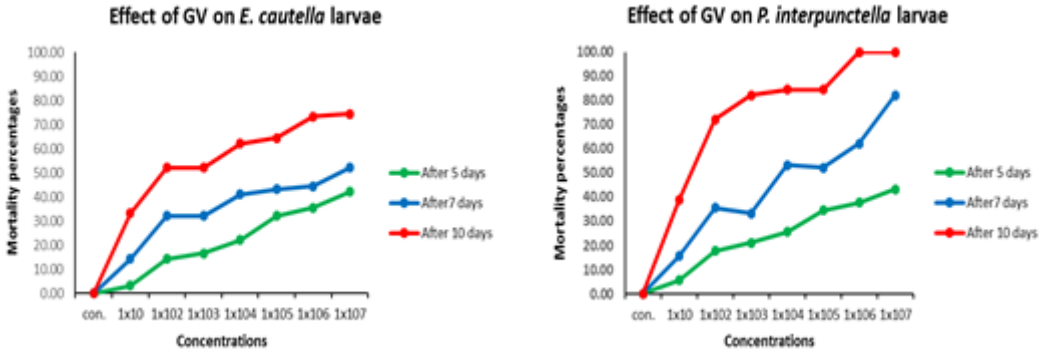


**Fig. 2. Efficacy of using virus GV against *E. cautella* and *P. interpunctella* larvae on stored date fruits. a) Effect of GV on *E. cautella* larvae, b) Effect of GV on *P. interpunctella* larvae.**

**3. Lethal concentration values and mortality regression lines by NPV and GV viruses.**

Lethal concentration values ($LC_{50}$ and $LC_{90}$) and parameters of mortality regression line for the *E. cautella* and *P. interpunctella* larvae treated with NPV and GV viruses on stored date fruits are presented in Table (1). The results showed that the concentration required to obtain 50% ($LC_{50}$) mortality for the NPV virus various

concentration were ($2.8\times10^6$ and $7.3\times10^3$) for *E. cautella* and *P. interpunctella* larvae respectively. While the LC50 of GV virus were ($1.3\times10^3$ &$2.1\times10^1$) for *E. cautella* and *P. interpunctella* larvae respectively. The same trend was recorded at the $LC_{90}$ level for the two tested viruses NPV and GV. Data showed clearly that the *P. interpunctella* larvae was more susceptible to the two viruses NPV and GV than the *E. cautella* larvae and virus GV is more efficient in controlling the larvae of the two insects *E. cautella* and *P. interpunctella* than NPV virus. The obtained correlation coefficient values of regression lines for *E. cautella* and *P. interpunctella* larvae indicated high significant correlation between the concentration and the larval mortality.

**Table 1. $LC_{50}$ and $LC_{90}$ values with their confidence limits for *E. cautella* and *P. interpunctella* larvae exposed to viruses NPV and GV.**

| Viruses | Insect species | $LC_{50}$ PIB/ml | $LC_{90}$ PIB/ml | Confidence limits(PIB/ml) | | | | Slope± SD | r |
|---|---|---|---|---|---|---|---|---|---|
| | | | | $LC_{50}$ | | $LC_{90}$ | | | |
| | | | | Lower | Upper | Lower | Upper | | |
| NPV | *E. cautella* | $2.8\times10^6$ | $4.2\times10^{12}$ | $5.8\times10^5$ | $2.7\times10^7$ | $5.8\times10^{10}$ | $1\times10^{16}$ | 0.208±0.032 | 0.984 |
| | *P. interpunctella* | $7.3\times10^3$ | $6.6\times10^{10}$ | $1.4\times10^3$ | $3\times10^4$ | $1.9\times10^9$ | $3.5\times10^{13}$ | 0.184±0.027 | 0.992 |
| GV | *E. cautella* | $1.3\times10^3$ | $2.9\times10^{10}$ | $1.5\times10^2$ | $6.7\times10^3$ | $5.1\times10^8$ | $6.8\times10^{13}$ | 0.174±0.029 | 0.988 |
| | *P. interpunctella* | $2.1\times10^1$ | $2.5\times10^3$ | 8.8 | $3.9\times10^1$ | $9.5\times10^2$ | $1.4\times10^4$ | 0.617±0.098 | 0.969 |

**4. Efficacy of combination of the two viruses NPV and GV against *E. cautella* and *P. interpunctella* larvae.**

The $LC_{50}$s of the two viruses NPV and GV were determined ($2.8\times10^6$ and $7.3\times10^3$) respectively after treated *E. cautella* and *P. interpunctella* larvae with NPV while it the case of GV virus recorded ($1.3\times10^3$ and $2.1\times10^1$) respectively. After mixing the $LC_{50}$s of the two viruses NPV and GV together and treating the larvae of two tested insects *E. cautella* and *P. interpunctella*, the corrected mortality percentages were (80.00, 85.56 and 95.56%) after exposer to (5,7and 10 d) respectively for *E. cautella* larvae of compared to the mortality percentage of $LC_{50}$ .While in the case of *P. interpunctella* larvae it were (84.44, 93.33 and 98.89%) after (5,7and 10 d) respectively compared to the mortality percentage of $LC_{50}$. Obtained data illustrated in Fig. (3), showed clearly that , When the $LC_{50}$ of the two viruses were mixed together the corrected mortality percentages resulting from treated *E.cautella* and *P. interpunctella* larvae at treatments increased compared to the LC50 for each virus and reduced the concentrations needed .Thus, mixing the two viruses (NPV+GV ) increased their efficacy for controlling *E.cautella* and *P. interpunctella*.

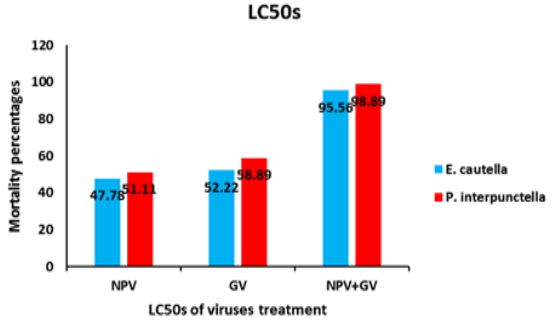


**Fig. 3. Comparison between the effect of LC50 of viruses NPV and GV either singly or in a mixed way on the mortality percentages of the tested insects.**

**5. Cytotoxicity of NPV and GV assay.**

As shown in Table (2), data results of percentage viability cells using different concentration were 100, 94 and 93% with concentration 10, 15 and 20% of initial concentration of NPV respectively. In case of GV, the percentage viability cells were 100, 98.5 and 92% with concentration 10, 15 and 20% of initial concentration respectively.

**Table 2. Cytotoxicity assay of NPV and GV on normal cells.**

| Sample | Concentration % | Cell viability % |
|---|---|---|
| NPV | 10 | 100 |
| | 15 | 94 |
| | 20 | 93 |
| GV | 10 | 100 |
| | 15 | 98.5 |
| | 20 | 92 |

**6. Biochemical Studies**

The effect of NPV and GV on biochemical contents and quality of treated date fruits Siwi-cultivar is tabulated in Table (3). Obtained data showed that total sugars, reducing and non- reducing sugars recorded (0.772 and 0.775), (0.723and 0.727) and (0.0486 and 0.0480) mg/g f.w. after treated with NPV and GV respectively compared to control (0.773,0.725 and 0.0480) mg/g f.w. with no significant differences. The same trend could be applied for amino acids and total indoles which were (0.490 and 0.520) and (0.190 and 0.190) mg/g f.w. after treated with NPV and GV respectively compared to control (0.487 and 0.193) mg/g f.w. Although total phenols exhibited no significant differences between NPV and control treatment (0.963 and 0.680) mg/g f.w. and no significant differences between GV and control treatment (0.643 and 0.680) mg/g f.w. but it recorded significant differences between NPV and GV treatment (0.963 and 0.643).

**Table 3. Effect of NPV and GV viruses on biochemical contents and quality of treated date fruits Siwi-cultivar.**

| Biochemical contents Treatment | Total sugar (mg/g f.w.) | Reducing sugar (mg/g f.w.) | Non-reducing sugar (mg/g f.w.) | Amino acids (mg/g f.w.) | Indoles (mg/g f.w.) | Phenols (mg/g f.w.) |
|---|---|---|---|---|---|---|
| Control | 0.773±0.003a | 0.725±0.003a | 0.048±0.00a | 0.487±0.02a | 0.193±0.021a | 0.680±0.02ab |
| NPV | 0.772±0.002a | 0.723±0.003a | 0.0486±0.00a | 0.490±0.01a | 0.190±0.01a | 0.963±0.02a |
| GV | 0.775±0.002a | 0.727±0.002a | 0.0480±0.00a | 0.520±0.02a | 0.190±0.021a | 0.643±0.015b |
| P. value | 0.0038 | 0. 0045 | 0.0013 | 0.0352 | 0.0352 | 0.0377 |
| L.S.D. | 0.1907 | 0.197 | 0.4219 | 0.1106 | 0.9651 | 0.0416 |

**7. Symptoms of NPV and GV isolates on *E. cautella* and *P. interpunctella* larvae**

The symptoms of NPV isolate on *E. cautella* and *P. interpunctella* larvae were shown in fig (4). The symptoms in *E. cautella* larvae were discoloration from brownish to dark brown and then to black and the larvae became liquefied. In *P. interpunctella* larvae, the color of larvae was turned from creamy to brownish and the moving were slowly and the body became liquefied.

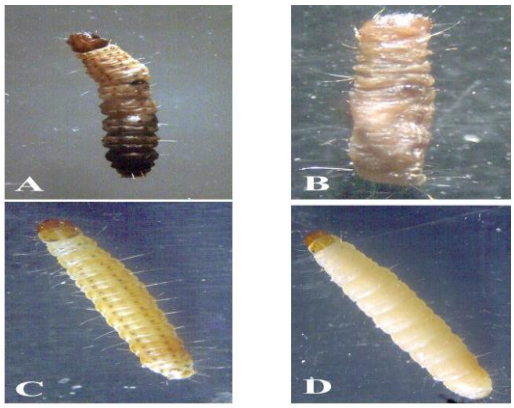


**Fig. 4. Symptoms of NPV on *E. cautella* and *P. interpunctella* larvae showed as discoloration on *E. cautella* larvae (A) and *P. interpunctella* larvae (B) compared with control *E. cautella* larvae (C) and control *P. interpunctella* larvae (D).**

As shown in fig (5), the larvae that treatment with GV showed many of symptoms. In *E. cautella* larvae, the noticed symptoms were larvae move slowly, the color of larva change from brownish to black. Finally, the body of the larva was burst and the internal body fluids come out. In case of *P. interpunctella*, the moving of larva were slowly and the color of larvae were changed from creamy color to grey then brown and in advanced infection the color became dark black.


**Fig. 5. symptoms of GV on *E. cautella* and *P. interpunctella* larvae that showing discoloration to black in *E. cautella* larvae (A) burst and liquefaction (B) change color to grey in *P. interpunctella* larvae (C) and change to brownish (D) comparing with *E. cautella* control larvae (E) and *P. interpunctella* control larvae (F).**

**Discussion**

The results of evaluating the effect of using seven concentration of NPV and GV against *E. cautella* and *P. interpunctella* larvae on stored date fruits at various exposure durations, 5, 7 and 10 days revealed that the corrected larval mortality percentages of *E cautella* and *P. interpunctella* increased by increasing viruses concentrations and increasing the exposure durations. This observation is in accordance with Thompson and Redlinger (1968) who observed that all larvae (100%) of *E. cautella* were dead after several days from treating with NPV. Hunter, *et al.* (1973) reported that the percentage average of mortality of *P. interpunctella* that treated with four different concentrations of NPV ($8x10^3$, $16x10^3$, $32x10^3$and $64x10^3$ PIB) were 12, 19, 24 and 45%. In the case of using GV our results in agreement with Hunter and Hoffmann (1970), who used five different concentrations of GV ($12x10^1$, $12x10^2$, $12x10^3$, $12x10^4$ and $12x10^5$ PIB) on *E. cautella*. They observed that, the average percentages of mortality were 1, 12, 36, 74 and 100% respectively. Sait, *et al.* (1998) reported that the fertility of lived male *P. interpunctella* larvae that infected with GV virus was reduced comparing with the control. In the present study *P. interpunctella* larvae were more susceptible to the two tested viruses *NPV* and GV than *E cautella* larvae. Further, the two tested insects were more susceptible to the GV virus than NPV virus concentrations, which is in harmony with Hunter, *et al.* (1972), who observed that, the pathogenicity of GV on *P. interpunctella* was high comparing with *E. cautella*.

The calculated $LC_{50}$ values in the present work were similar to Ignoffo (1963) when used four concentrations of NPV ($2.5x10^3$, $5x10^3$, $5x10^4$ and $5x10^5$ PIB) against *E. cautella* on cabbage-looper medium. $LD_{50}$ was 8.65 $x10^4$ PIB. On the other hand, Thompson and Redlinger (1968) calculated the $LD_{50}$ of NPV for *E. cautella* and it was $1.3x\ 10^6$ PIB. Tweeten, *et al.* (1977) used GV against *P. interpunctella* larvae and found that $LD_{50}$ of GV was 18x103PIB. Vail *et al.* (1993) used NPV that isolated from celery looper moth (*Anagrapha falcifera*) to infect *P. interpunctella*. They found that $LC_{50}$ of NPV against *P. interpunctella* was $5.1 \times 10^2$.

Our results indicated that a mixture of the two viruses (NPV+GV) increased its efficacy against *E. cautella.* and *P. interpunctella* .This results is in a harmony with Tanada and Hukuhara (1971) they observed direct increase in the number of the armyworm, *Pseudaletia unipuncta* infected larvae after fed them with the mixture of Capsules of a granulosis-virus (GV) and nuclear-polyhedrosis-virus (NPV). Several studies (e.g., Hodgson, *et al*., 2004; Simon, *et al*., 2005 ) have shown that combination of wild type viruses has always more pathogenic than any of the individual one, suggesting a positive interaction between them. Barrera, *et al.* (2021), mentioned that, the both viruses NPV and GV are individually infective to *Spodoptera ornithogalli* (Guenée) larvae but coexist in nature, producing mixed infections with a synergistic effect that improves the performance of the NPV and enables the transmission of the GV, which presents a slowly killing phenotype.

The symptoms of NPV and GV on *E. cautella* and *P. interpunctella* larvae were showed and illustrated in Figs. (5 and 6). It comes in agreement with those of Ignoffo (1963) who referred that the *E. cautella* larvae that grown on cabbage-looper medium that treated with NPV became sluggish, unable to eat after 4 days and their color changed from opaque white to pinkish to milky white. Thompson and

Redlinger (1968) reported that the most similar symptoms were observed on *E. cautella* larvae when infected with NPV that isolated from almond moths larvae *E. cautella*. The observed symptoms on larvae were divided into dark and light larvae while the light larvae have white patches on their dorsum. Hunter and Hoffmann (1970) showed that the infected *E. cautella* larvae with GV turned from pinkish to brown and then turned to dark. On the other hand, Vail *et al.* (1993) reported that *E. cautella* didn't show any symptoms and it was not susceptible to NPV that isolated from celery looper (*Anagrapha falcifera*) while *P. interpunctella* was susceptible to this isolate.

## CONCLUSION

The efficacy of biological control of two stored date fruits insect pests by using viruses NPV and GV were evaluated. Seven concentrations of each virus were tested to control these two insects at various exposure durations, 5, 7 and 10 days. Results revealed that the corrected larval mortality percentages of *E. cautella P. interpunctella* increased by increasing the viruses concentrations and/or exposure durations. *P. interpunctella* larvae were more susceptible to the 2 tested viruses**,** NPV and GV than *E. cautella* larvae and the two tested insects were more susceptible to the GV virus than NPV virus. Thus, mixing the two viruses (NPV+GV) increased their efficacy for controlling *E.cautella.* and *P. interpunctella* .

**List of abbreviations.**

NPVs: Nucleopolyhedroviruses.
GVs: Granuloviruses.
ARC: Agricultural Research Center.
r: Correlation coefficient of regression line.
SD: Standard deviation of the mortality regression line.
L.S.D. = Least Significant Difference.
Pr: probability Level.

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## المكافحة الحيوية لإثنين من آفات التمور الحشرية باستخدام الفيروسات الممرضة للحشرات (NPV وGV)

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### الملخص

يعتبر نخيل التمر من اهم انواع الفاكهة فى منطقة الشرق الاوسط حيث تتعرض الثمار للاصابة بالعديد من الافات مثل حشرة دودة البلح العامرى وحشرة البلوديا اللتان تسببان خسارة فى كمية وجودة التمر. ويهدف هذ البحث الى تقييم المكافحة الحيوية باستخدام فيروسي (NPV وGV) لهاتين الحشرتين. وقد تم اختبار ٧ تركيزات من كل فيروس (من ١x١٠$^{١}$ حتى (١ x١٠$^{٧}$) لمكافحة الحشرتين لمدد مختلفة ٥ و ٧ و ١٠ أيام . وقد اظهرت النتائج ان نسبة الموت المصححة ليرقات دودة البلح العامرى ويرقات البلوديا زادت بزيادة تركيز الفيروس او زيادة مدة التعريض فكانت في يرقات دودة البلح العامرى و فراشة البلوديا بعد المعاملة بتركيز ١ x١٠$^{٧}$ لفيروس NPV لمدة عشرة ايام ٥٤,٤٤ و ٧٣,٣٣ ٪ على التوالي مقارنة بالكنترول. بينما كانت في يرقات دودة البلح العامرى و فراشة البلوديا بعد المعاملة بتركيز ١ x١٠$^{٧}$ لفيروس GV لمدة عشرة ايام ٧٤,٣٣ و١٠٠ ٪ على التوالي مقارنة بالكنترول. كما أوضحت النتائج أيضا ان يرقات البلوديا اكثر حساسية للفيروسين المختبرين من يرقات دودة البلح العامرى. وكانت يرقات الحشرتين المختبرتين اكثر حساسية لتركيزات فيروس GV عن تركيزات فيروس NPV. وعندما تم خلط $LC_{50}$ للفيروسين معا زادت نسبة موت يرقات دودة البلح العامرى والبلوديا وسجلت ٩٥,٥٦ و ٩٨,٨٩ ٪ على التوالى مقارنة بنسبة موت يرقات الحشرتين عند التعرض $LC_{50}$ لكل فيروس على حدة وقل من تركيزات الفيروسات المطلوبة. كما أوضحت النتائج ان الفيروسين ليس لهما تأثير على الصفات الكيميائية للثمار المعاملة. وبذلك يكون خلط الفيروسين معا يؤدى الى زيادة الفاعلية فى مكافحة دودة البلح العامرى والبلوديا.