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First Report for Pathogenity of *Cydia Pomonella* Granulovirus and *Helicoverpa Armigera* Nucleopolyhedrovirus to Indian Meal Moth *Plodia Interpunctella* Hübner (Lepidoptera: Pyralidae) *in Vitro*

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Abstract

The purpose of this study was to examine the for the first time the effect of *Cydia pomonella* granulovirus and *Helicoverpa armigera* nucleopolyhedrovirus on the larvae of the Lepidopteran *Plodia interpunctella*. L_3 larvae were tested to see whether they were influenced by the infection of the two entomopathogenic viruses *Cydia pomonella* granulovirus and *Helicoverpa armigera* nucleopolyhedrovirus. The experiment lasted seven days. The results showed that the effect of the two Baculoviruses was statistically important in relation to the control. The effect of the virus *H. armigera* nucleopolyhedrovirus was greater than the effect of the virus *C. pomonella* granulovirus,

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and this led us to the assumption that the use of entomopathogenic viruses can play an important role in controlling *P. interpunctella* larvae. The recorded mortality after 7 days was for larvae treated with *C. pomonella* granulovirus 33.3 to 86.7%, with *H. armigera* nucleopolyhedrovirus 53.3 to 93.3% and control mortality was 0.7%. This information may appear particularly useful in the future control of the insect's populations in the warehouse.

Keywords: Entomopathogenic virus, *C. pomonella granulovirus*, *H. armigera nucleopolyhedrovirus*, *Plodia interpunctella*

1. Introduction

Stored product insects immediately infect and damage a product, and they can grow and reproduce in a warehouse or space that has been hosting agricultural products or food for a long time [1]. Most types of warehouse insects belong to the Coleopteran class followed by those of the Lepidopteran class. Insects are a major cause of post-harvest losses of stored foods and food products the world over. They not only consume these commodities but may also damage or contaminate them with insect fragments or whole bodies, feces, webbing, foul smelling metabolic products, and promote growth of a variety of microflora some of which pose serious health hazards (i.e., mycotoxins) to humans and livestock. Baculoviruses are natural control agents of a wide range of lepidopterous insect pests[2]. Due to their high specificity and virulence, several baculoviruses are used as biological insecticides world-wide Baculoviruses have a narrow host range, are very pathogenic, and have occlusion bodies which make them more biologically stable than some other families of entomopathogenic virus [2]

Plodia interpunctella Hübner is one of the most harmful insects in stored products and processed foods; it thus constitutes one of the most important pests in the world. It is a polyphagous insect whose diet determines its physiology and future generations [3, 4]. The stages of its life cycle include the egg, the larva, the nymph and the adult. The larvae

can complete their growth in six to eight weeks at temperatures ranging from 18°C to 35°C. The infant stage lasts fifteen to twenty days at 20°C. Adults mainly appear at night or early in the morning and survive for five or seven days at 30°C.

The treatment of *P. interpunctella* has undergone rapid changes in recent years. Unlike the system of previous years which was based almost exclusively on pesticide use, chemical control is now limited because of the latest levels of environmental pollution and the detection of chemical residues in products. Nowadays, the use of chemical pesticides is reduced, both for the protection of the environment and for the protection of the consumer. Hence, more environmentally friendly methods of dealing with insects have emerged [5-9]. These methods involve the use of predatory insects, growth regulators, bacteria, viruses, protozoa [10-12]. Larvae of Indian meal moth are susceptible to infection by a granulovirus, *P. interpunctella* GV (PiGV), which has been assessed as a potential control agent [13].

The pathogenicity caused by these microorganisms is not the same in all insects and differs even between the insect's developmental stages. Entomopathogenic viruses infect several different families and they exhibit very high selectivity, as some of them infect only one insect host. A virus-infected insect displays diminished activity for some time until death occurs. Although they do not cause acute and immediate mortality, viruses often cause dramatic reductions in their host populations.

Baculoviruses are large DNA and highly pathogenic viruses of insects [14]. They limit themselves to host cells in certain species of arthropods, meaning that the probability of becoming infectious to vertebrates, plants and non-targeted insects is close to zero. This is important for security reasons when registering a viral product. Additionally, they show zero residual accumulation and contamination. In organic farming, insect pathogens could be used as insecticides and thus function as a great tool for dealing with harmful enemies. In this paper, we examine for the first time, the effect of two insecticidal Baculoviruses for the control of the Lepidopteran *P. interpunctella*.

2. Materials and Methods

2.1 Bioassay procedure

Insect rearing was carried out at the University of Patras, Department of Pharmacy, LMBI Laboratory. Adult rearing of the Lepidopteran *P. interpunctella* (Lepidoptera: Pyralidae) took place in 25x45x30 cm cages. The frame of the cages, their sides, their back and their floor were wooden (MDF), while the two sides were covered with plastic mesh. The front of the cage was also covered with a small cross-section plastic mesh serving as an entrance to its interior. Each cage contained 15 to 20 pairs of adult insects. Sugar water was offered to adults with 1 cm cotton slices, placed in a Petri dish with water, on the floor of the cage.

In the cages with *P. interpunctella*, we placed transparent plastic cassettes containing 200 g of whole flour, 100 g of bran, 80 g of brewing yeast, 130 ml of pure honey and 100 ml of glycerol as artificial spawning substrate. The cassettes remained in the cages for two days and they were then placed in a separate dark place until the insects completed their biological cycle. The cassettes had first been covered with tulle to prevent the escape of the newly hatched larvae. The larvae were age distributed in transparent containers to avoid overcrowding which would impact larval development and food intake. The remaining food was stored at 4°C until reused. Throughout their development, the insects were kept in a room in stable conditions, at a temperature of 25±1°C, humidity of 60-70% h and photoperiod 16: 8 h light:dark.

The lepidopteran larvae were starved for 2 h. On sterile six well plates, food was placed roughly at the center. Each plate was then weighted separately. A solution of viruses was prepared of the insect pathogens *Cydia Pomonella* Granulovirus *(CpGV)* (Madex $6x10^{12}$ OB/ml produced by Hellafarm, Athens, Greece) and *Helicoverpa Armigera* Nucleopoyhedrovirus *(HearNPV) (*Helicovex SC $7.5x10^{12}$ OB/ml produced by Hellafarm, Athens, Greece*)*. The used doses were determined by 10 independent counts under phase

contrast microscopy (400x) in a Neubauer hemocytometer (TIEFE 0,100mm 1/400 9mm). The concentration was calculated with

$$\mathbf{OB\ ml^{-1} = D\ x\ X\ /\ N\ x\ K}$$

Where D = dilution of suspension dispensed into the haemocytometer, X =number of OB counted, N = number of small squares counted, and K = volume above a small square (in ml). The artificial feed was sprayed with the virus suspension and left for twenty minutes to dry naturally before placing it on each sub-plate. The solution was prepared inside a laminar flow chamber (Equip Vertical Air Laminar Flow Cabinet Clean Bench, Mechanical Application LTD, Athens, Greece). Laboratory-reared larvae (25 ± 1 ^{o}C, 65 ± 5% R.H., L:N 12:12) (PHC Europe/Sanyo/Panasonic Biomedical MLR-352-PE) were used for this study. Virus pathogenicity against 3th instar larvae of *P. interpunctella* was tested at four different doses using a Potter spray tower (Burkard Manufacturing Co. Ltd., Rickmansworth, Hertfordshire, U.K.) at 1 kgf cm^{-2}. The used doses were 10^{9} OB/ml, 10^{10} OB/ml, 10^{11}OB/ml and 10^{12}OB/ml of the pathogen made for each virus The larvae were placed on plastic six well plates where they were monitored daily for 7, 14 and 21 days. Six individuals' larvae L3 were repeated ten-time for each dose. The same procedure was performed for the control. The mortality and weight of larvae were measured daily. In order to determine the cause of death and to determine the pathogen, each dead larva was examined using a stereoscope.

2.2. Statistical analysis

The efficiency of all viral strains on larvae was calculated by Abbott's formula [15,16]. The IBM SPSS statistical package (IBM cop., IL, USA, version 23.0) was used to analyze data variance. The data were appropriately converted (arcsin) whenever it was deemed necessary, to meet the requirements of the parametric analysis for equal variations between treatments. The survival time of *P. interpunctella* larvae was calculated by Kaplan-Meier analysis compared with Log Rank test (Mantel-Cox) and Wilcoxon (Gehan). The average lethal concentration (LC50) was estimated by Probit analysis.

3. Results- Discussion

Figure 1. Dead *P. interpunctella* larvae L3 with symptoms of virus infection and bioassay procedure

Kaplan-Meier survival analysis (LogRank (Mantel-Cox) indicates that the average overall survival time of *P. interpunctella* infected with *C. pomonella* granulovirus was 93±13.1 h, with *H. armigera* nucleopolyhedrovirus it was 101±12 h, while the endpoint for the control was 166±2.5 h. Most often these infections arise after the insects have been stressed, or when treated with a virus [17]. The recorded mortality after 21 days was for larvae treated with *C. pomonella* granulovirus 33.3 to 86.7%, with *H. armigera* nucleopolyhedrovirus 53.3 to 93.3% and control mortality was 2% (Table 1, 3.).

Source	df	F	Sig.
Dose	3	5,665	,000
Virus species	2	30,465	,000
Virus species* Dose	6	6,576	,000
Error	592		
Total	540		
Corrected Total	539		

Table 1. Two way ANOVA Post Hoc (Bonferroni test to Independent Variable Mortality)

At the end of the experiment, the LC50 concentration of the *H. armigera* nucleopolyhedrovirus was greater than the effect of the *C. pomonella* granulovirus (Table 2). Khamiss et al. [18] obtained for an Egyptian isolate LC50 values of 2.8×10^{10} and 4.5×10^{11} OB/ml for *S. littoralis* larvae treated in second and fourth instars, respectively with high mortality.

Treatment	***LC_{50}***	***Lg $LC_{50} \pm Sd$***
C. pomonella granulovirus	7.5×10^8	8.40 ± 0.3054
H. armigera nucleopolyhedrovirus	1.7×10^9	9.23 ± 0.1831

Table 2. LC50 of virus for 3-instar *P. interpunctella* larvae

The mean fresh weight (w/w) of larvae at the end of the experiment was statistically significant and calculated at 0.00537±0.00384 g for the treatment with *C. pomonella* granulovirus, at 0.00417±0.00308 g for the treatment with *H. armigera* nucleopolyhedrovirus, and at 0.00718±0.00256 g for the control ($F = 155.167$, $df = 1$, $P < 0.001$) (Fig. 2.). In the present study, an attempt was made to study the entomopathogenic effects of *C. pomonella* granulovirus and *H. armigera* nucleopolyhedrovirus on young *P. interpunctella* larvae. Virus infections have a biological cost to the larvae by delaying development or reducing reproduction [19]. Comparison of their effect shows that the insect pathogens do not exhibit very high selectivity. This information may be particularly useful in future insect control and, if properly utilized, within the context of integrated insect management programs in the warehouse environments. Hughes et al. [20] reported that NPV infection was present in all life stages of a laboratory culture of *M. brassicae*. Spodoptera NPV has been reported to be more effective against first and second instar larvae than against older tobacco cutworm larvae because young larvae are

positively phototropic, very mobile and wander before they start feeding on the host plant or diet [21].

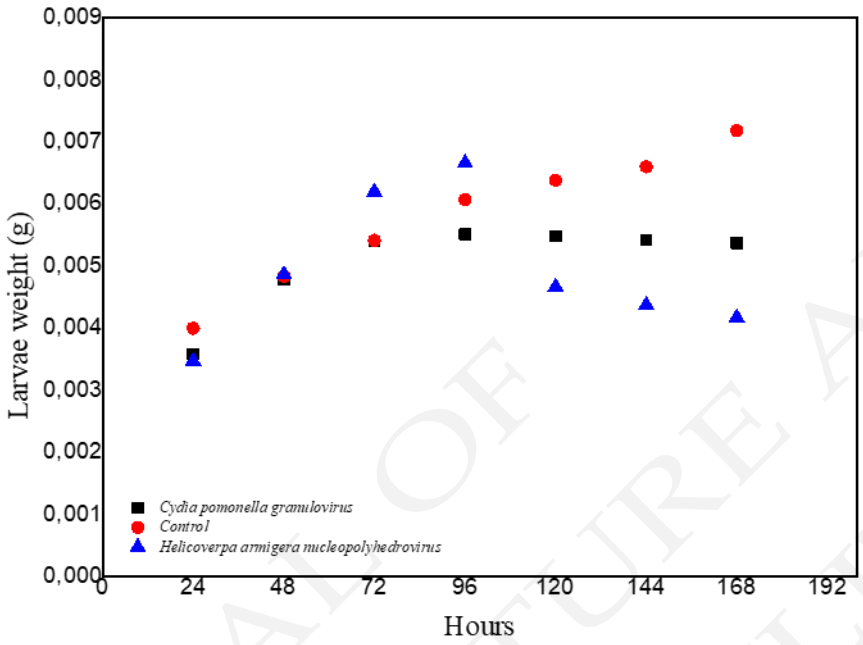

Figure 2. Overall change in weight of *P. interpunctella* larvae over time under laboratory conditions after insect pathogenic effects (25 o C, RH 70%) (n=60)

The idea of abandoning synthetic chemical pesticides and replacing them with more natural eco-friendly methods is increasingly being favored in recent years. To this end, many species of insect pathogens, like baculoviruses, have been used against specific pests [22] with good results. Viruses, particularly those belonging to the family Baculoviridae, are one of the most promising biological insecticides to date [23]. Several species have been reported to be important agents in the treatment of harmful insect pests, such as the insect pathogens *C. pomonella* granulovirus and *H. armigera* nucleopolyhedrovirus. Post-harvest annual losses of agricultural products caused by insects, microbial infections and other factors are estimated at around 10-25% worldwide. The organic control approach has introduced a new way of combatting pests with

biopesticides. This may have been due to the shorter feeding period and the later stage larvae studied by Sheppard et al.[24].

Treatment	**Mortality, % Observation duration (days)**		
	7	***14***	***21***
C. pomonella granulovirus Density			
10^9	3.3±1a	10.0±3c	33.3±1d
10^{10}	30.0±1d	43.3±2e	63.3±2g
10^{11}	33.3±2d	46.7±2e	66.6±3g
10^{12}	50.0±3f	66.7±3g	86.7±2k
H. armigera nucleopolyhedrovirus			
10^9	10.0±3b	23.3±2c	53.3±2f
10^{10}	33.3±2c	46.7±1	63.3±2g
10^{11}	33.3±2c	56.7±1	73.3±3g
10^{12}	56.7±4f	70.0±1g	93.3±1l
Control	0.0±0m	0.0±0m	0.7±1m

Table 3. Mortality (%) (mean ± sd) (F = 5.714, df = 12.380, P <0.001) of lepidopteran larvae after 7, 14, and 21 days due to the presence of entomopathogenic viruses (n = 60) at doses 10^9 OB/ml, 10^{10} OB/ml, 10^{11}OB/ml and 10^{12}OB/ml and control (ddH2O).

In summary, the *P. interpunctella* display great susceptibility to the *H. armigera* nucleopolyhedrovirus and *C. pomonella* granulovirus, larval infections caused deleterious effects on development of survivors, which may have implications to a persistent-infection mechanism. Except *P. interpunctella* granulovirus (PiGV)[25], we can use for the controlling of Indian meal moth the *H. armigera* nucleopolyhedrovirus and *C. pomonella* granulovirus. Our results also indicate that entomopathogenic viruses can be used as a no specific biopesticide with no range limitation.

From a practical point of view, infections may benefit pest control programs, given that insects that do not die from covert infection after consuming contaminated food have delayed effects which will be manifested in next generations of pest. Further research will seek to investigate the nature and biological implications of *H. armigera* nucleopolyhedrovirus and *C. pomonella* granulovirus of these effects.

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