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Research Article

Novel alphabaculovirus isolated from *Helicoverpa armigera* (Lep., Noctuidae) in Iran: characterization and pathogenicity

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Abstract. A novel alphabaculovirus, originally isolated from *Helicoverpa armigera* dead larvae, was collected from a tomato field in the North West region of Iran. Preliminary virus infection was revealed by microscopic observation of irregularly shaped polyhedral inclusion bodies, and the virus was subsequently confirmed by molecular and sequence analysis. Results revealed that the collected larvae contained *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV), which was named HearNPV-IR. Phylogenetic analysis based on partial single and concatenated sequences of *late expression factor 8* (*lef-8*), *late expression factor 9* (*lef-9*), and *polyhedrin* (*polh*) genes placed the HearNPV-IR isolate within the HearMNPVs clade, near the Russia isolate HearMNPV 3154. Concentration-mortality bioassays were conducted on 3rd instar larvae of *H. armigera*, and the mean lethal dose (LC_{50}) of HearMNPV-IR was estimated at 4×105 OBs/ml. The ST_{50} (median survival time) was 3- and 4-days post-infection (dpi) when the 3rd instar larvae were inoculated by OBs concentration equivalent to 3×109 and 3×108 OBs/ml, respectively. The highest dose resulted in 100% mortality at 5 dpi. The desirable insecticidal properties of HearMNPV-IR support its promising biocontrol agent against cotton bollworm.

Keywords: Microbial control, Insect pathology, Phylogenetic analysis, Biocontrol

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Introduction

Helicoverpa armigera Hübner is one of the main constraints to the production of several economically important crops worldwide. The host range and trophic preference of the pest vary in different world regions (CABI, 2014; Riaz *et al.*, 2021; Haile *et al.*, 2021; Anonymous, 2025). In Iran, *H. armigera* is considered a key pest of tomato and it causes damage primarily to maize, soybean, pea, and cotton crops (Avand-Faghih *et al.*, 2021). The complexity of *H. armigera's* life span, including reproduction, dispersal, host range, and adaptability to adverse environmental conditions, necessitates the implementation of an efficient integrated pest management (IPM) strategy to reduce crop losses (Riaz *et al.*, 2021). This IPM strategy relies on the application of several pest control measures including cultural practices, synthetic and microbial pesticides, pytopesticides, host plant resistance and genetically modified organisms (GM crops) expressing a microbial toxin or interference RNAs complementary to key genes crucial for complementation of the pest life cycle (Riaz *et al.*, 2021; Afrazeh & Jalali Sendi, 2025; Anonymous, 2025). However, extensive application of some of these control approaches, particularly chemical pesticides and GM crops have resulted in the emergence and prevalence of pesticide-resistant population of the pests (Ahmad *et al.*, 2019; Gutierrez-Moreno *et al.*, 2019; Legan *et al.*, 2024; Posos-Parra *et al.*, 2024a,b; Holman *et al.*, 2025), commonly by changing their life history parameters (Liu *et al.*, 2022; Hasnain *et al.*, 2023; Ahmad *et al.*, 2025; Lu *et al.*, 2026). Alterations such as loss of functions of transporter/receptor proteins, detoxification of chemical insecticides by the host enzymatic activity, regulation of gene expression, horizontal gene transfer, and

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symbiont microbiome in insect gut have also been shown in pesticide-resistant populations of several insects (Riaz *et al.*, 2021; Zheng *et al.*, 2024; Al Naggar *et al.*, 2025; Ahmad *et al.*, 2025; Lu *et al.*, 2026). Therefore, intelligent and environmentally safe pest control measures within the context of sustainable agriculture have been the subject of numerous research studies. Host-specific biocontrol agents, including bacteria, fungi, nematodes, viruses, and botanical-based insecticides, and particularly highly virulent variants/strains of insect pathogens, have been suggested as the most promising alternatives to chemical pesticides (Yang *et al.*, 2024; Afrazeh & Jalali Sendi, 2025; Holman *et al.*, 2025).

The insecticidal property of baculoviruses (family *Baculoviridae*), a group of viral pathogens of arthropods, in terms of specificity, virulence, and safety, has been widely studied since the last decades (Arrizubieta *et al.*, 2013, 2015; Magholifard *et al.*, 2014, 2017; Eroglu *et al.*, 2018; Costa *et al.*, 2019; Martinez-Castillo *et al.*, 2022; Kenis *et al.*, 2023; Pandi *et al.*, 2024; Yang *et al.*, 2024; da Silva *et al.*, 2025; Garcia-Munguia *et al.*, 2025). The *Baculoviridae* family is divided into four genera, including *Alpha-, Beta-, Gamma-,* and *Delta-baculoviruses* (Simmond *et al.*, 2024). Baculoviruses belonging to the genus *Alphabaculovirus* (lepidopteran-specific nucleopolyhedroviruses, NPVs) comprise pathogenic isolates with a restricted and narrow host range and infect only closely related insect species pests such as *Anticarsia gemmetalis* Hübner and *Cydia pomonella* Linnaeus (Clem & passarelli, 2013; Costa *et al.*, 2019). Virions of NPVs contain morphologically single (SNPV) or multiple nucleocapsids (MNPV), and in both cases, many occluded virions (ODVs) are occluded within each occlusion body (OB). The MNPVs have been isolated only from Lepidoptera. It is suggested that the MNPV phenotype has an advantage over the SNPV due to accelerated primary infection and systemic progression (Washburn *et al.*, 2003). Phylogenetic classification of baculoviruses has been mainly based on the sequences of their conserved genomic domains such as *late expression factor 8* (*lef-8*), *late expression 9* (*lef-9*) and *polyhedrin* (*polh*) genes (Jehle *et al.*, 2006). The biocontrol properties of Baculovirus heliothis was first reported from China in 1975 (Ignoffo, 1999). Subsequently, NPVs isolated from cotton bollworm in several countries, such as Brazil (Costa *et al.*, 2019; da Silva *et al.*, 2025), T*ü*rkiye (Eroglu *et al.*, 2018), Iran (Mehrvar *et al.*, 2008a; Shahbazi *et al.*, 2020), and the Iberian Peninsula (Figueiredo *et al.*, 2009), and in-depth studies suggested their potential as ecologically safe pesticides for commercial production (Baillie & Bouwer, 2012; Garcia-Munguia *et al.*, 2025). In addition, NPVs isolated from other species of Noctuidae, including *Spodoptera frugiperda* in Colombia (Barrera *et al.*, 2011), the Philippines (Lavina *et al.*, 2001), China (Lei *et al.*, 2020), Mexico (Martinez-Castillo *et al.*, 2022), India (Pandi *et al.*, 2024), and from *S. exigua* in Mexico (Zamora-Aviles *et al.*, 2017) have been reported. It is worth noting that nowadays genotypic variants from a significant number of naturally occurring HearNPVs have been identified, biologically purified, and some variants have been used for high-scale production of commercial biopesticides (Munoz & Caballero, 2000; Rowley *et al.*, 2011; Barrera *et al.*, 2011; Baillie & Bouwer, 2012; Arrizubieta *et al.*, 2015; Costa *et al.*, 2019; Lei *et al.*, 2020; Shahbazi *et al.*, 2020; da Silva *et al.*, 2025).

Generally, native isolates or genotypic variants of NPVs are more effective at managing local insect populations than exotic isolates (Luna-Espino *et al.*, 2018; Garcia-Banderas *et al.*, 2020; Lei *et al.*, 2020; Martinez-Castillo *et al.*, 2022; Pandi *et al.*, 2024). Indeed, the application of exotic isolates or genotypic variants of NPVs may cause adverse effects on local isolates of the viruses (Munoz *et al.*, 1998; Munoz & Caballero, 2000) and useful insects, whereas adaptability, effectiveness, and resilience of native isolates against local pest populations have been reported (Barrera *et al.*, 2011; Luna-Espino *et al.*, 2018; Lei *et al.*, 2020; Martinez-Castillo *et al.*, 2022; Pandi *et al.*, 2024). In fact, identifying indigenous isolates with high virulence across different ecological regions is necessary. In addition, the simultaneous presence of genotypic variants with different pathogenic properties has been reported in field and/or laboratory isolates of NPVs (Baillie & Bouwer, 2012; Shahbazi *et al.*, 2020). Therefore, accurate characterization of genotypic variant mixture of viral isolates and biological purification are critical for host-virus interactions. Although the HearNPV isolates have been reported from several pest species feeding on tomato, cotton and tobacco in Iran, and are applied to pure and practical studies thereof (Mehrvar *et al.*, 2008a, b; Mehrvar, 2009, 2013; Mehrvar & Saber, 2022; Kalantari *et al.*, 2013, 2018; Allahyari *et al.*, 2019, 2020; Magholifard *et al.*, 2014, 2017; Gifani *et al.*, 2015; Shazdehahmadi *et al.*, 2016; Moshtaghi *et al.*, 2013; Shahbazi *et al.*, 2020; Valizadeh *et al.*, 2020, 2024); however, their characterization at the species levels (HearMNPV/HearSNPV) has only been carried out in a few numbers of reports (Shahbazi *et al.*, 2020; Mehrvar & Saber, 2022; Valizadeh *et al.*, 2024). In this study, we describe the isolation of a HearNPV from a natural population of *Helicoverpa armigera* larvae from tomato crops in East-Azarbaijan province, Iran, which we

designated H. armigera nucleopolyhedrovirus-Iran (HearNPV-IR). Microscopic properties clarified this wild-type isolate. Further molecular dissections using genomic approaches targeting highly conserved Baculovirus domains, such as *the polh*, *lef-8*, and *lef-9* sequences, and phylogenetic studies placed the HearNPV-IR isolate within the HearMNPV clade. Biological studies of this isolate against the third-instar larvae of *H. armigera* showed that the HearMNPV-IR isolate can be introduced as promising biocontrol agent for appropriate control strategies of *H. armigera* in the future.

Materials and methods

Insect rearing

The *H. armigera* colony was established with pupae received from Tarbiat-Modares University, Tehran, Iran. It was maintained in a growth chamber at constant environmental conditions (25±1°C, 70±5% RH and a photoperiod of 16:8 h light: dark) and reared on a pinto bean-based semisynthetic diet (Naseri *et al.*, 2009). To avoid covert infections (da Silva *et al.*, 2025), the colony was checked by polymerase chain reaction (PCR) amplification of the viral *polh* gene (Shahbazi *et al.*, 2020).

Virus isolation, purification, and propagation

Two NPV isolates were originally obtained from *Helicoverpa armigera* larvae from traditional tomato fields located in East-Azarbaijan province (38°05' N and 46°46' E), Iran, in 2014. Larvae were individually stored in 1.5 ml plastic micro tubes at -20 °C in the Seed and Plant Certification and Registration Research Institute, Karaj, Iran. Occlusion bodies (OBs) were extracted from cadavers, and the fresh OB stocks for each isolate were obtained by multiplying them in newly molted 5th instar larvae of *H. armigera* using the plug-diet method as described elsewhere (Shahbazi *et al.*, 2020). Briefly, groups of 40 fifth-instar overnight-starved larvae were allowed to feed from a diet plug inoculated with 5×105 OB/ml. After 24 h, the larvae were transferred to individual cups containing virus-free diet and reared under standard conditions until death. Occlusion bodies from cadavers were purified as described above.

Microscopic studies

Visualization and quantification of viral OBs were carried out under 400-1000× magnification by light microscope (Micros, Austria) and a Neubauer hemocytometer. The refractive protein crystals of OBs were visible under phase contrast illumination of the light microscope. The visibility of the crystalline structures was further enhanced by staining the OBs with methylene blue. For this purpose, the appropriate volume of OBs suspension was mixed with diluted methylene blue. A volume of 10 µl of the stained OB suspension was loaded into both halves of the hemocytometer using a pipette and allowed to stand for 15 min to facilitate sedimentation of the particles onto the chamber floor, where the bright OB structures were clearly visible.

Isolation of viral DNA and preliminary detection of virus

Purified viral OBs, extracted from individual larvae, were used to extract viral genomic DNA (gDNA) using the SinaPure Viral Kit (SinaClone, Iran) according to the manufacturer's instructions. The extracted DNA was quantified using a spectrophotometer (NanoDrop ND-1000 UV/Vis, USA). Further confirmation of the virus in both isolates was performed by PCR amplification of the viral *polh* gene as a marker (Christian *et al.*, 2001; Jehle *et al.*, 2006; Shahbazi *et al.*, 2020).

Phylogeny and Kimura-2 parameter analysis of virus isolate

Partial single and concatenated sequences of the *polh*, *lef-8*, and *lef-9* genes (Jehle *et al.*, 2006) of an isolate (hereafter HearNPV-IR) were targeted for the construction of a phylogenetic tree. Amplification of these core genes was performed by PCR using the following primer sets (Table 1): rPol-f/rPol-r (Christian *et al.*, 2001), prL8.1/prL8.2, and prL9.1/prL9.2 (Lange *et al.*, 2004). PCR products were purified using a gel DNA recovery kit (Vivantis, South Korea) and sequenced (Bioneer, South Korea). Multiple-sequence alignments of single sequences of the three genes and their concatenated sequences, treated as a single sequence, were performed using

the MUSCLE program (Nai *et al.*, 2017). The phylogenetic tree was constructed using the minimum-evolution (ME) method in MEGA6 (Lange *et al.*, 2004; Jehle *et al.*, 2006). Confidence levels for the branching points were determined using 1000 bootstrap replicates. Distance matrices between HearNPV-IR and other closely related species were determined for partial *lef-8*, *lef-9*, and *polh* gene sequences using the pairwise distance calculation in MEGA version 6.0, applying the Kimura-2-parameter (K-2-P) model. Homologous sequences of *H. armigera* GV were also included in the K-2-P analysis.

Biological activity of virus isolates against laboratory colony

Concentration-mortality response (LC_{50}) and time-mortality of HearMNPV-IR were performed by using seven different viral concentrations (3×103to 3×109 OBs/ml) in 3rd instar larvae of *H. armigera*. Bioassays were carried out in two separate experiments, each with three replications of 10 third-instar larvae. The 3rd instars newly molted and overnight-starved were allowed to feed from a diet plug inoculated with the virus isolate at the following concentrations (3×103, 3×104, 3×105, 3×106, 3×107, 3×108, and 3×109 OBs/ml/larvae). After 24 h and feeding the whole diet, the larvae were transferred to new individual trays containing a virus-free diet. Sterile water containing food dye, at the same concentration as used for virus-contaminated diet, was applied on diet and used as negative control. Larvae were incubated in a growth chamber under constant environmental conditions (26 ± 1 °C, 70 ± 5% RH, and a photoperiod of 16:8 h light: dark), and mortality was recorded at 10 days post-infection (dpi) in 24 h intervals. Baculovirus infection in dead larvae was diagnosed by syndrome assessment, including "wilt" and integument discoloration, and by light microscopy. The mean larval mortality at each concentration was subjected to probit analysis to determine the median lethal concentration (LC_{50}) using the POLO-PC program (Le Ora Software, 1987). Time-to-mortality results for larvae were analyzed using Kaplan-Meier estimates (Kaplan & Meier, 1958) for survival curve analysis in SPSS statistical program 26 (Anonymous, 2019).

Results

Microscopic structure and virus detection

The refractile protein crystals of the HearNPV-IR isolate were observed under phase-contrast illumination. The polyhedral structure and irregular shape of OBs were obvious at 400× and 1000× magnification under light microscopy. The *polh* gene sequence derived from the amplified fragment by universal primer set (rpol-f/rpol-r) confirmed NPV infection (Fig. 1).

Phylogenetic and Kimura-2 parameter analysis of virus isolates

Partial sequences of highly conserved genes of *polh*, *lef-8*, and *lef-9* among baculoviruses were used as targets for PCR to characterize lepidopteran NPVs. Amplified PCR products from both isolates were directly sequenced. Comparison of the amplified sequences from both isolates revealed 100% identity across all three genes (data not shown). Therefore, one sequence of each gene for HearNPV-IR was deposited in GenBank (NCBI) under the accession numbers OP751377 (*polh*), OP571375 (*lef-8*), and OP751376 (*lef-9*). Phylogenetic trees were constructed based on single and concatenated partial sequences of *lef-8*, *lef-9* and *polh* genes to determine the taxonomic relationship between the newly detected HearNPV-IR isolate and closely related NPVs (Table 2). The results indicated that HearNPV-IR clustered with other HearMNPV isolates and was located close to *the H. armigera* MNPV Russia isolate 3154 in all phylograms (Fig. 2). To precisely clarify the relationship of *Helicovrapa*-derived NPVs, the K-2-P distance between the aligned *polh*, *lef-8* and *lef-9* nucleotide sequences was performed (Table 3); if the value of the nucleotide locus distance between two NPVs is less than 0.015, they are considered the same species, but a distance of more than 0.050 defines those isolates as different species. Additionally, for the K-2-P values between 0.015 and 0.05, more complementary data is needed to determine the viral species (Jehle *et al.*, 2006; Nai *et al.*, 2017). The distances between HearNPV-IR and other HearMNPVs were less than 0.015 for single *lef-8* and *lef-9* sequences, but only the *polh* sequence distance was equal to 0.015. The distances between HearNPV-IR and other HearMNPVs were less than 0.015, whereas the distances for all three genes between HearNPV-IR, the SNPVs group, and HearGV exceeded apparently much more than 0.050. Therefore, based on the data presented in Table 3, the HearNPV-IR isolate belongs to the HearMNPV group.

Table 1. Primers used for amplification of NPVs conserved genomic regions from *Helicoverpa armigera*

Primer	Sequence (5'→3')	Amplified fragment size (bp)
PrL8.1 PrL8.2	GTAAAACGACGGCCAGTTYTTYCAYGGNGA AACAGCTATGACCATGGNAYRTANGGRTCY	800
PrL9.1 PrL9.2	CAGGAAACAGCTATGACCAARAAYGGITAYGCBG TGTAAAACGACGGCCAGTTTGTCDCCRTCRCARTC	350
rPol-f rPol-r	TTICCIATTGTIAACGAICAAGA ATGGGITTGTAIAAGTTITCCCA	400

Biological activity of HearNPV-IR isolate against *H. armigera*

The infectivity of the HearNPV-IR isolate was studied on freshly molted 3rd-instar larvae of *the H. armigera* laboratory colony under controlled growth chamber conditions. To test the time response of *H. armigera*, a survival-time analysis was conducted on 3rd-instar larvae inoculated with different concentrations of OBs from HearNPV-IR. Survival began to decline at 2 dpi in the three higher OB concentrations, and 100% mortality was observed in 3rd instar larvae at 5 to 7 dpi. The median survival time (ST_{50}) was 3 and 4 dpi for larvae receiving OBs at concentrations equivalent to 3×109 and 3×108 OBS/ml, respectively. The survival curve reached ST_{50} at approximately 144 h after inoculation for the fourth (3×106) and fifth (3×105) dilutions of polyhedral suspension. All larvae were alive at 10 dpi in the control treatments (Fig. 3).

Discussion

Naturally occurring pathogenic baculoviruses of *H. armigera* could provide valuable sources for manufacturing and improvement of local microbial insecticides. Elcar, a Heliothis virus-based product, was the first baculovirus-derived biocontrol agent registered in 1975, although it was not continued (Mondal *et al.*, 2021). More recently, several indigenous variants of *H. armigera* NPV identified from China (HearNPV-C1, HearNPV-G4), Spain (HearNPV-Sp1), Africa (HearNPV-NNg1) and Australia (HearNPV-Aus, HearNPV-AC53) as promising candidates for the development of local biopesticides against native insect hosts (Arrizubieta *et al.*, 2015; Williams *et al.*, 2022; Kenis *et al.*, 2023; Garcia-Munguia *et al.*, 2025).

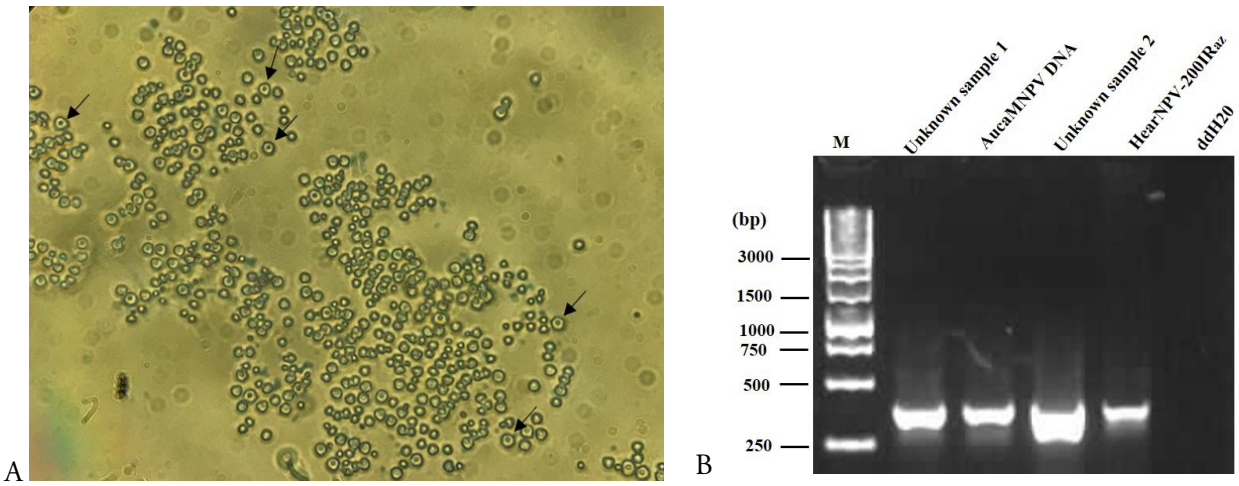

Fig. 1. Microscopic and molecular detection of unknown NPV from *Helicoverpa* sp. dead larvae. (A) Microscopic observation of liquefaction extracted from *Helicoverpa armigera* larvae at 1000× magnification. Black arrows indicate the polyhedral occlusion bodies (OBs) of the virus. (B) PCR detection of partial *polyhedrin* gene (400 bp) by universal primer pair. M indicates DNA ladder 1Kb (Fermentas) and *Autographa californica* multiple nucleopolyhedrovirus (M25054) and HearNPV-200IR_{az} (MN840078) are positive controls. ddH_2O represents negative control of PCR.

Table 2. Accession numbers for the *lef-8*, *lef-9*, and *polh* genomic sequences of several HearMNPV and HearSNPV isolates retrieved from GenBank and used for phylogenic studies

Isolate	Origin	Accession number		
		Lef-8	*Lef-9*	*polh*
HearMNPV-IR	Iran	OP751375	OP751376	OP751377
HearMNPV-120	Poland	HQ246040	HQ246032	HQ246024
HearMNPV-443	India	HQ246042	HQ246034	HQ246026
HearMNPV-3154	Russia	HQ246047	HQ246039	HQ246031
HearMNPV-3153	China	HQ246046	HQ246038	HQ246030
HearMNPV-1072	China	HQ246044	HQ246036	HQ246028
HearSNPV-TR	Türkiye	MG870624	MG870625	MH161372
HearSNPV-3010	China	HQ246121	HQ246148	HQ246094
HearSNPV-2588	India	HQ246120	HQ246147	HQ246093
HearSNPV-138	Poland	HQ246100	HQ246127	HQ246073
HearGV	USA	EU255577	EU255577	EU255577

It is known that the application of exotic commercial products originated from exogenic virus variants may cause adverse effects on the biological control of local host biotypes (Munoz *et al.*, 1998; Munoz & Caballero, 2000). However, this phenomenon is important for reducing the overall pathogenicity of virulent variants and preventing epizootic development, as shown by the case of defective variants US2C and US2E in the Spod-X commercial biopesticide against *Spodoptera exigua* in the USA (Munoz & Caballero, 2000). Additionally, this phenomenon enables access to NPVs for subsequent transmission cycles, a phenomenon known as biopesticide sublethal effects. In addition, native host biotypes may show varying susceptibility to viral OBs originating from different geographic niches (Figueiredo *et al.*, 2009).

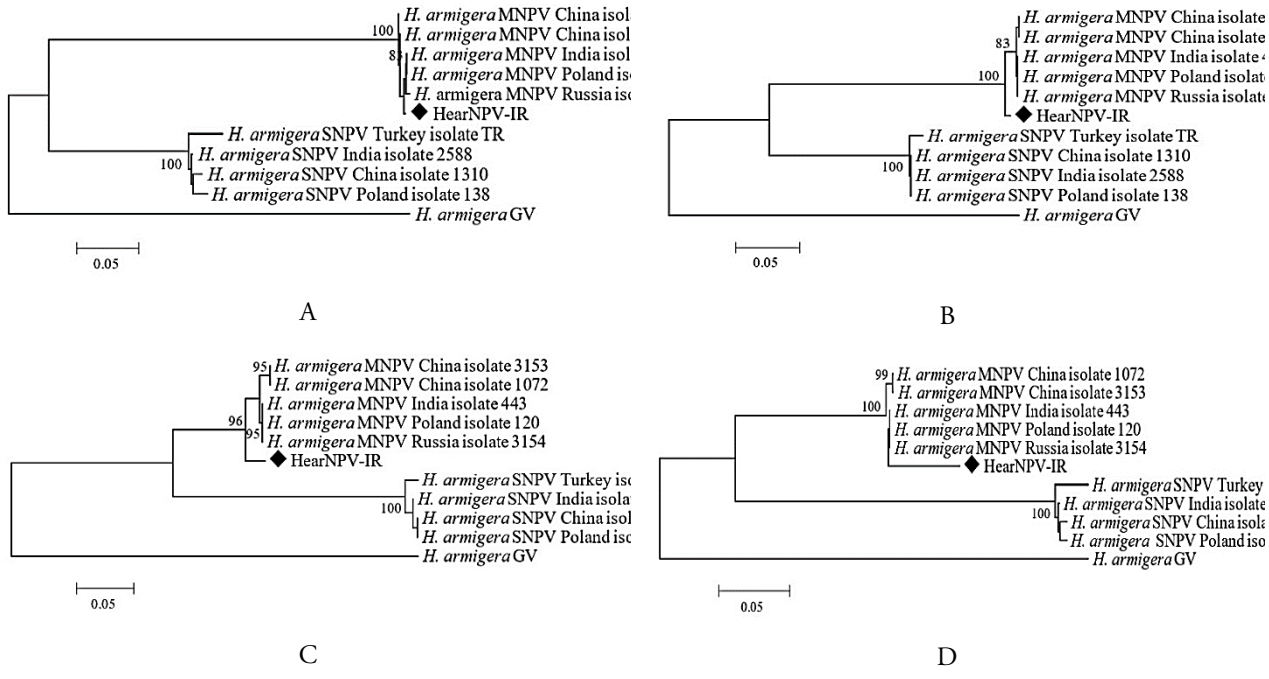

Fig. 2. Phylogenetic position of HearNPV-IR among the other closely related NPV isolates. Minimum evolution phylogram is based on partial sequences of polh (A), lef-8 (B), lef-9 (C) genes and their concatenated sequences (D). Numbers at the nodes indicate bootstrap value analyses.

Table 3. Pairwise Kimura-2-parameter distances of the aligned partial sequences of individual *polh* (A), *lef-8* (B), and *lef-9* (C) genes of HearMNPV-IR to published sequences in GeneBank. (A)

lef-8		1	2	3	4	5	6	7	8	9	10	11
1	HearMNPV-IR											
2	HearMNPV-3154	0.009										
3	HearMNPV-3153	0.005	0.012									
4	HearMNPV-443	0.003	0.006	0.006								
5	HearMNPV-120	0.003	0.006	0.006	0.000							
6	HearMNPV-1072	0.005	0.011	0.002	0.005	0.005						
7	HearSNPV-TR	0.434	0.436	0.426	0.438	0.438	0.429					
8	HearSNPV-3010	0.412	0.413	0.404	0.416	0.416	0.407	0.034				
9	HearSNPV-2588	0.404	0.405	0.396	0.407	0.407	0.399	0.034	0.011			
10	HearSNPV-138	0.420	0.421	0.412	0.424	0.424	0.415	0.039	0.017	0.012		
11	HearGV	0.768	0.767	0.765	0.769	0.769	0.760	0.429	0.386	0.394	0.402	

(B)

lef-9		1	2	3	4	5	6	7	8	9	10	11
1	HearMNPV-IR											
2	HearMNPV-3154	0.008										
3	HearMNPV-3153	0.012	0.004									
4	HearMNPV-443	0.008	0.000	0.004								
5	HearMNPV-120	0.008	0.000	0.004	0.000							
6	HearMNPV-1072	0.012	0.004	0.000	0.004	0.004						
7	HearSNPV-TR	0.304	0.310	0.317	0.310	0.310	0.317					
8	HearSNPV-3010	0.291	0.297	0.304	0.297	0.297	0.304	0.012				
9	HearSNPV-2588	0.291	0.297	0.304	0.297	0.297	0.304	0.012	0.000			
10	HearSNPV-138	0.291	0.297	0.304	0.297	0.297	0.304	0.012	0.000	0.000		
11	HearGV	0.534	0.543	0.534	0.543	0.543	0.534	0.461	0.468	0.468	0.468	

(C)

polh		1	2	3	4	5	6	7	8	9	10	11
1	HearMNPV-IR											
2	HearMNPV-3154	0.015										
3	HearMNPV-3153	0.027	0.011									
4	HearMNPV-443	0.015	0.000	0.011								
5	HearMNPV-120	0.015	0.000	0.011	0.000							
6	HearMNPV-1072	0.027	0.011	0.000	0.011	0.011						
7	HearSNPV-TR	0.289	0.278	0.296	0.278	0.278	0.296					
8	HearSNPV-3010	0.290	0.280	0.298	0.280	0.280	0.298	0.023				
9	HearSNPV-2588	0.284	0.274	0.292	0.274	0.274	0.292	0.019	0.004			
10	HearSNPV-138	0.290	0.280	0.298	0.280	0.280	0.298	0.023	0.000	0.004		
11	HearGV	0.688	0.675	0.664	0.675	0.675	0.664	0.706	0.735	0.723	0.735	

Therefore, isolation of local wild-type viruses, *in vivo*/*in vitro* purification of their genotypic variants, comparative pathogenicity evaluation of variants, and selection of variants with suitable insecticidal properties against indigenous pests are necessary for developing effective virus-based biopesticides (Lei *et al.*, 2020; Pandi *et al.*, 2024). In this study, two field isolates of NPV were obtained from a natural population of the invading pest *Helicoverpa armigera* in Iran using microscopic and molecular detection approaches. Visual observation of OBs revealed a typical polyhedral morphology with variable diameters, easily visible at 1000× magnification. However, detailed information on virus particles and morphology is typically needed, and future studies should use higher-resolution approaches such as transmission electron microscopy (TEM). This simplified diagnosis by light microscopy was also performed in several previous studies for visualization of virus structures in liquefactions from insect cadavers (Lavina *et al.*, 2001; Grzywacz *et al.*, 2005; Ferrelli *et al.*, 2016; Nai *et al.*, 2017; Eroglu *et al.*, 2018; Costa *et al.*, 2019). Simple and primitive detection of unknown NPVs with PCR utilizing specific primers

based on *polyhedrin* (or *granulin*) gene sequences has been commonly used in laboratories (Jehle *et al.*, 2006; Arrizubieta *et al.*, 2013; Krejmer-Rabalska *et al.*, 2019; Lei *et al.*, 2020; Dou *et al.*, 2024; Pandi *et al.*, 2024; Erlandson *et al.*, 2024). We amplified the *polh* gene fragments from gDNA of both isolates using commonly used *polh* gene primers (Jehle *et al.*, 2006). Further sequencing of the amplicons confirmed that the isolated NPVs belong to the baculovirus family. Therefore, both isolates were named HearNPV-IR provisionally according to the host from which they were isolated. Further amplification and sequencing of the Baculovirus highly conserved genes *lef-8*, *lef-9*, and *polh* from both isolates confirmed complete (100%) sequence identity between the two isolates. Therefore, one isolate (isolate 1) was used for phylogenetic and biological studies. The Kimura-2-parameter (K-2-P) distance model is commonly used to estimate genetic relatedness and taxonomic placement among closely related NPVs (Nai *et al.*, 2017; Lei *et al.*, 2020; Erlandson *et al.*, 2024). The K-2-P distances between the aligned *polh*, *lef-8* and *lef-9* nucleotide sequences indicated that the virus obtained from diseased larvae is a typical isolate of *H. armigera* multiple nucleopolyhedrovirus (HearMNPV).

Table 4. Summary of previous studies dealt with *Helicoverpa armigera* nucleopolyhedrovirus isolates in Iran

Reference	Studied pest/Research topic	Virus isolate[a]	Origin[b]	LC_{50}/LT_{50}	Virus characterization[c]
Mehrvar *et al.*, 2008a	*Helicoverpa armigera* /Phylogenetic study	HaNPV	Bangalore, India	0.02859 OB mm^{-2} of diet surface/97.8 h	PCR-RFLP
Mehrvar *et al.*, 2008b	*H. armigera*/Virus formulation	HaNPV	Bangalore, India	1×10^5 OB ml^{-1}/99.6 h	ND
Mehrvar, 2009	*H. armigera*/Virus persistence	HaNPV	Bangalore, India	ND/ND	ND
Mehrvar, 2013	*H. armigera*/Mass production optimization	HaNPV	East Azarbaijan, Iran	ND/ND	ND
Moshtaghi *et al.*, 2013	*H.armigera*/Temperature effect on pathogenicity	HaMNPV	IRIPP, Iran	5×10^5 OB/ml^{-1}/6.58 days at 30 °C	ND
Kalantari *et al.*, 2013	*H.armigera*/Insecticidal property in co-application with *Bacillus thuringiensis*	HaSNPV	IRIPP, Iran	9.2×10^3 OB ml^{-1}/ND	ND
Magholifard *et al.*, 2014	*Spodoptera litteralis*/ Insecticidal activity	HaNPV	IRIPP, Iran	6×10^4 OB ml^{-1}/19.7 days	ND
Shazdehahmadi *et al.*, 2016	*H. armigera*/ Phylogenetic study	HaNPV	Mazandaran/Golestan, Iran	ND/ND	RAPD
Gifani *et al.*, 2015	*H. armigera*/ Virus formulation	HaNPV	Henan Jiyuan Baiyun, China	ND/ND	ND
Magholifard *et al.*, 2017	*Plutella xylostella*/ Insecticidal activity	HaNPV	Henan Jiyuan Baiyun, China	3.8×10^4 OB ml^{-1}/114.23 h	ND
Kalantari *et al.*, 2018	*H. armigera* and *P. xylostella*/ Insecticidal activity	HaNPV	Henan Jiyuan Baiyun, China	9.2×10^3 OB ml^{-1}/5 days (*H. armigera*); 3.8×10^4 OB ml^{-1}/4.8 days (*P. xylostella*)	ND
Allahyari *et al.*, 2019	*H. armigera*/ Insecticidal property in co-application with *Bacillus thuringiensis*	HaNPV	IRIPP, Iran	5.4×10^6 OB ml^{-1}/ND	ND
Shahbazi *et al.* 2020	*Helicoverpa armigera* /Phylogenetic study	HaSNPV	East Azarbaijan, Ardebil, Khorasan Razavi, North Khorasan, Golestan, Iran	ND/ND	Molecular dissection
Valizadeh *et al.*, 2020	*Ephestia kuehniella*/ Virus formulation	HaNPV	Golestan and Mazandaran, Iran	3.31×10^5 OB ml^{-1}/ND	ND
Allahyari *et al.*, 2020	*H. armigera*/ Insecticidal activity on *Habrobracon hebetor*	HaNPV	IRIPP, Iran	2.54×10^4 OB ml^{-1}/ND	ND
Mehrvar & Saber, 2022	*H. armigera*/Virus formulation	HaSNPV	SPCRI, Iran	5×10^4 OB ml^{-1}/4.21 days	Molecular dissection
Valizadeh *et al.*, 2024	*H. armigera* /Virus formulation	HaNPV-IR18	Shahid Madani University, Iran (Source: SPCRI, Iran)	2.71×10^5 OB ml^{-1}/144 h	ND

[a]HaNPV: *Helicoverpa armigera* nucleopolyhedrovirus; HaMNPV: *H. armigera* multiple nucleopolyhedrovirus; HaSNPV: *H. armigera* single nucleopolyhedrovirus. [b]IRIPP: Iranian Plant Protection Research Institute; SPCRI: Seed and Plant Certification and Registration Research Institute. [c]PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism; RAPD: Randomly amplified polymorphic DNA; ND: Not determined.

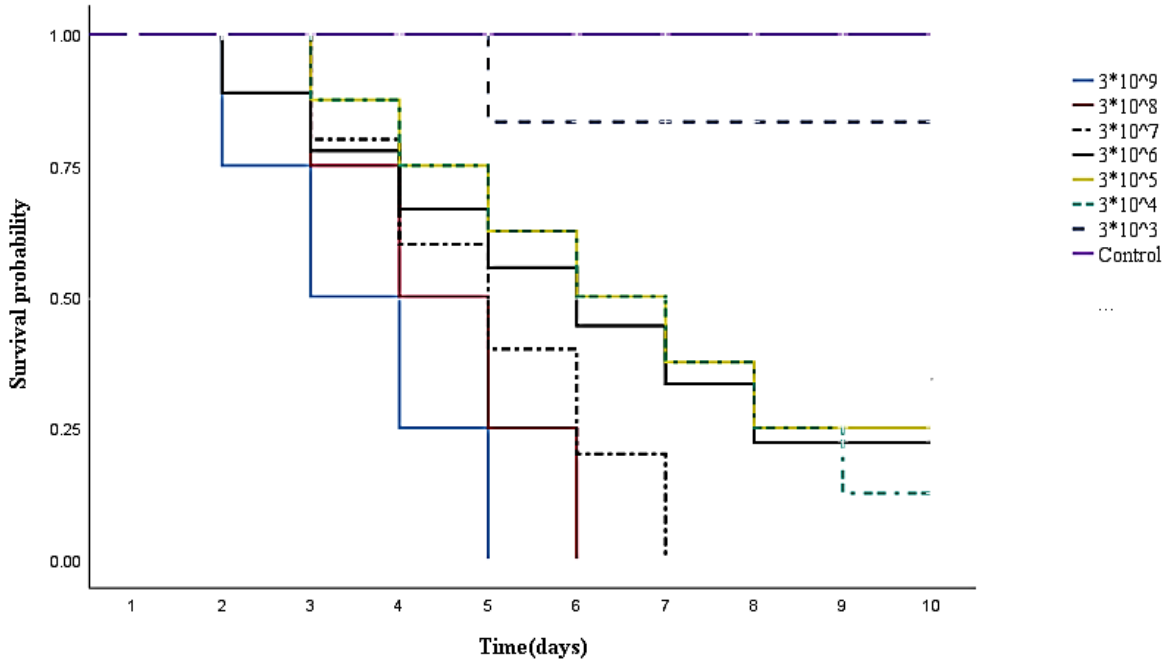

Fig. 3. Kaplan–Meier survival curve for *Helicoverpa armigera* 3rd instar larvae after feeding on diet plug inoculated with different occlusion body concentrations of HearNPV-IR isolate at 10 days post infection.

Also, the ME phylogram was constructed from single and concatenated partial sequences of the three genes, and the clustering of HearMNPV-IR with other HearMNPV isolates confirmed the results of the K-2-P analysis. The Iranian HearMNPV-IR isolate was most closely related to the HearMNPV-3154 isolate from Russia. It has been documented that Helicoverpa NPVs obtained from different species and geographic regions are geographical variants of the same viral species (Jehle *et al.*, 2006; Rowley *et al.*, 2011; Arrizubieta *et al.*, 2013). Although a significant number of biocontrol research activities using HearNPV have been carried out in Iran, studies are largely limited to either biologically uncharacterized endemic isolates or imported isolates from international research institutes/commercial companies (Summarized in Table 4). Indeed, the overall pathogenicity parameters of a single viral isolate in a specific host species is orchestrated by collaborative interactions of multiple genotypic variants, including virulent, defective, haplotype, and recombinant variants (Munoz & Caballero, 2000; Baillie & Bouwer, 2012; Shahbazi *et al.*, 2020; Pandi *et al.*, 2024; Garcia-Munguia *et al.*, 2025) or even different virus species (Washburn *et al.*, 2003; Beperet *et al.*, 2021; Arrizubieta *et al.*, 2022). Therefore, using genetically and biologically homogenous isolates and strains is strictly recommended for future studies.

The pathogenicity of HearMNPV-IR isolates 1 was tested against laboratory-reared, newly molted 3rd-instar larvae of *H. armigera* under growth chamber conditions. The LC_{50} value of the isolate (4 x 10 ^5 OB/ml) was comparable to those reported in the screening for biological activity of HearNPV-01 by Eroglu *et al.* (2018) in Türkiye and in the biological control program of *H. armigera* using the HearSNPV-S1 isolate in Spain (Arrizubieta *et al.*, 2013). However, compared with the infectivity of MNPVs reported from other countries, the HearMNPV-IR showed a higher LC_{50} value. These include the former USSR HearMNPV-SP1 on Spanish *H. armigera* (Arrizubieta *et al.*, 2022), Nicaraguan *Spodoptera frugiperda* MNPV (SfMNPV)-NIC on *H. armigera* from Honduras (Simon *et al.*, 2004), and Colombian SfMNPV-COL (Barrera *et al.*, 2013), Indian SfMNPV-NBAIR (Pandi *et al.*, 2024), and Chinese SfMNPV-HUB (Lei *et al.*, 2020) isolates on local *S. fugiperda* colonies. It is worth noting that we assayed the HearMNPV-IR on *H. armigera* 3rd instar larvae, whereas the 2nd instars were used in the above-mentioned reports, except for the SfMNPV-HUB, which was applied on the 4th instars of the host. Williams & Payne (1984) showed that although the LC_{50} and LT_{50} values of SNPVs and MNPVs are independent of larval weight, they are affected by larval stage (Williams & Payne, 1984; Magholifard *et al.*, 2014).

Therefore, our study on the host 3rd instars could somehow explain the high LC_{50} value of HearMNPV-IR. Survival time and mortality response of treated larvae are critical parameters to assess the effectiveness and virulence of virus isolates. Surprisingly, the LT_{50} value of HearMNPV-IR (72-144 h) was shorter than those of SfMNPV-NIC (131 h), SfMNPV-COL (167 h), and HearMNPV (143 h) (Simon *et al.*, 2004; Barrera *et al.*, 2013; Arrizubieta *et al.*, 2022). Unlike the single nucleopolyhedrovirus envelopes that contain a single rod-shaped virus particle, multiple nucleopolyhedroviral particles are enveloped in groups that could carry different genotypic variants of a certain viral species or even different species of viruses, particularly in mixed-infected host insects (Wahburn *et al.*, 2003; Beperet *et al.*, 2021; Arrizubieta *et al.*, 2022).

Also, compared to HearMNPVs, HearSNPVs display lower LC_{50} and LT_{50} values and higher OB production rates (Luna-Espino *et al.*, 2018; Lei *et al.*, 2020; Garcia-Munguia *et al.*, 2025). Arrizubieta *et al.* (2022) showed significant enhancement in the pathogenicity parameters of HearMNPV in larvae of *H. armigera*, *S. frugiperda*, and *Mamestra brassicae* when coinfected with HearSNPV. Further quantitative PCR (qPCR) analysis revealed a small fraction of HearSNPV genome compared to that of HearMNPV (Arrizubieta *et al.*, 2022). The presence of HearSNPV isolates circulating in tomato fields of distinct geographical regions, including the North West of Iran, where the HearMNPV-IR was isolated, has been reported in our previous study as well (Shahbazi *et al.*, 2020). Therefore, although we did not purify the HearMNPV-IR isolate by *in vitro* or *in vivo* procedures, the presence of genotypic variants or other NPV species (e.g., HearSNPV) that confer a fast-kill phenotype (lower LT_{50}) could not be ruled out. It is well known that the biological activity of the NPV species isolated from different geographic areas differs from each other in terms of LC_{50}, LT_{50}, and/or OB production rates within the host body (Luna-Espino *et al.*, 2018; Lei *et al.*, 2020; Garcia-Munguia *et al.*, 2025). Also, the virulence of a single isolate differs from that of single genotypic variants or experimental mixtures of selected variants of the same isolate (Luna-Espino *et al.*, 2018; Lei *et al.*, 2020; Martinez-Castillo *et al.*, 2022; Pandi *et al.*, 2024). The latter is well-defined for the two defective variants, US2E and US2C, in the genotypic variant composition of the SeMNPV-US2wt field isolate used for commercializing Spod-X biopesticide (Munoz & Caballero, 2000). Taking all together, further purification of HearMNPV-IR isolate is necessary to elucidate its genotypic variants composition and pathogenic parameters. In summary, the HearMNPV-IR was clearly identified as a pathogenic, fast-killing isolate, demonstrating a desirable lethal concentration and rapid kill rate against the 3rd instars of the pest. Therefore, it offers valuable opportunities for future detailed studies and for its use as an active bioagent in developing virus-based biopesticides for successful biological control of *H. armigera* in the Middle East, particularly in Iran. Although the sequences of the Baculovirus conserved genes *polh*, *lef-8*, and *lef-9* showed no detectable differences between the two HearMNPV isolates (1 and 2) in this study, future investigations using whole-genome sequencing and comprehensive comparisons of their biological activities are strongly recommended.

Author's Contributions

Raheleh Shahbazi: Investigation, Data analysis, Writing original draft. **Masoud Naderpour**: Conceptualization, Supervision, Methodology, Formal analysis, writing original draft, Reviewing and editing manuscript, Project management and funding acquisition. **Reza Talaei-Hassanloui**: Methodology, Formal analysis, Reviewing manuscript.

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Data Availability Statement

All data supporting the findings of this study are available within the paper. The specimens examined in this study are deposited in the first author's collection at the Seed and Plant Certification and Registration Research Institute, Karaj, Iran and are available by the curator upon request.

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Ethics Approval and Consent to Participate

Insects were used in this study. All applicable international, national, and institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by the author.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Generative AI statement

The authors declare that no Generative AI tools were used in the writing, analysis, or preparation of this manuscript.

REFERENCES

Afrazeh, Z. & Jalali Sendi, J. (2025) Eco-friendly control of *Helicoverpa armigera* using synergistic mixtures of thymol and eucalyptol. *Scientific Reports*, 15, 26974. https://doi.org/10.1038/s41598-025-12752-7.

Ahmad, S., Cheema, H. M. N., Khan, A. A., Khan, R. S. A. & Ahmad, J. N. (2019) Resistance status of *Helicoverpa armigera* against *Bt* cotton in Pakistan. *Transgenic Research*, 28, 199–212. https://doi.org/10.1007/s11248-019-00114-9.

Ahmad, S., Muzammil, K., Siddiqui, S. & Hasan, F. (2025) Re-evaluation of recommended insecticides for resistance development, cross-resistance pattern, and underlying mechanisms in *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Ecotoxicology*, 34, 1753–1767. https://doi.org/10.1007/s10646-025-02948-y.

Allahyari, R., Aramideh, S., Safaralizadeh, M. H., Rezapanah, M. R. & Michuad, J. P. (2019) Synergy between parasitoids and pathogens for biological control of *Helicoverpa armigera* in chickpea. *Entomologia Experimentalis et Applicata,* 168, 70–75. https://doi.org/10.1111/eea.12866.

Allahyari, R., Aramideh, S., Michuad, J. P., Safaralizadeh, M. H. & Rezapanah, M. R. (2020) Negative life history impacts of *Hebrobracon hebetor* (Hymneoptera: Braconidae) that develop in bollworm larvae inoculated with *Helicoverpa armigera* nucleopolyhedrovirus. *Journal of Economic Entomology*, 113, 1648–1655. https://doi.org/10.1093/jee/toaa066.

Al Naggar, Y., Fahmy, N. M., Alkhaibari, A. M., Al-Akeel, R. K., Alharbi, H. M., Mohamed, A., Eleftherianos, I., El-Seedi, H. R., Giesy, J. P., & Alharbi, H. A. (2025) Mechanisms and genetic drivers of resistance of insect pests to insecticides and approaches to its control. *Toxics*, 13, 681. https://doi.org/10.3390/toxics13080681.

Anonymous. (2019) IBM Statistics for Windows. Version 26.0, IBM Corporation, Armonk, NY, USA.

Anonymous. (2025) 2026 insect control guide for agronomic crops. Mississippi State University Extension, Available at: https://extension.msstate.edu/sites/default/files/document/2025-10/P2471_web_2026.pdf

Arrizubieta, M., Williams, T., Caballeroa, P. & Simon, O. (2013) Selection of a nucleopolyhedrovirus isolate from *Helicoverpa armigera* as the basis for a biological insecticide. *Pest Management Science*, 70, 967–976. http://doi: 10.1002/ps.3637.

Arrizubieta, M., Simon, O., Williams, T. & Caballero, P. (2015) A novel binary mixture of *Helicoverpa armigera* single nucleopolyhedrovirus genotypic variants has improved insecticidal characteristics for control of cotton bollworms. *Applied* and *Environmental Microbiology*, 81, 3984 –3993. http:// doi: 10.1128/AEM.00339-15.

Arrizubieta, M., Simon, O., Ricarte-Bermejo, A., Lopez-Ferber, M., Williams, T. & Caballero, P. (2022) Coocclusion of *Helicoverpa armigera* single nucleopolyhedrovirus (HearSNPV) and *Helicoverpa armigera* multiple

nucleopolyhedrovirus (HearMNPV): Pathogenicity and stability in homologous and heterologous hosts. *Viruses*, 14, 687. https://doi.org/10.3390/v14040687.

Avand-Faghih, A. A., Sheikhigarjan, A., Bagheri, A., Askari-Siyahooei, M. & Famil, M. (2021) Damage control of *Helicoverpa armigera* on tomato by plant volatiles-based attract and kill approach. *Applied Entomology* and *Phytopathology*, 89, 75–94. https://doi.org/10.22092/jaep.2020.351194.1363.

Baillie, V. L., & Bouwer, G. (2012) High levels of genetic variation within *Helicoverpa armigera* nucleopolyhedrovirus populations in individual host insects. *Archives of Virology*, 157, 2281–2289. http://doi: 10. 1007/s00705-012-1416-6.

Barrera, G., Simon, O., Villamizar, L., Williams, T. & Caballero, P. (2011) *Spodoptera frugiperda* multiple nucleopolyhedrovirus as a potential biological insecticide: genetic and phenotypic comparison of fields isolates from Colombia. *Biological Control*, 58, 113–120. https://doi.org/10.1016/j.biocontrol.2011.04.009.

Barrera, G., Williams, T., Villamizar, I., Caballero, P. & Simon, O. (2013) Deletion genotypes reduce occlusion body potency but increase occlusion body production in a Colombian *Spodoptera frugiperda* nucleopolyhedrovirus population. *Plos One*, 8, e77271. doi.org/10.1371/journal.pone.0077271.

Beperet, I., Simon, O., Lopez-Ferber, M., Lent, J. V., Williams, T. & Caballero, P. (2021) Mixtures of insect pathogenic viruses in a single virion: towards the development of custom-designed insecticides. *Applied and Environmental Microbiology*, 87, e02180–20. https://doi.org/10.1128/AEM.02180-20.

CABI. (2014) Crop Protection Compendium. Commonwealth Agricultural Bureau International (CABI), Wallingford, UK.

Clem, R. J. & Passarelli, A. L. (2013) Baculoviruses: Sophisticated pathogens of insects. *PLOS Pathogens*, 9, e1003729. http://doi: 10.1371/journal.ppat.1003729.

Costa, V. H. D., Soares, M. A., Dimate, F. A. R., Sa, V. G. M. & Zanincio, J. C. (2019) Genetic identification and biological characterization of baculovirus isolates from *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Brazil. *Florida Entomologist*, 102, 59–64. https://doi.org/10.1653/024.102.0109.

Christian, P. D., Gibb, N., Kasprzak, A. B. & Richards, A. (2001) A rapid method for the identification and differentiation of *Helicoverpa nucleopolyhedroviruses* (NPV Baculoviridae) isolated from the environment. *Journal of Virology Methods*, 96, 51–65. https://doi.org/10.1016/S0166-0934(01)00318-4.

da Silva, L. A., de Camargo, B. R., Fisch, A. A. F. M. S., Santos, B., Ardisson-Araújo, D. M. P. & Ribeiro, B. M. (2025) Identification and detection of known and new viruses in larvae of laboratory-reared fall armyworm, *Spodoptera frugiperda*. *Journal of Invertebrate Pathology*, 210, 108290.

Dou, T., Bai, S., Gao, F., Tian, L., Zhu, J., Gu, X., Yang, X., Hao, Y., An, S., Liu, X. & Yin, X. (2024) Improved effects of *Helicoverpa armigera* nucleopolyhedrovirus integrated with *Campoletis chlorideae* against *H. armigera* and impact of the virus on the parasitoid. *Pest Management Science*, 80, 1145-1152. 10.1002/ps.7845.

Erlandson, M., Baldwin, D., Vlak, J. M. & Theilmann, D. (2024) Genomics of alphabaculovirus isolates infecting *Mamestra* species from North America and Eurasia. *Journal of Invertebrate Pathology*, 203, 108063. https://doi.org/10.1016/j.jip.2024.108063.

Eroglu, G. B., Demir, I. & Demirbag, Z. (2018) A novel alphabaculovirus isolated from the cotton bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae): characterization and pathogenicity. *Biologia*, 73, 545–551. https://doi.org/10.2478/s11756-018-0053-2.

Ferrelli, M., Taibo, C., Fichetti, P., Sciocco-Cap, A. & Arneodo, J. (2016) Characterization of a new *Helicoverpa armigera* nucleopolyhedrovirus variant causing epizootic on a previously unreported host, *Helicoverpa gelotopoeon* (Lepidoptera: Noctuidae). *Journal of Invertebrate Pathology*, 138, 89–93. https://doi.org/10.1016/j.jip.2015.08.009.

Figueiredo, E., Munoz, D., Murillo, R., Mexia, A. & Caballero, P. (2009) Diversity of Iberian nucleopolyhedrovirus wild-type isolates infecting *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Biological Control*, 50, 43-49. https://doi.org/10.1016/j.biocontrol.2009.02.005.

Garcia-Banderas, D., Tamayo-Mejia, F., Pineda, S., de la Rosa, J. I. F., Lasa, R., Chavarrieta-Yanez, J. M., Gervasio-Rosas, E., Zamora-Aviles, N., Morales, S. I., Ramos-Ortiz, S., Valle, J. & Martinez-Castillo, A. M. (2020) Biological characterization of two *Spodoptera frugiperda* nucleopolyhedrovirus isolates from Mexico and evaluation of one isolate in a small-scale field trial. *Biological Control*, 149, 104316. https://doi.org/10.1016/j.biocontrol.2020.104316.

Garcia-Munguia, A., M., Garcia-Munguia, C. A., Guerra-Avila, P. L., Sanchez-Mendoza, A., Rubalcava-Castillo, F. A., Garcia-Munguia, A., Robles-Lopez, M. R., Cisneros-Guzman, L. F., Martinez-Alba, M. G., Olvera-Gonzalez, E., Robles-de la Torre, R. R. & Garcia-Munguia, O. (2025). Baculovirus-based biocontrol: synergistic and antagonistic interactions of PxGV, PxNPV, SeMNPV, and SfMNPV in integrative pest management. *Viruses*, 17, 1077. https://doi.org/10.3390/v17081077.

Gifani, A., Marzban, R., Safekordi, A., Ardjmand, M. & Dezianian, A. (2015) Ultraviolet protection of nucleopolyhedrovirus through microencapsulation with different polymers. *Biocontrol Science and Technology*, 25, 814-827. http://dx.doi.org/10.1080/09583157.2015.1018814.

Gutierrez-Moreno, R., Mota-Sanchez, D., Blanco, C. A., Whalon, M. E., Teran-Santofimio, H., Rodriguez-Maciel, J. C. & DiFonzo, C. (2019) Field-evolved resistance of the fall armyworm (Lepidoptera: Noctuidae) to synthetic insecticides in Puerto Rico and Mexico. *Journal of Economic Entomology*, 1–11. doi: 10.1093/jee/toy372.

Grzywacz, D., Rabindra, R. J., Brown, M., Jones, K. A. & Parnell, M. (2005) The *Helicoverpa armigea* Nucleopolyhedrovirus Production Manual. Natural Resources Institute, p. 107. http://www.fao.org/docs/eims/upload/agrotech/2011/hanpvmanual-pt1.pdf.

Haile, F., Nowatzki, T. & Storer, N. (2021) Overview of pest status, potential risk, and management considerations of *Helicoverpa armigera* (Lepidoptera: Noctuidae) for U.S. soybean production. *Journal of Integrated Pest Management*, 12, 1–10. doi: 10.1093/jipm/pmaa030.

Hasnain, A., Zhang, S., Chen, Q., Xia, L., Wu, Y., Gong, C., Liu, X., Jian, P., Zheng, L. & Wang, X. (2023) Effects of Chlorantraniliprole on the life history traits of fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Frontiers in Physiology*, 14:1155455. doi: 10.3389/fphys.2023.1155455.

Holman, S., Grundy, P., Spafford, H. & Furlong, M. (2025) Lethal and sublethal effects of cotton expressing single and pyramided proteins of *Bacillus thuringiensis* (*Bt*) on *Helicoverpa armigera* (Lepidoptera: Noctuidae), *Spodoptera litura* (Lepidoptera: Noctuidae), and *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Journal of Economic Entomology*, 118, 1702–1710. https://doi.org/10.1093/jee/toaf089.

Kalantari, M., Marzban, R., Imani, S. & Askari, H. (2013) Effects of *Bacillus thuringiensis* isolates and single nuclearpolyhedrosis virus in combination and alone on *Helicoverpa armigera*. *Archives of Phytopathology and Plant Protection,* 47, 42–50. https://doi.org/10.1080/03235408.2013.802460.

Kalantari, M., Magholifard, Z. & Marzban, R. (2018) Virulence determination of nuclear polyhedrosis virus on cotton bollworm *Helicoverpa armigera* and diamond back moth, *Plutella xylostella*. *Journal of Applied Research in Plant Protection,* 7, 37–47.

Kaplan, E. L. & Meier, P. (1958) Nanparametric estimation from incomplete observations. *Journal of American Statistical Association*, 53, 457–481. https://doi.org/10.1080/01621459.1958.10501452.

Ignoffo, C. M. (1999) The first viral pesticide: past, present, and future. *Journal* of *Industrial Microbiology* and *Biotechnology*, 22, 407–417.

Jehle, J. A., Lnage, M., Wang, H., Hu, Z. H., Wang, Y. & Hauschild, R. (2006) Molecular identification and phylogenetic analysis of baculoviruses from Lepidoptera. *Virology*, 346, 180–193. http:// doi.org/ 10.1016/j.virol.2005.10.032.

Kenis, M., Benelli, G., Biondi, A., Calatayud, P.-A., Day, R., Desneux, N., Harrison, R. D., Kriticos, D., Rwomushana, I., van der Berg, J., Verheggen, F., Zhang, Y.-J., Agboyi, L. K., Ahissou, R. B., Ba, M. N., Bernal, J., Bueno, A. D. F., Carriere, Y., Carvalho, G. A., Chen, X.-X., Cicero, L., du Plessis, H., Early, R., Fallet, P., Fiaboe, K. K. M., Firake, D. M., Georgen, G., Groot, A. T., Guedes, R. N. C., Gupta, A., Hu, G., Huang, F. N., Jaber, L. R., Malo, E. A., McCarthy, C. B., Meagher, R. L., Jr, Mohamed, S., Sanchez, D. M., Nagoshi, R. N., Negre, N., Niassy, S., Ota, N., Nyamukondiwa, C., Omoto, C., Palli, S. R., Pavela, R., Ramirez-Romero, R., Rojas, J. C., Subramanian, S., Tabashnik, B. E., Tay, W. T., Virla, E. G., Wang, S., Williams, T., Zang, L.-S., Zhang, L. & Wu, K. (2023) Invasiveness, biology, and management of the fall armyworm, *Spodoptera frugiperda*. *Entomologia Generalis,* 43, 187–241. doi.org/10.1127/entomologia/2022/1659.

Kreimer-Rabalska, M., Rabalska, L., Jukes, M., D., Souza, M., L., Moore, S., D. & Szewczyk, B. (2019) New method for differentiation of granuloviruses (Betabaculoviruses) based on real time polymerase chain reaction (Real-Time PCR). *Viruses*, 11, 115. http://doi.rog/10.3390/v11020115.

Lange, M., Wang, H., Hu, Z. & Jehle. J. A. (2004) Towards a molecular identification and classification system of lepidopteran-specific baculoviruses. *Virology Journal,* 325, 36–47. http://doi.org/10.1016/j.virol.2004.04.023

Lavina, B. A., Padua, L. E., Wu, F. Q., Shirata, N., Ikeda, M. & Kobayashi, M. (2001) Biological characterization of a nucleopolyhedrovirus of *Spodoptera litura* (Lepidoptera; Noctuidae) isolated from the Philippines. *Biological Control*, 20, 39–47. https://doi.org/10.1006/bcon.2000.0877.

Legan, A. W., Allan, C. W., Jensen, Z. N., Degain, B. A., Yang, F., Kerns, D. L., Benowitz, K. M., Fabrick, J. A., Li, X., Carriere, Y., Matzkin, L. M. & Tabashnik, B. E. (2024) Mismatch between lab-generated and field-evolved resistance to transgenic *Bt* crops in *Helicoverpa zea*. *PNAS*, 121, e2416091121. https://doi.org/10.1073/pnas.2416091121.

Le Ora Software. (1987) POLO-PC user's guide to probit or logit analysis. Berkeley, CA.

Lei, C., Yang, J., Wang, j., Hu, J. & Sun, X. (2020) Molecular and Biological characterization of *Spodoptera frugiperda* multiple nucleopolyhedrovirus field isolate and genotypes in China. *Insects*, 11, 777. http://doi.org/10.3390/insects11110777.

Liu, Z.-K., Li, X.-L., Tan, X.-F., Yang, M.-F., Idrees, A., Liu, J.-F., Song, S.-J. & Shen, J. (2022) Sublethal effects of Emamectin Benzoate on fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Agriculture*, 12, 959. https://doi.org/10.3390/agriculture12070959.

Lu, P., Li, R., Guo, X., Tang, J., Tong, Y., Zhang, S., Su, Q., Qu, C. & Wang, R. (2026) Resistance mechanisms and fitness outcomes of tetraniliprole, one novel diamide insecticide, in a near-isogenic strain of *Spodoptera litura* (Lepidoptera: Noctuidae). *Pesticide Biochemistry and Physiology*, 216, 106834. https://doi.org/10.1016/j.pestbp.2025.106834.

Luna-Espino, J. C., Castrejón-Gómez, V. R., Pineda, S., Figueroa, J. I. & Martinez, A. M. (2018) Effect of four multiple nucleopolyhedrovirus isolates on the larval mortality and development of *Spodoptera exigua* (Lepidoptera: Noctuidae): determination of virus production and mean time to death. *Florida Entomologist*, 101, 153–159. http://doi.org/10.1653/024.101.0201.

Magholifard, Z., Abbasipour, H. & Marzban, R. (2014) Effects of *Helicoverpa armigera* nucleopolyhedrovirus (HaNPV) on the larvae of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). *Plant Protection Science*, 50, 184–189. http://10.17221/78/2013-PPS

Magholifard, Z., Hesami, S., Marzban, B. & Salehi Jouzani, G. (2017) Pathogenic effects of three nucleopolyhedrovirus, *Spodoptera littoralis* NPV, *Helicoverpa armigera* NPV, *Spodoptera litura* NPV on life stages of Egyptian cotton leafworm *Spodoptera littoralis*. *Plant Pests and Diseases,* 85, 203–218. https://10.22092/JAEP.2018.109796.1158

Martinez-Castillo, A. M., Zamora-Aviles, N., Camargo, A. H., De la Rosa, J. I. F., Pineda, S. & Ramos-Ortiz, S. (2022) Biological activity of two Mexican nucleopolyhedrovirus isolates and sublethal infection effects on *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae). *Florida Entomologist*, 105, 179–184. https://doi.org/10.1653/024.105.0301.

Mehrvar, A., Rabindra, R. J., Veenakumari, K. & Narabenchi, G. B. (2008a) Molecular and biological characteristics of some geographic isolates of nucleopolyhedrovirus of *Helicoverpa armigera* (Lep.: Noctuidae). *Journal of Entomological Society of Iran*, 28: 39-60.

Mehrvar, A., Rabindra, R. J., Veenakumari, K. & Narabenchi, G. B. (2008b) Evaluation of adjuvants for increased efficacy of HearNPV against *Helicoverpa armigera* (Hubner) using sunset machine. *Journal of Biological Sciences,* 8, 534–541. doi.org/10.3923/jbs.2008.534.541.

Mehrvar, A. (2009) Persistence of different geographical isolates of *Helicoverpa armigera* nucleopolyhedrovirus in two types of soils under different conditions. *Journal of Biological Sciences*, 9, 264–267. http://10.3923/jbs.2009.264.267.

Mehrvar, A. (2013) Virus yield parameters in mass production of three Iranian geographic isolates of *Helicoverpa armigera* nucleopolyhedrovirus. *Acta Entomologica Sinica*, 56, 1229–1234.

Mehrvar, A. & Saber, M. (2022) Timely inoculation of *Helicoverpa armigera* nucleopolyhedrovirus improves its efficiency in combination with benzoylphenylurea compounds. *Journal of Asia-Pacific Entomology*, 25, 101894. https://doi.org/10.1016/j.aspen.2022.101894

Moshtaghi, F. M., Jalali Sendi, J. & Rezapanah, M. R. (2013) A laboratory investigation on the effect of environmental temperature on virulence of *Helicoverpa armigera* multiple nucleopolyhedrovirus. *Journal of Animal Researches*, 26, 372–383. http://20.1001.1.23832614.1392.26.4.3.0.

Munoz, D., Castillejo, J. I. & Caballero, P. (1998) Naturally occurring deletion mutants are parasitic genotypes in a wild-type nucleopolyhedrovirus population of *Spodoptera exigua*. *Applied and Environmental Entomology*, 64, 4372-4377. 10.1128/AEM.64.11.4372-4377.1998.

Munoz, D. & Caballero, P. (2000). Persistence and effects of parasitic genotypes in a mixed population of the *Spodoptera exigua* nucleopolyhedrovirus. Biological Control, 19, 259–264. https://doi.org/10.1006/bcon.2000.0864.

Mondal, P., Kumar, A. & Tammana. (2021) Nuclear polyhedrosis virus: An overview. *Bulletin* of *Environment*, *Pharmacology* and *Life Sciences*, 10, 12–19.

Nai, Y. S., Huang, Y. F., Chen, T. H. & Wang, C. H. (2017) Determination of nucleopolyhedrovirus' taxonomic position, in: Shields, V.D.C (Ed.) *Biological Control of Pest and Vector Insects*. Intech publisher, pp. 169–195. http://doi.org/10.5772/66634.

Naseri, B., Fathipour, Y., Moharramipour, S. & Hosseininaveh, V. (2009) Comparative life history and fecundity of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on different soybean varieties. *Entomological Science,* 12, 147–154. http://dx.doi.org/10.1111/j.1479-8298.2009.00310.x.

Pandi, R. K., Gopalsamy, S., Onkarappa, D., Thiruvengadam, V., Muthugounder, M. & Nand, S. S. (2024) Characterization and bioassay of indigenous isolates of the fall armyworm, *Spodoptera frugiperda* nucleopolyhedrovirus in India. *Egyptian Journal of Biological Pest Control*, 34, 15. https://doi.org/10.1186/s41938-024-00779-3.

Posos-Parra, O., Mota-Sanchez, D., Pittendrigh, B. R., Wise, J. C., DiFonzo, C. D. & Patterson, E. (2024a) Characterization of the inheritance of field-evolved resistance to diamides in the fall armyworm (*Spodoptera frugiperda*) (Lepidoptea: Noctuidae) population from Puerto Rico. *Plos One*, 19, 0295928. https://doi.org/10.1371/journal.pone.0295928.

Posos-Parra, O. A., Pittendrigh, B. R., Wise, J. C., DiFonzo, C., Patterson, E. & Mota-Sanchez, D. (2024b) Inheritance and resistance mechanisms of field-evolved resistance to pyrethroids in a fall armyworm (*Spodoptera frugiperda* J.E.Smith) (Lepidoptea: Noctuidae) strain from Puerto Rico. *Insects*, 15, 912. https://doi.org/10.3390/insects15120912.

Riaz, S., Johnson, J. B., Ahmad, M., Fitt, G. P. & Naiker, M. (2021) A review on biological interactions and management of the cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Nuctoidae). *Journal of Applied Entomology*, 145, 467–498. http://10.1111/jen.12880

Rowley, D. L., Popham, H. J. R. & Harrison, R. L. (2011) Genetic variation and virulence of nucleopolyhedroviruses isolated worldwide from the heliothine pests *Helicoverpa armigera*, *Helicoverpa zea*, and *Heliothis virescens. Journal of Invertebrate Pathol*ogy, 107,112–126. https://doi.org/10.1016/j.jip.2011.03.007

Simon, O., Williams, T., Lopez-Ferber, M. & Caballero, P. (2004) Genetic structure of a *Spodoptera frugiperda* nucleopolyhedrovirus population: high prevalence of deletion genotypes. *Applied and Environmental Microbiology*, 70, 5579–5588. doi.org/10.1128/AEM.70.9.5579–5588.2004.

Simmond, P., Adriaenssens, E. M., Lefkowitz, E. J., Oksanen, H. M., Siddeli, S. G., Zerbini, F. M., Alfenas-Zerbini, P., Aylward, F. O., Dempsey, D. M., Dutilh, B. E., Freitas-Astua, J., Garcia, M. L., Hendrickson, R. C., Hughes, H. R., Junglen, S., Krupovic, M., Kuhn, J. H., Lambert, A. J., Lobocka, M., Mushegian, A. R., Penzes, J., Munoz, A. R., Robertson, D. L., Rpux, S., Rubino, L., Sabanadzovic, S., Smith, D. B., Suzuki, N., Turner, D., Doorslaer, K. V., Vandamme, A.-M. & Varsani, A. (2024) Changes to virus taxonomy and the ICTV statutes ratified by the International Committee on Taxonomy of Viruses (2024). *Archives of Virology*, 169, 236. http://10.1007/s00705-024-06143-y

Shahbazi, R., Talaei Hassanloui, R., Naderpour, M., Mehrvar, A., Dizaji, A. & Fotouhi, F. (2020) Collecting and partial characterization of Iranian *Helicoverpa armigera* SNPV isolates based on *polh* gene. *Iran Journal of Plant Protection Science*, 51, 195–207. http://doi.org/10.22059/ijpps.2020.302425.1006943.

Shazdehahmadi, M., Assemi, H., Shahadatimogadam, Z., Sajjadi, A. & Rezapanah, M. R. (2016) An investigation on phylogenetic classification of *Helicoverpa armigera* nucleopolyhedrovirus (HaNPV), isolated from tobacco fields of north of Iran, by RAPD markers. *Journal of BioControl in Plant Protection*, 3, 123–129. http://10.22092/BCPP.2016.105939.

Valizadeh, B., Samarfard, S., Jalali Sendi, J. & Karbanowicz, T. P. (2020) Developing an *Ephestia kuehniella* hemocyte cell line to assess the bio-insecticidal potential of microencapsulated *Helicoverpa armigera* nucleopolyhedrovirus against cotton bollworm (Lepidoptera: Noctuidae) larva. *Journal of Economic Entomology*, 113, 2086–2095.http://10.1093/jee/toaa148

Valizadeh, B., Afrazeh, Z. & Jalali Sendi, J. (2024) A novel *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV-IR18 isolate) formulation on hemocyte cultures of cotton bollworm (*Helicoverpa armigera* Hubner). *Journal of Asia-Pacific Entomology*, 27, 10186. https://doi.org/10.1016/j.aspen.2023.102186.

Washburn, J. O., Trudeau, D., Wong, J. F. & Volkman, L. E. (2003) Early pathogenesis of *Autographa californica* multiple nycleopolyhedrovirus and *Helicoverpa zea* single nucleopolyhedrovirus in *Heliothis virescens*: a comparison of the 'M' and 'S' strategies for establishing fatal infection. *Journal of General Virology*, 84, 343-351. http://10.1099/vir.0.18701-0.

Williams, C. F. & Payne, C. C. (1984) The susceptibility of *Heliothis armigera* larvae to three nuclear polyhedrosis viruses. *The Annals of Applied Biology*, 104, 405-412. https://doi.org/10.1111/j.1744-7348.1984.tb03021.x.

Williams, T., Lopez-Ferber, M. & Caballero, P. (2022) Nucleopolyhedrovirus coocclusion technology: a new concept in the development of biological insecticides. *Frontiers in Microbiology*, 12, 810026. http://doi.org/doi.org/10.3389/fmicb.2021.810026.

Yang, Y., Dai, J., Zhang, G., Singh, D., Zhang, X. & Liang, Z. (2024) *Mamestra brassicae* multiple nucleopolyhedroviruses prevents pupation of *Helicoverpa armigera* by regulating juvenile hormone titer. *Insects*, 15, 202. https://doi.org/10.3390/insects15030202.

Zamora-Aviles, N., Murillo, R., Lasa, R., Pineda, S., Figueroa, J. I., Bravo-Patino, A., Diaz, O., Corrales, J. L. & Martinez, A. M. (2017) Genetic and biological characterization of four nucleopolyhedrovirus isolates collected in Mexico for the

control of *Spodoptera exigua* (Lepidoptera: Noctuidae). *Journal of Economic Entomology*, 110, 1465–1475. https://doi.org/10.1093/jee/tox130.

Zheng, J., Chen, X., Xie, Y., Zhang, Y., Huang, Y., Wu, P., Lv, J. & Qiu, L. (2024) Knocking out of UDP-Glycosyltransferase gene *UGT2B10* via CRISPR/Cas9 in *Helicoverpa armigera* reveals its function in detoxification of insecticides. *Journal of Agriculture and Food Chemistry*, 72, 20862–20871. https://doi.org/10.1021/acs.jafc.4c05055.

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آلفاباکولوویروس جدید از ایران روی *Helicoverpa armigera* (Lep., Noctuidae): شناسایی و بیماری زایی

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چکیده: در پژوهش حاضر از لاروهای مرده و بیمار *Helicoverpa armigera* جمع‌آوری شده از مزارع گوجه فرنگی در مناطق شمال غربی کشور، ویروس چندوجهی هسته‌ای از جنس آلفاباکولوویروس جداسازی و شناسایی گردید. آلودگی به ویروس ابتدا با مشاهده میکروسکوپی اشکال نامنظم و درخشان پلی‌هدرین استخراج شده از مایعات سلولی لارو مشخص و سپس با آنالیزهای مولکولی و توالی‌یابی تایید شد. نتایج بررسی‌ها گونه *Helicoverpa armigera* nucleopolyhedrovirus را در لاروهای جمع‌آوری شده اثبات و به اختصار HearNPV-IR نامگذاری شد. مطالعات تبارزایی بر اساس بخشی از توالی‌های منفرد و به‌هم پیوسته ژن‌های *late expression factor 8* (*lef-8*)، *late expression factor 9* (*lef-9*) و *polyhedrin* (*polh*) مشخص کرد که جدایه HearNPV-IR در شاخه HearMNPV و در ارتباط نزدیک با جدایه HearMNPV 3154 از روسیه قرار دارد. پاسخ غلظت–مرگ و میر HearMNPV-IR روی لارو سن ۳ *H. armigera* بررسی و متوسط LC_{50} آن ۱۰^۵ ×۴ پلی‌هدر/میلی‌لیتر تخمین زده شد. متوسط زمان بقاء در غلظت‌های ۱۰^۹×۳ و ۱۰^۸ ×۳ پلی‌هدر/میلی‌لیتر، به ترتیب ۳ و ۴ روز پس از بیماریزایی محاسبه گردید. بالاترین دوز ویروس، در مدت زمان ۵ روز منجر به مرگ و میر ۱۰۰ درصدی لاروهای آفت شد. نتایج بدست آمده بیانگر ویژگی‌های مطلوب حشره‌کشی جدایه HearMNPV-IR به عنوان عامل کنترل بیولوژیک علیه کرم غوزه پنبه است.

کلمات کلیدی: بیماری شناسی حشرات،کنترل میکروبی، شجره شناسی، کنترل زیستی

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