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Isolation characterization and pathogenicity of Helicoverpa armigera single nucleopolyhedrovirus isolate from Türkiye

Türkiye'den Helicoverpa armigera single nükleopolihedrovirüs izolatının izolasyonu, karakterizasyonu ve patojenitesi

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Abstract

The cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), is a major pest of agricultural crops, such as cotton, horticultural plants, a range of vegetable crops and forest trees. Many dead and live larvae and pupa samples of *H. armigera*, an extremely polyphagous pest, were collected from Adana in Türkiye. It was observed that cadaveric integuments were fragile, and their body tissues were also liquefied. Studies with phase-contrast microscopy revealed a very intense baculovirus infection in cadavers. Transmission electron microscopy exhibited that the new baculovirus had one nucleocapsid in an envelope in the occlusion bodies. Therefore, the isolate was named *Helicoverpa armigera* single nucleopolyhedrovirus-D (HearSNPV-D). The phylogenetic analysis according to the *polyhedrin* and *late expression factor*-8 genes appeared that the new isolate was in close relationship with HearSNPV China and *Türkiye* isolate. The new isolate was tested on six different insects including its own host *Helicoverpa armigera*, *Hyphantria cunea*, *Malacosoma neustria*, *Lymantria dispar*, *Thaumetopea pityocampa* and *Spodoptera exigua* larvae. Bioassay studies showed that HearSNPV-D was virulent to *H. armigera* and had a narrow host range. The LC_{50} value was determined as 0.7×10^5 and 1.9×10^7 OBs/ml for *H. armigera* and *S. exigua*, respectively. Non-significant mortality was observed in non-host species by HearSNPV-D, which may reveal the highly specific nature of HearSNPV-D. Therefore, HearSNPV-D can be used as a specific, environmentally friendly control agent for *H. armigera*.

Özet

Pamuk kurdu, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), pamuk, bahçe bitkileri, çeşitli sebze bitkileri ve orman ağaçları gibi tarımsal ürünlerde önemli bir zararlıdır. Polifag bir zararlı olan *H. armigera*'nın çok sayıda ölü ve canlı larva ve pupa örneği Türkiye'de Adana'dan toplanmıştır. Kadavra derilerinin kırılgan olduğu ve vücut dokularının da sıvılaştığı görüldü. Faz-kontrast mikroskobu ile yapılan çalışmalar, kadavralarda çok yoğun bir bakülovirüs enfeksiyonu ortaya çıkardı. Transmisyon elektron mikroskobu, yeni bakülovirüsün oklüzyon yapılarında, zarf içinde bir nükleokapside sahip olduğunu gösterdi. Bu nedenle izolata *Helicoverpa armigera* single nükleopolihedrovirüs-D (HearSNPV-D) adı verildi. Polihedrin ve geç ekspresyon faktörü-8 genlerine göre yapılan filogenetik analiz, yeni izolatın HearSNPV Çin ve Türkiye izolatı ile yakın ilişki içinde olduğunu ortaya koydu. Yeni izolat, kendi konakçısı *Helicoverpa armigera*'nın yanısıra *Hyphantria cunea*, *Malacosoma neustria*, *Lymantria dispar*, *Thaumetopea pityocampa* ve *Spodoptera exigua* olmak üzere toplam altı farklı böcek üzerinde test edildi. Biyoanaliz çalışmaları, HearSNPV-D'nin *H. armigera*'ya karşı öldürücü olduğunu ve dar bir konakçı aralığına sahip olduğunu gösterdi. LC_{50} değeri *H. armigera* ve *S. exigua* için sırasıyla 0.7×10^5 OBs/ml ve 1.9×10^7 OBs/ml olarak belirlendi. HearSNPV-D tarafından konakçı olmayan türlerde anlamlı olmayan ölüm gözlemlendi, bu da HearSNPV-D'nin oldukça spesifik doğasını ortaya çıkarmıştır. Bu nedenle HearSNPV-D, *H. armigera* için spesifik, çevre dostu bir kontrol ajanı olarak kullanılabilir.

INTRODUCTION

The cotton bollworm *Helicoverpa armigera* (Hübner) is a major pest of agricultural crops, such as cotton, horticultural plants, a range of vegetable crops and forest trees. It is a polyphagous pest and many of its host plants are economically important crops in Africa, Asia, Europe and Oceania (https://gd.eppo.int/taxon/HELIAR/distribution) (Tay et al. 2013). The cotton bollworms are an important group that makes damage causing loss of millions of dollars every year in the world (Haile et al. 2021, Sharma 2005). Larvae generally feed on reproductive and

vegetative structures of a wide variety of agricultural crops (Dias et al. 2019). Chemical control is the most commonly used method in the control of *H. armigera*, but it has not been a continuous success due to the development of insecticide resistance (Tatchell 1997, Martin et al. 2005, Yang et al. 2013). There are two commercial preparations based on *Bacillus thuringiensis* (Cherry et al. 2003) and nucleopolyhedrovirus (NPV), which are widely used in the world for the microbial control of *H. armigera*. However, it was stated that *H. armigera* developed resistance against *B. thuringiensis* (Luttrell and Jackson 2012, Yang et al. 2013). For this reason, there has been a need for the development of baculovirus-derived products in the control of *H. armigera*, especially in our country and all over the world.

Baculoviruses are double-stranded DNA viruses that infect insects and cause their death. Two Baculovirus genera, Nucleopolyhedrovirus (NPVs) and Granulovirus (GVs) (Rodríguez et al. 2012) are used as biological control materials in agriculture as a good alternative to synthetic insecticides for the pathogen organisms (Bilimoria 1991, Nawaz et al. 2019). The host spectrum of baculoviruses is narrow, usually limited to a single insect genus or species (Black et al. 1997, Cunningham 1998). For this reason, it is preferred as an effective and reliable biological control material. Morphological and molecular characteristics of baculovirus isolates obtained from various insects were defined and their virulence characteristics were determined (Gencer et al. 2018, Gencer et al. 2022, Eroglu et al. 2019).

In this study, a new single nucleopolyhedrovirus variant (HearSNPV-D) causing epizootic in *H. armigera* was isolated from the dead larvae and pupae of the pest in *Türkiye*. The virus was characterized according to ultrastructural morphology and the sequence of the coding regions of *polh* and *lef*-8 genes and analyzed phylogenetically. Besides, the host range of the virus was investigated on lepidopteran species representing different families.

MATERIAL AND METHODS

Field Collections

For virus screening, cotton bollworm (*H. armigera*) larvae were collected from different host plants from Adana, *Türkiye* province between June and August in 2019. Samples found to be dead spontaneously in nature were placed in separate microcentrifuge tubes and brought to the laboratory. All living larvae found were brought to the laboratory and their development observed. Of the live larvae fed on fresh lettuce leaves, those that died during development were stored at -20 °C for use in virus screening.

Extraction of Occlusion Bodies

Purification of occlusion bodies (OBs) was done with 30% sucrose gradient. Larvae were crushed completely in a homogenizer and transferred to a new tube by filtering through a double layer of sterile cheesecloth. Homogenate was centrifuged at 5000 rpm for 30 min. The supernatant was discarded, and the pellet was dissolved in 1 ml of dH_2O. Thawed pellet was centrifuged under the same conditions by gently adding 1 ml of virus solution onto 3 ml of 30% sucrose. The pure virus band separated from the sucrose was carefully removed and transferred to a new tube. Virus sample was washed to completely remove sucrose and centrifuged for 15 min. The resulting pellet was dissolved in 5 ml of dH_2O and stored at -20 °C (O'Reilly et al. 1992).

Microscopic Examinations

Light Microscopy

Preparations between slides and coverslips were prepared from insect homogenates. OBs of baculoviruses were observed in all preparations at 100 X magnification under a microscope (Nikon Eclipse LH-M100C-1). OB structures were then photographed (Nikon Coolpix 5000).

Scanning Electron Microscopy

Virus isolate was dried in an oven at 37 °C overnight by dropping 20 µl on separate stabs. It was then covered with gold powder on a sputter coater (Quorum Technology SC7620-CF) and examined under a scanning electron microscope (Zeiss EVO LS10).

Transmission Electron Microscopy

The OBs (500 µl) was precipitated at 13000 rpm for 5 minutes. Phosphate salt buffer (PBS) was added to the OB structures in the pellet and centrifuged twice. Supernatant was removed and 2.5% glutaraldehyde (2ml) in 0.1 M PBS was added to the pellet and left to fixation at +4 °C overnight. After primary fixation, cells were washed with PBS twice. Subsequently, 2 ml of 1% osmium tetroxide was added and secondary fixation was carried out at +4 °C for 1 hour. Then, it was washed 2 times with PBS. With the help of a Pasteur pipette, 5% molten agar was added to the pellet in a 50 ml tube and dropped onto the slide. The frozen agar pieces were cut into small pieces with the help of a scalpel tip and uranyl acetate was added and incubated at +4 °C for 15 minutes. Then, cells embedded in agar were passed through a series of 30%, 50%, 70% and 90% (twice) alcohol for 10 minutes at +4 °C, respectively. Samples were kept in pure propylene oxide at +4 °C for 10 minutes. A 1:1 ratio of propylene oxide/epon mixture was added to it, and it was kept at +4 °C for 10 minutes. Pure epon mixture was added and kept at +4 °C for 48 hours and embedded in araldite. Sections of 60 nm thickness were taken with an ultramicrotome (LEICA ULTRACUT R), and the sections were placed on copper grids. Staining was performed with uranyl acetate-lead citrate dye and examined under transmission electron microscopy (JEOL JEM 1220).

Reproduction of OBs

The isolate, determined as baculovirus according to the sequence analysis, was amplified. For the propagation process, 100 3rd instar healthy green worm larvae were used. For the reproduction of the isolate, the larvae, which were fasted for 16 hours, were infected with the droplet feeding method. According to this method, a 1:1 ratio of droplet dye (20% sucrose and 2% red food coloring) and virus suspension were mixed and 1 µl was given to each larva (Hughes et al. 1986). Infected larvae were individually placed in 25 ml plastic containers and 100 mg of artificial diet was given to the larvae. It was then incubated in a climate cabinet (26 °C, 60% humidity) for 14 days. Mortality in larvae was checked daily and nutrients were replenished every other day. Larvae that died as a result of infection were collected and stored at -20 °C until virus purification.

DNA Isolation

For viral DNA extraction, 100 µl of isolate was taken and 3 X DAS (0.3 M Na_2CO_3, 0.5 M NaCl, 0.03 M EDTA; pH 10.5) solution was added and incubated on a rotator at 40 rpm for 30 minutes at room temperature. It was then centrifuged at 20300 x g for 30 minutes at room temperature and the supernatants were discarded. The pellet was dissolved with 300 µl of lysis buffer (10 mM Tris, 100 mM EDTA, 1% SDS; pH 8.0) and 25 µl of proteinase K and incubated at 50 °C overnight. Subsequently 40 µl RNase was added on it and incubated at 37 °C for 30 minutes and then kept on ice for 1 minute. Then, 100 µl of protein precipitation solution was added to the solution and kept on ice for an additional 5 minutes. At the end of the period, it was centrifuged for 10 min at 16000 x g at +4 °C. The supernatant was taken into a new tube and 500 µl of isopropanol was added and centrifuged at +4 °C for 2 minutes at 16000 x g. The supernatant was decanted, and the pellet was washed twice with 70% ethanol (EtOH). The DNA pellet was dried for 20 minutes at 37 °C, allowing EtOH to evaporate, and 30 µl of dH_2O was added and kept at 55 °C for 10 minutes (Nakai and Kunimi 1997). The purity and amount of isolated DNA was measured with Nanodrop (Thermo Scientific).

Polymerase Chain Reaction and Phylogenetic Analysis

DNA was amplified by PCR using primers belong to the conserved partial sequences of *polyhedrin* (*polh*), and *late expression factor*-8 (*lef*-8) genes of baculoviruses (Jehle et al. 2006). The PCR reaction was performed with GoTaq enzyme according to the manufacturer's standard protocol using the GoTaq DNA Polymerase Kit (Promega). PCR reaction include 3 µl (10 ng/µl) template DNA, 10 µl

(10X) PCR buffer, 1 µl (10 mM) dNTP mix, 1 µl each of the forward and reverse primers (10 µM), 6 µl $MgCl_2$ (25 mM), 0.25 µl GoTaq DNA polymerase (0,5 µl) and dH_2O up to 50 µl. The reaction cycle is 2 min at 94 ºC for denaturation. After waiting for primer attachment, 0.5-1 min at 42-58 ºC, 35 cycles at 72 ºC for elongation and 7 minutes at 72 ºC for the final elongation step. The samples were electrophoresed for 30 minutes on a 1% agarose gel containing 0.5 µg/ml ethidium bromide. The fragments cut from the agarose gel and cleaned with the Nucleospin Extract II DNA Purification (Macherey-Nagel) kit according to the manufacturer's protocol and sent to Macrogen Inc. (The Netherlands) for sequencing. The sequences were compared with Biotechnology Information Center (NCBI) using the BLAST tool. The nucleotide sequences were submitted to NCBI database. All genes were added sequentially (*polh* / *lef*-8) for phylogenetic analysis and data were edited in BioEdit 7.0.5.3 (Hall 1999). Neighbor-Joining analysis and Bootstrap method (1000 repetitions) in MEGA 7 7.0.26 (Kumar et al. 2016) program were used for phylogenetic tree drawing. Additionally, the granulin gene from Hyphantria cunea granulovirus (HycuGV) and Helicoverpa armigera granulovirus (HearGV) were used as out group.

Insect Rearing

Lab-reared cultures of *Helicoverpa armigera* (Hübner, 1805) (Lepidoptera: Noctuidae), *Spodoptera exigua* (Hübner, 1808) (Lepidoptera: Noctuidae), and field collected *Hyphantria cunea* (Drury, 1773) (Lepidoptera: Erebidae), *Malacosoma neustria* (L., 1758) (Lepidoptera: Lasiocampidae), *Thaumetopea pityocampa* (Tams, 1924) (Lepidoptera: Thaumetopoeidae) and *Lymantria dispar* (L., 1758) (Lepidoptera: Erebidae) larvae were tested in bioassays. *H. armigera* and *S. exigua* were maintained on artificial diet (266 g wet beans, 4 g ascorbic acid, 1.25 g sorbic acid, 2.5 g methyl 1-4 hydroxybenzoate, 3 g wheat germ, 14 g agar-agar, 35 g yeast and 800 ml distilled water) under laboratory conditions (Bergomaz and Boppré 1986). *H. cunea*, *M. neustria, T. pityocampa* and *L. dispar* were fed with fresh leaves of mulberry, rose, pine and oak, respectively, which host plants most favored by these insects in natural conditions and from which they were collected for trial. *H. armigera* and *S. exigua* cultures were grown in the laboratory at 26 ± 1 °C, 75% relative humidity and 16:8 (L:D) h period (Mardani-Talaee et al. 2016).

Insecticidal Activity Tests

The median lethal concentration (LC_{50}) was calculated in third stage larvae of all insect hosts. The pathogenicity of HearSNPV-D isolate was carried out by the droplet-feeding process (Hughes et al. 1986). After the third instar larvae were starved for 6 hours, they were fed with the virus mixture (sucrose, blue food coloring dye and virus isolate in different dilutions). Viral suspensions were administered to contain from 10^4 to 10^8 OBs/ml. Virus-free solution was also used as control. Thirty larvae from each insect were used individually. After the larvae were allowed to be fed with viral solutions for 2 hours, the larvae continued to be fed with their own food under laboratory at 26 ± 1 °C, 75% relative humidity and 16:8 (L:D) h period. The experimental setup was checked daily, the deads were removed, and the experiment was continued until the larvae became pupae. Experiments were performed independently 3 times. The data recorded because of the biotest were statistically analyzed using the SPSS program. Dose mortality rates were plotted using the Veusz 3.4 software.

RESULTS

Microscopy

Phase-contrast and light microscopies of samples taken from cadavers from both regions revealed abundant OBs with bright crystalline structures in all examination areas (Figure 1A). Observations in scanning electron microscopy showed that the isolates had an irregular shape, like HearNPVs (Figure 1). The dimensions of the embedded structures of HearSNPV-D were determined as 1.15 ± 0.47 µm. Transmission electron images revealed that HearSNPV-D contain single virions in an envelope and were multiple nucleocapsids (Figure 1B). It was observed that the capsid number of the embedded isolate was single, thus it was understood that the isolate was a single nucleopolyhedrovirus. The capsid lengths of the strain were measured as 178 × 64 nm (Figure 1C).

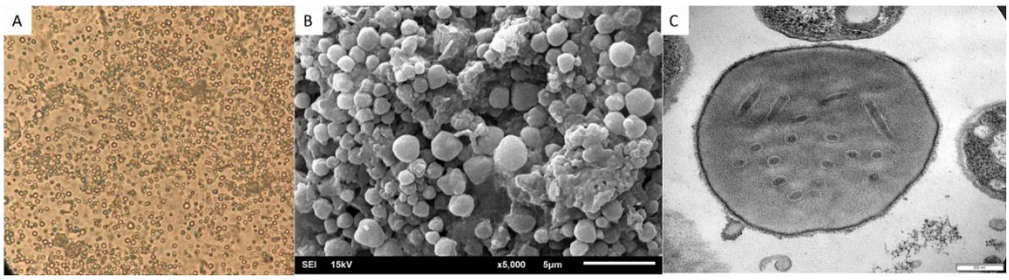

Figure 1. Micrographs of new HearSNPV-D isolate from *H. armigera* larvae. A: The OBs are seen as bright crystal structures (100X). B: Scanning electron micrograph of OBs. C: Transmission electron micrograph of cross section of OBs.

Phylogenetic Analysis

Partial gene sequences of the conserved *polh* (426 base pairs) and *lef*-8 (800 base pairs) genes of *Helicoverpa / Heliothis* NPV samples were added one after another and subjected to phylogenetic analysis together with the other baculoviruses deposited GenBank. Our results indicated that the Turkish HearSNPV-D isolate separate in a clade with the HearSNPV (MK507817) *Türkiye* isolate and HearSNPV (AF271059) China isolate (Figure 2). Accession numbers were received for HearSNPV-D *polh* (OQ656459) and *lef*-8 (OQ656458) partial genes. The H. armigera GV (USA) and H. cunea GV (*Türkiye*) isolates were used as the outgroup (Figure 2).

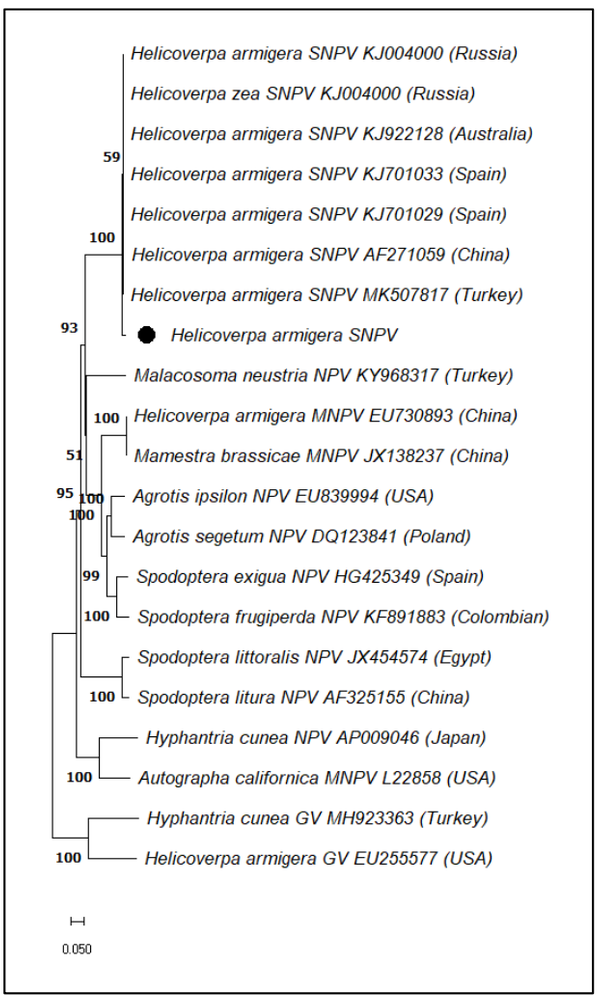

Figure 2. A phylogenetic tree (neighbor joining) according to the nucleotide sequences of concatenated partial polh and lef-8 genes. Bootstrapscores were showed with numbers on branches. Black square indicates the location of the HearSNPV-D

Biological Activity Trials

The biotest results showed that isolate had lethal effects on pests. Increased isolate concentrations resulted with increase in pest mortality (Figure 3). Isolate produced the highest mortality effect on its own host. Fourth days after application, larvae of *H. armigera* infected with isolate showed typical symptoms of baculoviral infection and began to die. The mortality of HearSNPV-D on *H. armigera* was recorded as 93% at the end of the test at 1×10^8 OBs/ml concentration. The HearSNPV-D isolate produced varying degrees of mortality on the other hosts used in the study. Fourteen days later, the 10^8 OBs/ml concentration of HearSNPV-D caused 93%, 53%, 37%, 33%, 31% and 26% mortality on *H. armigera*, *S. exigua*, *L. dispar*, *H. cunea* and *T. pityocampa* and *M. neustria*, respectively ($p < 0.01$) (Figure 3). As a result of probit analysis, it was shown that the LC_{50} value of HearSNPV-D was determined as 0.7×10^5 and 1.9×10^7 OBs/ml for *H. armigera* and *S. exigua*, respectively.

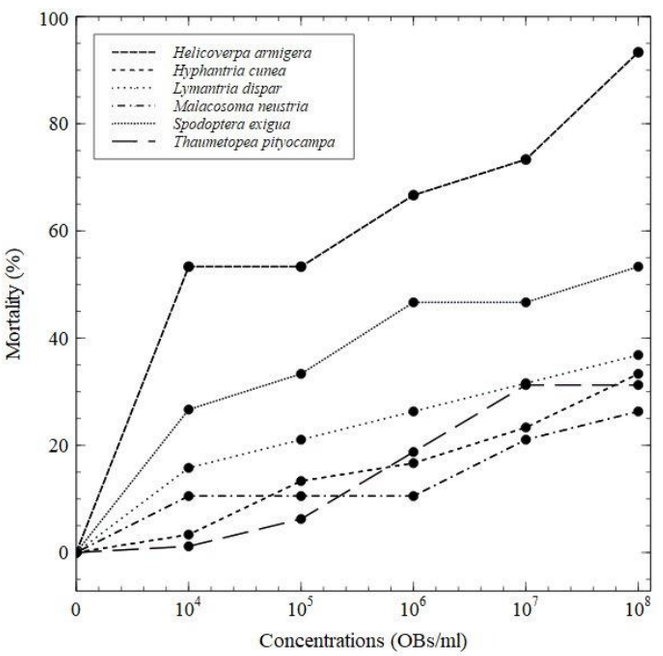

Figure 3. Mortality of insect larvae as a result of HearSNPV-D infections

DISCUSSION

Baculovirus infections are frequently observed on insect populations in nature (Adams and McClintock 1991, Cooper et al. 2003, Il'inykh and Ul'yanova 2005). These infections are important in keeping pest populations below the economic damage threshold (Fuxa et al. 1993, Cory and Myers 2003). Baculoviruses, which are among the biological control factors, stand out in that they are specific to the type or genus of the target organism, have high virulence and do not cause harm to non-target organisms. Thus, the development of an effective baculovirus-derived biopesticide against harmful insects is an important need for both the world and our country. *Greenworm* (Helicoverpa/Heliothis, Lepidoptera: Noctuidae) species are polyphagous agricultural pests that are cosmopolitan in all countries of the world, including our country. The isolate was examined under light and electron microscopes. As a result of light microscopy examinations, inclusion bodies of nucleopolyhedrovirus were observed brightly in isolate. In scanning electron microscopy studies, OBs were observed to have irregular morphology and their sizes were measured.

In the literature, it has been stated that the OB structures of NPVs are in the 0.4-5 µm size range and their morphological structures are irregular (Federici 1995, Harrison and Hoover 2012). In one study, it was noted that the OB dimensions of SNPVs in general are 1.02 ± 0.04 µm (Sudhakar and Mathavan 1999). In this study, it was determined that all HearNPV isolate examined fit a typical NPV characteristic size, shape and morphology. Compared to the OB sizes of other Heliothis NPV isolates in the literature, it is smaller than the isolates of India (1.6-2.4 µm) (Somasekar et al. 1993), China (2 µm) (Tang et al. 2012), and larger than isolates from Argentina (0.6-1.2 µm) (Ferrelli et al. 2016). It is almost the same size as the *Helicoverpa armigera* isolate (HearSNPV-D) isolated from *Helicoverpa peltigera* (single isolate HearNPV-TR) (0.73-1.66 µm) (Eroglu et al. 2019). Another single HearNPV isolate (0.89 µm) (Jacob and Subramanian 1972) is small size than our isolate HearSNPV-D. Many factors may be effective in the OB size difference between different geographic isolates of the same virus. One of them is related to the environmental and physical conditions of the host cells during infection (Kioukia et al. 1995).

It has been reported in the literature that the nucleocapsid sizes of baculoviruses are 250-300 nm in

length and 30-60 nm in diameter (Jehle et al. 2006). Considering the nucleocapsid sizes detected in HearNPV studies in the literature, the nucleocapsid of the Indian isolate (277.7 × 41.6 nm) (Kumar et al. 2011), Chinese isolate (230 × 50 nm) (Tang et al. 2012) *Türkiye* isolates (HearNPV-O1 /279 × 50 nm; HearNPV-TR /184 × 41 nm) (Eroglu et al. 2018, 2019) were larger than the nucleocapsid of HearSNPV-D isolate (178 × 64 nm). It has been reported that isolates with multiple and large nucleocapsid numbers may have advantages such as having a wider host range and earlier initiation of infection on the host (Volkman et al. 1995, Washburn et al. 1999). It has been found that the initiation of infection in isolates containing single nucleocapsid in the host takes 8 hours longer than isolates containing multiple nucleocapsids (Washburn et al. 2003).

Analyzes based on partial sequences of *polh* and *lef*-8 genes in nucleopolyhedroviruses are also widely used in phylogenetic studies of baculovirus strains and isolates. In the co-phylogenetic analysis of the *polh* and *lef*-8 genes, we found that the new isolate was clustered most closely with other *Helicoverpa armigera* single nucleopolyhedrovirus isolates and showed homology with Malacosoma neustria nucleopolyhedrovirus isolate in the tree a little further from them according to the degree of phylogenetic relationship.

Today, the use of biological agents has become important due to the harmful effects of chemical insecticides on environment. Among these agents, baculoviruses have an important in the control of insects. Geographic baculovirus strains may have different pathogenicity on local insect populations under different conditions (Shapiro and Robertson 1991, Caballero et al. 1992, Cory et al. 2005, Haase et al. 2015). Similarly, different host species or geographically different populations may have different susceptibility to some viral isolates (Milks 1997, Ribeiro et al. 1997). Biological activity trials were used to explain the pathogenicity of HearSNPV-D isolate in six lepidopteran insect host including its host. One of the characteristics of the nucleopolyhedroviruses is a narrow host range. These viruses often show the highest mortality effect on their own host, and lower effects on insect species related to their host. In a previous study, HearNPV-TR isolate (isolated from *Helicoverpa peltigera*) was prepared at different concentrations, and a dose trial was performed on second instar Heliothis larvae in *Türkiye*. As a result of the trials, LC_{50} values were calculated as 0.95×10^4 (*H. peltigera*), 1.9×10^4 (*H. armigera*), 8.6×10^4 (*H. viriplaca*) and 9.2×10^4 (*H. nubigera*), respectively (Eroglu et al. 2019). In another study, it is stated that the LC_{50} value of HearNPV India isolate tested against second instar *H. armigera* larvae is 2.3×10^4 OBs/ml (Kumar et al. 2011). The LC_{50} value of HearSNPV-D was determined as 0.7×10^5 OBs/ml for third instar *H. armigera* larvae. The reduction of lethality in late instars has been demonstrated in biotest studies on *H. armigera* larvae (Eroglu et al. 2018). It is stated that the LC_{50} value of HearNPV India isolate tested against third instar *H. armigera* larvae is 1.5×10^5 OBs/ml (Kumar et al. 2011). Thus, according to LC_{50} values, HearSNPV-D isolate was found to have a higher virulence than the Indian isolate. This is due to the isolation of viruses from different geographies and the difference in their genes that affect virulence (Williams et al. 2011).

These results showed that HearSNPV-D was extremely effective on the larvae of its host. These examples mentioned in the literature and the results we found in our study indicate that HearNPV strains isolated from different ecological conditions of the world have serious mortality effects on the *H. armigera* and are an important natural suppressor of this pest populations. In addition to the virulence studies of HearNPV, several studies have been conducted regarding its host range (Eroglu et al. 2019, Abid et al. 2022). In one of these studies, Abid et al. (2022) reported mortality and infectivity of H. armigera NPV isolated from Pakistan on second instar *H. armigera*, *Spodoptera litura*, *S. exigua*, *Pectinophora gossypiella* and *Trichoplusia ni* larvae. Daily mortality graphs are also shown in this study for deaths with not high mortality rates. In our study, the infectivity of the virus was tested against the larvae of *Hyphantria cunea*, *Malacosoma neustria*, *Lymantria dispar*, *Thaumetopea pityocampa* and *Spodoptera exigua*. Mortality has been observed in established biotests but is very low. A mortality rate of over fifty percent was observed in *S. exigua* larvae, suggesting that this virus may be effective in this insect. Therefore, the virus may be effective if tested at high

concentration on *S. exigua* larvae, or it may be effective on early instar *S. exigua* larvae.

CONCLUSION

Previously, *Helicoverpa armigera* multiple nucleopolyhedrovirus isolate from *H. armigera* and *H. armigera* single nucleopolyhedrovirus from *H. peltigera* have been isolated in *Türkiye*. Our isolate is the first single nucleopolyhedrovirus isolated from *H. armigera* from *Türkiye*. In conclusion the morphology and gene analysis of *Helicoverpa armigera* single nucleopolyhedrovirus-D Turkish isolate and the virulence tests showed that isolate is an NPV with high host specificity. However, it has been suggested that it may also have infectivity on *S. exigua* and further studies on this may be planned. Our newly found isolate can be used as a potential biological control agent against *H. armigera*, and biopesticides effective against *H. armigera* can be produced from this isolate in future studies.

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