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Isolation and characterization of novel Spodoptera exigua nucleopolyhedrovirus strains in Turkey and their potential for use in biological control

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Summary. – The beet armyworm (*Spodoptera exigua* Hübner) is a polyphagous pest that causes significant economic losses and has a large host range that contains 170 plant species from 35 families in many countries. Feeding with leaves and fruits, *S. exigua* larvae cause plant growth to slow down and decrease, and significant crop losses. While various cultural, chemical, and biological methods are used for pest control, their effectiveness is low and the pest is still harmful to agricultural areas. In this study, we isolated two novel Spodoptera exigua multiple nucleopolyhedrovirus (SeMNPV) strains from *S. exigua* larval cadavers collected from provinces of Igdir and Sanliurfa in Turkey, and named them SeMNPV-I and SeMNPV-U, respectively. Light microscopy confirmed that both cadavers died from baculovirus infection. Scanning electron microscope observations showed that the polyhedral occlusion bodies (OBs) of the SeMNPV-I and SeMNPV-U were irregularly shaped. Transmission electron microscopy revealed that OBs of SeMNPV-I and SeMNPV-U were occupied with several virions in which multiple nucleocapsids were packaged by a viral envelope. Sequences and phylogenetic analyzes of *polh*, *lef-8* and *lef-9* revealed that these strains are closely related to baculoviruses isolates from *Spodoptera* species. Dose-response experiments with the isolates at 10^5–10^9 OBs/ml concentrations against *S. exigua* larvae led to varied mortality between 47% to 100%. The results of this study revealed that two local SeMNPV strains both have the potential to be used to control *S. exigua*.

Keywords: Beet armyworm; Spodoptera exigua; nucleopolyhedrovirus; insecticidal effect; biological control

Introduction

The beet armyworm, *Spodoptera exigua* (Hübner, 1808) (*Lepidoptera: Noctuidae*), is a polyphagous pest that has an extremely wide host range containing thirty-five families, and occurs as a serious pest of vegetable, field, and flower crops such as welsh onion, maize, tobacco, cotton, and others (Smagghe *et al.,* 2003). This pest, originating from southeast Asia, is now spreading all over the world (CAB International 2000; Zheng *et al.,* 2011). It is also one of the most important agricultural pests that cause serious economic losses in Turkey (Sunulu *et al.,* 2020). Old caterpillars usually affect the growing parts of the plants. They create large holes in the leaves and sometimes can eat the leaf up to the midrib. Flowers and buds are also attacked by the pest. High-population attack of the insect has important consequences for plant growth and crop

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Abbreviations: LC_{50} = lethal concentration 50; lef-8 = late expression factor-8; lef-9 = late expression factor-9; NPV = nucleopolyhedrovirus; OBs = occlusion bodies; PCR = polymerase chain reaction; polh = polyhedrin; SeMNPV = Spodoptera exigua multiple nucleopolyhedrovirus

yield. If there is a significant caterpillar population, damage can spread to the stems and the fruit in worst cases.

The beet armyworm has some parasitoids and predators that control its population under natural conditions (Ruberson *et al.,* 1994). It is known that the pest also has important microbial natural enemies including fungal and bacterial agents (Eski *et al.*, 2018). Nucleopolyhedroviruses are considered to be one of the most important natural death factors of the pest. Despite all these facts, until recently, mostly chemical methods and factors have been used in the control of pests. Their intensive use brought along the problem of developing serious resistance to chemical insecticides used against *S. exigua* (Ahmad and Arif, 2010; Sayyed *et al.,* 2012). Biological insecticides such as *Bacillus thuringiensis*, *Beauveria bassiana* and nucleopolyhedroviruses (NPVs) have also been investigated in the control of this pest and it has been determined that they have different effects (Bianchi *et al.,* 2002; Thamthiankul *et al.,* 2004; Naimov *et al.,* 2014). Although there are some studies on the control of the pest, its damage to agricultural products and its effects continue. The presence and effectiveness of microbial enemies are quite advantageous in the fight against pests without the need for chemical insecticides. Insect viruses, and especially baculoviruses, are very common, extraordinary, and useful microbial agents with insect specificity. Turkey is one of the countries, where baculoviruses are found most intensely and are widely detected and studied, due to its climatic characteristics and insect diversity (Toprak *et al.,* 2005; Demir *et al.,* 2013, 2014; Bayramoglu *et al.,* 2018a,b; Gencer *et al.*, 2018a,b, 2019).

Baculoviruses are important biological agents in the control of many insect pests with high specificity and virulence, and some of these viruses are used as bioinsecticides (Hunter-Fujita *et al.,* 1998; Moscardi, 1999; Mahendra and Avinash, 2012). Baculoviruses belong to the family *Baculoviridae* and have a double-stranded circular DNA genome. *Baculoviridae* family is grouped into four genera as *Alphabaculovirus* (NPV infecting Lepidoptera), *Betabaculovirus* (GV infecting Lepidoptera), *Gammabaculovirus* (NPV infecting Hymenoptera) and *Deltabaculovirus* (NPV infecting Diptera) (Harrison *et al.,* 2018). They have an important role in the regulation of insect populations and are used as biological agents in the control of pests in agricultural products. (Szewczyk *et al.,* 2006; Sun and Peng, 2007).

Spodoptera exigua multiple nucleopolyhedrovirus (SeMNPV) is a virus species belonging to the *Alphabaculovirus* genus isolated from many different places in the world (Hara *et al.,* 1995; Hunter-Fujita *et al.,* 1998; Herniou *et al.,* 2011; Luna-Espino *et al.,* 2018). Numerous strains have been reported for this virus, isolated from different geographical regions (Kondo *et al.*, 1994; Muñoz *et al.*, 1999; Murillo *et al.,* 2006; Chen *et al.,* 2019). SeMNPV is very exciting due to its effective mortality and short kill time (Smits *et al.,* 1987; Ijkel *et al*., 1999). SeMNPV, which is an active ingredient in products such as SPOD-X®, SPEXIT®, and VIR-EX®, is used in the control of *S. exigua* both in greenhouse and field conditions (Bianchi *et al.,* 2002; Lasa *et al.,* 2007; Zamora-Avilés *et al.,* 2017).

Providing appropriate and effective microbial agents in biological control requires the identification of local isolates and strains from different geographical regions (Figueiredo *et al.,* 2009). In some NPV systems, the native virus tends to be more pathogenic to local populations than to foreign ones. In this study, we identified two SeMNPV isolates found in *S. exigua* larval cadavers that died in natural conditions, collected from two different regions in Turkey. We aimed to characterize both of these isolates on both morphological and molecular level and to investigate their biological activity on *S. exigua* larvae.

Materials and Methods

Virus detection, isolation, and propagation. Infected larvae of *S. exigua* (Hübner, 1808) (Lepidoptera: Noctuidae), were collected from corn fields in provinces of Igdır and Sanlıurfa, in Eastern Anatolian Region and Southeastern Anatolian Region in Turkey, respectively. The presence of viral infections was first detected by examining with phase-contrast and dark field microscopy (400X, Nikon Eclipse E600). Each of the dead larvae collected from two different regions was crushed in sterile water and filtered through two layers of cheesecloth. The filtrate containing the virus was spread on fresh lettuce leaves, which were sterilized with 5% (v/v) sodium hypochlorite (NaClO) and washed sterile distilled water. After the viral content on the surface of fresh lettuce leaves dried, they were placed in boxes containing individually starved *S. exigua* larvae to avoid cannibalism. After the infected leaves were consumed, the larvae were provided with fresh surface sterilized lettuce leaves and stored until virus symptoms were displayed. The purification of the occlusion bodies (OBs) of the virus was carried out according to the method described by Muñoz *et al.* (1997) with some modifications. Purification of strains was carried out as described by Ishii *et al.* (2003) and stock concentrations were calculated with using a hemocytometer. The isolates were designated as SeMNPV-I (Igdır) and SeMNPV-U (Sanlıurfa).

Electron microscopy. Viral suspensions were spread on a coverslip (12 × 12 mm) to observe the embedded structures of the virus under the scanning electron microscope (SEM). The samples were coated with gold using the sputter coater (Quorum Technology SC7620-CF) and examined under the SEM microscope EVO LS10 (Carl Zeiss, Brighton, Germany). The sizes of virus samples were measured using SmartSEM software (Carl Zeiss, Brighton, Germany). Pure virus OBs were

collected for transmission electron microscopy (TEM), washed with 0.1 molar (M) cacodylate buffer and precipitated. The pellet was fixed with Karnovsky's fixative (2% glutaraldehyde, 2% paraformaldehyde in a 0.05 M pH7.2 cacodylate buffer + 0.001 M calcium chloride) and osmium tetroxide (OsO_4), then embedded in resin. Ultrathin sections stained with uranyl acetate Reynold's lead citrate were examined under the electron microscope JEOL JEM 1220 (Gencer *et al.*, 2018).

Viral DNA extraction. For the extraction of viral DNA, OBs were removed by incubating the viral suspension with an equal volume of dissolving buffer (0.1 M sodium carbonate, 0.01 M ethylenediaminetetraacetic acid (EDTA), 0,17 M sodium chloride) at 37°C overnight (Reed *et al.*, 2003). Then, DNA isolation was proceeded using the kit (MasterPureTM DNA Purification Kit, Epicentre, an Illumina® Company) in accordance with the manufacturer's instructions. The concentrations of both viral DNA samples were determined by measuring with the nanodrop (Thermo Scientific, NanoDrop 2000). The density of viral DNA was determined using a spectrophotometer and the purity of the DNA was vindicated by measuring the 260/280 rate. A rate ≥ 1.8 is basically free of protein.

Phylogenetic analysis. To partially amplify *polh*, *lef-8* and *lef-9* genes, specific primer pairs were used for polymerase chain reaction (PCR). Primers sets for the *polh* (F: TGTAAAACGACG GCCAGTNRCNG ARGAYCCNTT; R: CAGGAAACAGCTATGACCDG GNGCRAAYTCYTT), *lef-8* (F: CAGGAAACAGCTATGACCCAYGGH GARATGAC; R: CAGGAAACAGCTATGAC CAYRTASGGRTCYTC SGC) and *lef-9* (F: TGTAAAACGACGGCCAGTTTGTCDC CRTCR CARTC and R: CAGGAAACAGCTATGACCAARAAYGGITAYGCBG) genes used in this study were preliminarily described by Jehle *et al.* (2006). After the PCR reactions were prepared, they were placed in a Bio-Rad thermocycler device and the annealing temperature for *polh*, *lef-8* and *lef-9* primers was set as 53, 48 and 49°C, respectively (Bayramoglu *et al.*, 2018b). PCR products were subjected to electrophoresis in agarose gel using TAE buffer (0.04 M Tris-acetate, 0.001 M EDTA). PCR products were sent to Macrogen company (Macrogen, The Netherlands) for sequence analysis and the sequences were deposited in GenBank. Additionally, a phylogenetic tree was built for SeMNPV-I and -U based on the concatenated amino acid sequences of these three genes using MEGA version 11 (Tamura *et al.*, 2007). Bootstrap analysis was used for testing the robustness of the phylogenetic tree. Phthorimaea operculella granulovirus (NC004062), Mythimna unipuncta granulovirus (NC033780), Cydia pomonella granulovirus (NC004062) and Neodiprion lecontei nucleopolyhedrovirus (NC005906) were used as out-groups in the phylogenetic analysis.

Biological activity experiments. Insects. Lab-reared cultures of *S. exigua* larvae were maintained on an 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). *S. exigua* culture was grown in the laboratory at 26 ± 1°C, 75% relative humidity and 16:8 (Light:Dark) hour (h) period (Mardani-Talaee *et al.*, 2016). *Dose-response experiments.* The median lethal concentration (LC_{50}) was calculated in third stage larvae of *S. exigua*. The pathogenicity of SeMNPV-I and -U strains was determined by the droplet-feeding process (Hughes and Wood, 1981). After the third instar larvae were starved for 6 hours, they were fed with the prepared mixture (sucrose, blue food coloring dye and virus isolates in different dilutions). Viral suspensions were administered to contain from 10^5 to 10^9 OBs/ml. The virus-free solution was used as a control. Twenty larvae from *S. exigua* were used individually. After the larvae were allowed to be fed with viral solutions for 2 h, they continued to be fed with their own food under laboratory conditions at 26 ± 1°C, 75% relative humidity and 16:8 (Light: Dark) h period. The experimental setup was checked daily, dead larvae were removed, and the experiment was continued until the larvae became pupae. Experiments were performed independently 3 times. The data of the bioassays were statistically analyzed using the SPSS program. Dose mortality rates were plotted using the Veusz 3.4 software.

Results

Microscopy

Phase-contrast and dark field microscopy of samples taken from cadavers from both regions revealed abundant polyhedral occlusion bodies (OBs) with bright crystalline structures in all examination areas (Fig. 1a,c,e,g). Observations in scanning electron microscopy showed that OBs had an irregular shape, like SeMNPVs (Fig. 1b,f). The diameter of the embedded structures of the strains were determined as 1.68 ± 0.54 and 1.61 ± 1.50 µm for SeMNPV-I and -U, respectively. Transmission electron images revealed that both strains contain multiple nucleocapsids in an envelope (Fig. 1d,h). For SeMNPV-I and -U isolates it was observed that the number of capsids in the embedded structure ranged from 1 to 4 and 1 to 6, respectively. It was observed that OBs of SeMNPV-I had 12 virions and that of SeMNPV-U had 17 virions. The capsid lengths of the strains were measured as 0.26 (0.21–0.34) mm for SeMNPV-I and 0.25 (0.16–0.30) mm for SeMNPV-U.

Phylogenetic analysis

Phylogenetic analyzes consisting of nucleotide sequences belonging to three gene regions (*polh*, *lef-8* and *lef-9*) were performed to indicate the taxonomic position of newly detected SeMNPV strains with other baculoviruses deposited GenBank. Our results indicated that the Turkish SeMNPV-I and -U strains separate in a clade with the Spain SeMNPV (HG425329) strain (Fig. 2). Accession numbers of the sequences SeMNPV-I and -U are: *polh* (OM417086

and OM417087), *lef*-8 (OM275431 and OM275432) and *lef*-9 (OM417084 and OM417085), respectively.

Biological activity trials

The biotest results showed that both strains had lethal effects on the *S. exigua* larvae. Increasing doses of both strains resulted in an increase in the mortality rates of the pest used in the study (Fig. 3). On the fourth day after application, some of *S. exigua* larvae inoculated with both strains showed typical symptoms of baculoviral infection and began to die. The mortality rates of the two SeMNPV strains on *S. exigua* larvae showed that SeMNPV-U was more effective (i.e. deadlier) than SeMNPV-I. The morta-

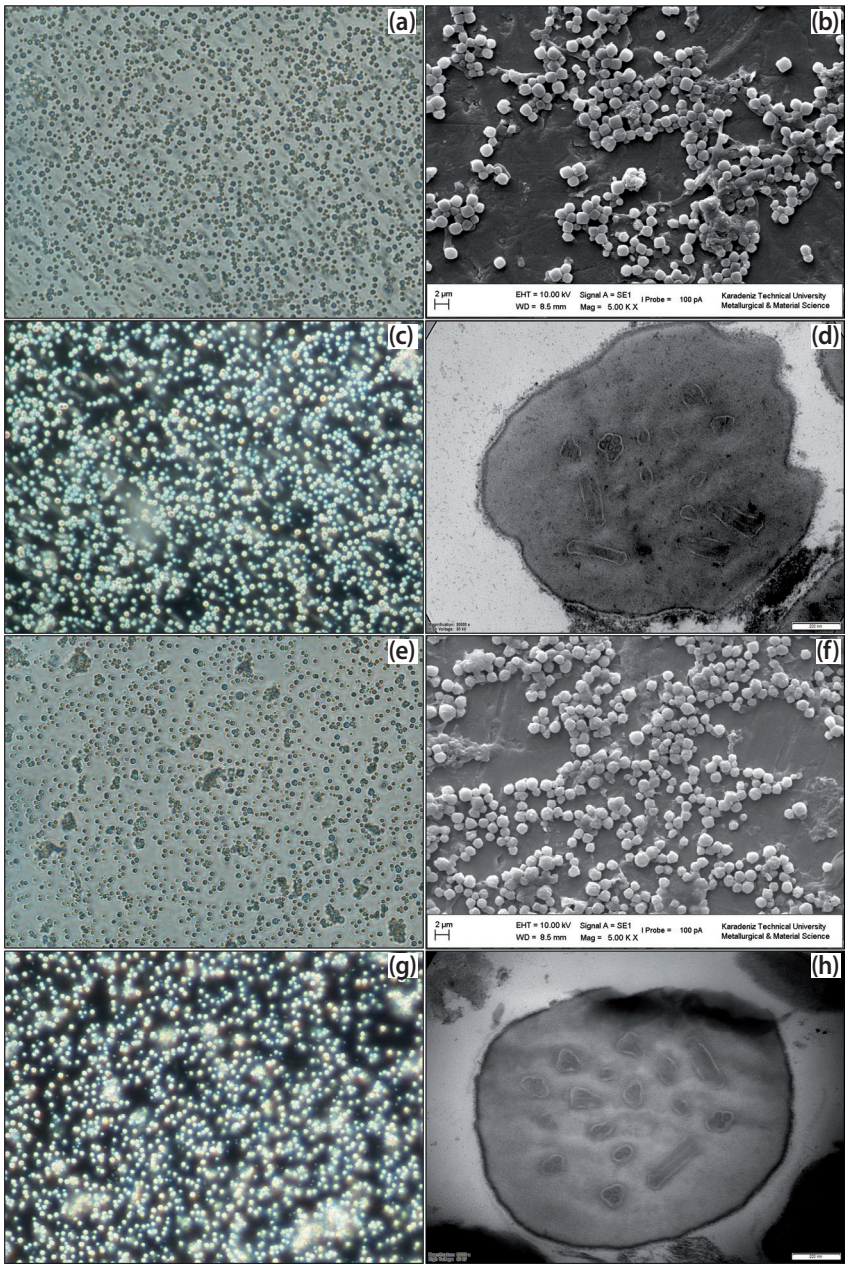

Fig. 1

Light and electron micrographs of new SeMNPV-I (a and d) and -U (e and h) strains from *S. exigua* larvae
The OBs are seen as bright crystal structures (40X). (**a)** and (**e)**: Phase-contrast microscopy; (**c)** and (**g)**: Dark-field microscopy. Electron micrographs of new SeMNPV-I (**b)** and (**d**) and -U (**f)** and (**h**) strains from *S. exigua* larvae. (**b)** and (**f)**: Scanning electron micrograph of OBs, (**d)** and (**h)**: Transmission electron micrograph of cross section of OBs.

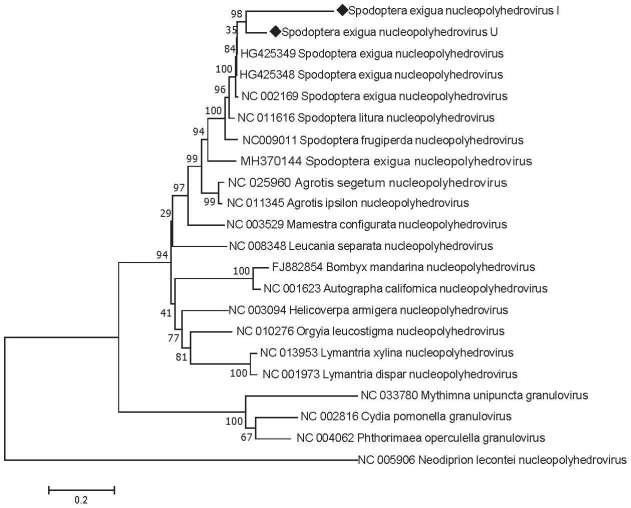

Fig. 2

A phylogenetic tree (neighbor joining) according to the amino acid sequences of concatenated partial *lef*-8, *lef*-9 and *polh* genes
Bootstrap scores were shown with numbers on branches. Black square indicates the location of the SeMNPV-I and -U.

lity of SeMNPV-U on *S. exigua* was recorded as 100% at the end of the tests at the infectious dose of 1×10^9 OBs/ml. At some concentrations, the SeMNPV-U was approximately 10% more effective than SeMNPV-I. It was observed that there was a decrease in adult emergence in individuals that turned into pupae at the end of the biotests.

The LC_{50} analyzes of the strains against *S. exigua* larvae are shown in Table 1. As a result of probit analysis, it was shown that the LC_{50} value of SeMNPV-I (1.8×10^6 OBs/ml) was higher than that of the SeMNPV-U (1.9×10^5 OBs/ml) isolate against *S. exigua* larvae.

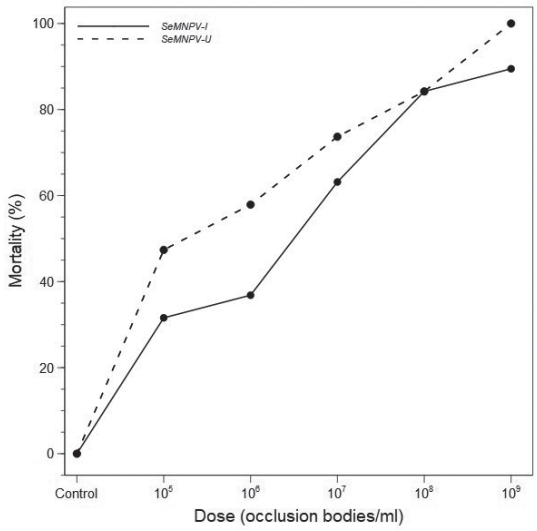

Fig. 3

Mortality of insect larvae resulting from SeMNPV-I and SeMNPV-U infections

Discussion

Today, the use of biological agents has become important due to the harmful effects of chemical insecticides on the environment. Among these agents, baculoviruses have an important position in the control of insects. Geographically distant 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). In the data of the present study, we reported the first isolation of two new SeMNPV strains from larval cadavers of *Spodoptera exigua* from two different regions in Turkey and their characterization according to their morphological and molecular properties. In addition, the pathogenicity of these strains was determined in *S. exigua* larvae.

Light microscopy results of samples from cadavers showing typical signs of baculovirus infection agree with previous isolation and characterization studies. The viral infection starts in the midgut cells in insects, then spreads to the whole body, and finally, both budded (BV) and embedded (OB) virus forms are formed. In baculovirus infection, after death external symptoms are: the outer skin ruptures easily, releasing the liquefied body content, and typical fragile hypodermis. As internal symptoms, the gut of larvae infected with nucleopolyhedroviruses becomes white, several tissues are infected such as fat body, tracheal matrix cells and hypodermal cells (Eberle *et al.*, 2012). Microscopy of samples taken from such cadavers reveals the presence of intense OBs in the study area of the microscope. Macroscopic observations and the presence of dense OBs in cadavers are the first evidence of a baculovirus infection.

Scanning electron microscopy observations showed that the OBs of SeMNPV-I and -U were irregularly shaped, proteinaceous occlusion bodies and about 1.68 and 1.61 µm in diameter, respectively. Transmission electron micrograph of OBs of new SeMNPV strains showed that virions were rod-shaped and randomly embedded in the polyhedron, and contained 1-6 nucleocapsids for SeMN-

Table 1. Median lethal concentrations (LC_{50}) of SeMNPV-I and SeMNPV-U on *S. exigua* larvae

Isolates	LC_{50} (OBs/ml)	Slope ± SE	df	X^2
SeMNPV-I	1.8×10^6 (0.2–11.3)	0.480 ± 0.407	3	0.712
SeMNPV-U	1.9×10^5 (0.1–22.2)	0.364 ± 0.542	3	0.933

PV-I and 2-4 nucleocapsids for SeMNPV-U. Baculoviruses are characterized according to inclusion bodies and the number of virions. OBs of the nucleopolyhedrovirus are polyhedral with a diameter of 0.6–2.0 µm. The length of the baculovirus virions is 230-385 nm. Previous studies on nucleopolyhedrovirus of the beet armyworm revealed that the OBs of SeMNPVs were 0.8–2.05 µm in diameter (Gelernter and Federici, 1986; Khattab, 2013; Chen *et al*., 2019). Steinhaus (1949) reported that a SeMNPV strain from California, USA, had virion size of 270 nm. According to the morphology and size of the OBs and the virions, the new SeMNPV strains are compatible with nucleopolyhedroviruses and especially SeMNPV strains in the literature.

Sequence analyzes of *polh*, *lef-8* and *lef-9* partial sequences in blast showed that the strains were similar to Spodoptea exigua nucleopolyhedrovirus isolates in GenBank database. In a previous congressional statement, SeMNPV-TR1 isolate was recorded from Turkey but isolated from a different host - *Helicoverpa armigera* (Gurkan *et al*., 2019). In this study, three gene sequences of the virus detected from *H. armigera* showed similarity to SeMNPV. In our study, two SeMNPV isolated from the host *S. exigua* were for the first time recorded in Turkey.

Baculoviruses have 38 core genes that play very important roles in their replication cycle (Javed *et al*., 2017). Phylogenetic analyzes performed by combining these genes reveal the genetic similarity of baculovirus isolates and strains. A phylogenetic analysis using 38 core genes of a Chinese strain of SeMNPV (SeMNPV-QD) determined that the new isolate was included in Group II and SeMNPV-QD clustered together with SeMNPV-US1, SpliNPV-II (*S. litura*), SfMNPV (*S. frugiperda*), Agrotis segetum nucleopolyhedrovirus (AgseNPV), Agrotis segetum nucleopolyhedrovirus B (AgseNPV-B), Agrotis ipsilon nucleopolyhedrovirus (AgipNPV) (Chen *et al*., 2019). A similar phylogenetic analysis of a nucleopolyhedroviruses from Turkey showed that the *Spodoptera* nucleopolyhedroviruses (SpliNPV-II, SeMNPV and SfMNPV) used had very close homology (Gencer *et al*., 2018a; 2018b). Analyzes based on partial sequences of *polh*, *lef*-8 and *lef*-9 genes in nucleopolyhedroviruses are also widely used in phylogenetic analyzes of baculovirus strains and isolates. Demir *et al*. (2014) showed that SeMNPV clustered most closely with Helicoverpa armigera nucleopolyhedrovirus (HearNPV) and Orgyia pseudotsugata nucleopolyhedrovirus (OpSNPV) for the *polh* gene and with Mamestra configurata nucleopolyhedrovirus 1 (MacoNPV-1), Mamestra configurata nucleopolyhedrovirus 2 (MacoNPV-2) and Mamestra brassicae multiple nucleopolyhedrovirus (MbMNPV) for the *lef*-8 gene. These examples show that strains in baculoviruses and isolates from closely related species have the closest homology in phylogenetic analyzes and cluster together. In the co-phylogenetic analysis of the *polh*, *lef*-8 and *lef*-9 genes, we found that the new isolates were together in the same branch, clustered most closely with other *Spodoptera* strains, and showed homology with *Agrotis* isolates in the tree a little further from them according to the degree of phylogenetic relationship.

The biological activity of SeMNPV-I and -U isolates were performed against their host. These viruses often show the highest mortality effect on their host, and lower effects on insect species related to their host. In a previous study, Demir *et al*. (2014) showed that ManeNPV from *Malacosoma franconicum* has the highest mortality against its host and *M. neustria*, which ManeNPV was first detected. In another study, Gelernter and Federici (1986) reported that SeMNPV-608 from California, USA, was extremely effective on the larvae of its host. A study conducted by Chen *et al*. (2019) revealed that another SeMNPV strain also had a 100% death effect on its host. These examples mentioned in the literature and the results we found in our study indicate that SeMNPV strains isolated from different ecological conditions of the world have serious mortality effects on the beet armyworm and are an important natural suppressor of this pest population. In addition to the virulence studies of SeMNPV, several studies have been conducted regarding its host range. In one of these studies, Vlak *et al*. (1981) reported that a SeMNPV strain isolated from a greenhouse in Netherlands caused infection in *S. exigua* larvae, but not in the congeneric species *S. littoralis*, another member of the Noctuidae family. The same study revealed that cross-infection of SeMNPV to other noctuids (such as *Mamestra brassicae* and *M. oleracea*), or members of other families, such as *Ostrinia nubilalis* (Crambidae), *Gallaria mellonella* (Pyralidae), *Plutella maculipennis* (Plutellidae), *Adoxophyes orana* (Tortricidae) and *Laspeyresia pomonella* (Tortricidae), did not occur. Another previous study showed *S. exigua* was the only species infected by SeMNPV-608 among noctuids such as *S. exigua*, *Heliothis virescens*, *Trichoplusia ni* and *S. frugiperda* (Gelernter and Federici, 1986). In a study testing strain of SeMNPV (#1, #2 and #5) isolated from Japan against *S. exigua*, *S. litura*, *Plutella xylostella* and *Bombyx mori* larvae showed that while SeMNPV #1 infects only *S. exigua* larvae, SeMNPV #2 and #5 infect *S. exigua*, *S. litura*, *Plutella xylostella* (Plutellidae) larvae, but no strains were infective against *B. mori* (Kondo *et al*., 1994). In another study with similar results, Chen *et al*. (2019) revealed that the SeMNPV-QD strain, which infects *S. exigua* larvae, did not infect other lepidopteran insects, although some are members of the same family such as *S. litura*, *Agrotis ipsilon*, *A. segetum* (Noctuidae), or closely related species (*Hyphantria cunea*, Artiidae; *Stilpnotia salicis*, Lymantriidae; *Bombyx mori*, Bombycidae).

All studies, including ours, show that SeMNPV is the most important natural microbial enemy of *S. exigua* larval populations, although some strains have varying degrees of virulence on the pest. In order to determine the usability of SeMNPV-I and -U strains in biological control applications, their field applications must be performed and the resistance of the strains to abiotic factors must be determined. According to the success of these studies, the viral biopesticide formulation of the strains should be prepared, its ecotoxicological tests should be performed and the formulation should be licensed.

In conclusion, morphological and molecular identification of Spodoptera exigua nucleopolyhedrovirus Turkish strains was performed. It was subsequently determined that these two isolates had high activity against their host. Phylogenetic analysis revealed that SeMNPV-I and -U belong to Group II in the family *Baculoviridae*. Our newly found isolates can be used as a good biological control agent against *S. exigua*. In future studies, effective biopesticides can be produced from these isolates against *S. exigua*. All results provide a theoretical basis for the future application of new strains.

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