Original article (Orijinal araştırma)

Insecticidal activities of wild type and recombinant invertebrate iridescent viruses on five common pests

Yaban tip ve rekombinant omurgasız iridesan virüslerinin beş yaygın zararlı üzerindeki böcek öldürücü aktiviteleri

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Abstract

Invertebrate iridescent virus 6 (IIV6) can infect a broad range of pest insect species. Viruses with new features created by recombinant DNA technology can be used effectively as biological control agents. Previously, recombinants have been constructed: IIVs harboring *green fluorescent protein* gene (*gfp*) in place of IIV6 157L ORF (rCIV-Δ157L-gfp) and a scorpion *Androctonus australis* (Linnaeus, 1758) insect toxin gene (*AaIT*) fused with *gfp* (rCIV-Δ157L/gfp-AaIT). In this study, wild type IIV6 and the two recombinants, were evaluated for their ability to cause infections on *Helicoverpa armigera* (Hübner, 1805) (Lepidoptera: Noctuidae), *Spodoptera littoralis* (Boisduval, 1883) (Lepidoptera: Noctuidae), *Lymantria dispar* (Linnaeus, 1758) (Lepidoptera: Erebidae), *Euproctis chrysorrhoea* (Linnaeus, 1758) (Lepidoptera: Erebidae) and *Tenebrio molitor* (Linnaeus, 1758) (Coleoptera: Tenebrionidae) larvae. This study was performed at Karadeniz Technical University, Department of Biology during 2018 and 2019. Five different concentrations (10^3, 10^4, 10^5, 10^6 and 10^7 $TCID_{50}$/ml) of viruses were used to inject each insect larvae. All larvae, infected with rCIV-Δ157L/gfp-AaIT, became paralyzed, except *S. littoralis*. The LC_{50} of insect larvae infected by rCIV-Δ157L/gfp-AaIT were determined as 0.3 x 10^7, 0.7 x 10^5, 0.2 x 10^5, 0.15 x 10^5, 0.7 x 10^4 $TCID_{50}$/ml on *S. littoralis*, *T. molitor*, *L. dispar*, *H. armigera* and *E. chrysorrhoea*, respectively. LT_{50} values, calculated according to the highest virus concentrations, were found as 10.5, 6.2, 4.7, 7.5 and 5 d on *S. littoralis*, *T. molitor*, *L. dispar*, *H. armigera* and *E. chrysorrhoea*, respectively, for rCIV-Δ157L/gfp-AaIT. This study showed that recombinant IIV6 has increased pathogenicity on some insects from Lepidoptera and Coleoptera.

Keywords: Invertebrate iridescent virus, insecticidal activity, recombinant virus

Öz

Omurgasız iridesan virüs 6 (IIV6), çeşitli zararlı böcek türlerini düşük oranda enfekte edebilir. Rekombinant DNA teknolojisi ile oluşturulan yeni özelliklere sahip virüsler biyolojik kontrol ajanları olarak etkin bir şekilde kullanılabilir. Daha önce IIV6 157L ORF'si yerine birisi yeşil floresan protein geni (rCIV-Δ157L-gfp) ve diğeri de gfp ile birleştirilmiş bir akrep *Androctonus australis* (Linnaeus, 1758) böcek toksin geni (rCIV-Δ157L/gfp-AaIT) ihtiva eden iki rekombinant IIV6 inşa edilmişti. Bu çalışmada, yaban tip IIV6 ve iki rekombinant virüs, *Helicoverpa armigera* (Hübner, 1805) (Lepidoptera: Noctuidae), *Spodoptera littoralis* (Boisduval, 1883) (Lepidoptera: Noctuidae), *Lymantria dispar* (Linnaeus, 1758) (Lepidoptera: Erebidae), *Euproctis chrysorrhoea* (Linnaeus, 1758) (Lepidoptera: Erebidae) ve *Tenebrio molitor* (Linnaeus, 1758) (Coleoptera: Tenebrionidae) larvalarında enfeksiyon oluşturma yeteneği açısından değerlendirildi. Çalışma 2018 ve 2019 yılları arasında Karadeniz Teknik Üniversitesi Biyoloji Bölümü'nde gerçekleştirildi. Her bir böcek larvasını enjekte etmek için virüslerin beş farklı konsantrasyonu (10^3, 10^4, 10^5, 10^6 ve 10^7 $TCID_{50}$/ml) kullanıldı. rCIV-Δ157L/gfp-AaIT ile enfekte olmuş *S. littoralis* dışında tüm larvalar felç oldu. rCIV-Δ157L/gfp-AaIT ile enfekte olmuş böcek larvalarının LC_{50}'si sırasıyla, *S. littoralis*, *T. molitor*, *L. dispar*, *H. armigera* ve *E. chrysorrhoea* üzerinde 0.3 x 10^7, 0.7 x 10^5, 0.2 x 10^5, 0.15 x 10^5, 0.7 x 10^4 $TCID_{50}$/ml olarak belirlenmiştir. En yüksek virüs konsantrasyonlarına göre hesaplanan LT_{50} değerleri, rCIV-Δ157L/gfp-AaIT için *S. littoralis*, *T. molitor*, *L. dispar*, *H. armigera* ve *E. chrysorrhoea*'da sırasıyla 10.5, 6.2, 4.7, 7.5 ve 5 gün olarak bulundu. Bu çalışma, rekombinant IIV6'nın Lepidoptera ve Coleoptera takımlarına ait bazı böceklerde patojeniteyi artırdığını göstermiştir.

Anahtar sözcükler: Omurgasız iridesan virüs, insektisidal aktivite, rekombinant virüs

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Introduction

A large portion of the economy is derived from agricultural products in many developing countries. Lepidoptera and Coleoptera larvae constitute the majority of pest insects damaging a wide variety of food, fiber, oilseed, forage and horticultural plants. Due to the emergence of insect populations resistant to currently used products, the demand for new insecticides is increasing.

The Iridoviridae family comprises large, icosahedral, double-stranded DNA viruses that infect a wide range of both vertebrate and invertebrate hosts. The family includes two subfamilies: Alphairidovirinae and Betairidovirinae. Alphairidovirinae comprises *Megalocytivirus*, *Ranavirus* and *Lymphocystivirus* genera whose members infect vertebrates. Betairidovirinae comprises *Chloriridovirus* and *Iridovirus* genera whose members infect invertebrates (Chinchar et al., 2017). The members of the *Iridovirus* genus had been described in insects and crustaceans. The type species of the *Iridovirus* genus is invertebrate iridescent virus 6 (IIV6) which can infect numerous insect species with agricultural importance (Henderson et al., 2001; Jakob et al., 2002; Jenkins et al., 2011). IIV6, as well as the other invertebrate iridescent viruses (IIVs), can produce either covert or patent infections. Mild infections are not lethal and not obvious to the naked eye but may cause extended development time, reduced fecundity and longevity (Marina et al., 2003). Patent infections are almost invariably lethal. The color of patently infected insects turns into an obvious iridescent color that typically ranges from violet, blue, green or orange. However, IIVs often cause a low prevalence of patent infections in nature.

IIV6 has very low *per os* infectivity but is highly infectious by injection. The range of insect species naturally infected by IIV6 includes a few members from Lepidoptera, and Orthoptera orders (Williams, 2008). However, the range of insect species that can be infected with IIV6 by injection is extensive (Mitsuhashi, 1967; Fukuda, 1971; Jensen et al., 1972; Ohba, 1975; Henderson et al., 2001). Virus transmission in nature may be through cannibalism, endoparasitic wasps or parasitic nematodes, which all do not occur commonly for all insects. Low infectivity and difficulties in transmitting the virus to susceptible hosts in the nature limit the usage of IIV6 as a biological control agent (Williams et al., 2005). Currently there are very few data available about the usage of iridoviruses in biocontrol studies. In a study performed by Hunter et al. (2003) the root weevil *Diaprepes abbreviates* (Linnaeus, 1758) (Coleoptera: Curculionidae) was infected with IIV6, in order to evaluate its potential in biocontrol studies. IIV6 infection in *D. abbreviates* caused both patent and sublethal infections in both larvae and adults. In another study, IIV6 infected *Phyllophaga vandinei* (Smyth, 1916) (Coleoptera: Scarabaeidae), scarab beetle, larvae or adults showed 30% mortality. Moreover, covert IIV6 infection changed feeding and mating behavior in *P. vandinei* adults (Jenkins et al., 2011).

The infectivity of the viruses that cause covert infections can be enhanced through bioengineering. A recombinant IIV6 (rCIV-Δ157L/gfp-AaIT) harboring a scorpion *Androctonus australis* (Linnaeus, 1758) (Scorpiones: Buthidae) insect toxin (*AaIT*) gene was previously constructed (Ozgen et al., 2014; Nalcacioglu et al., 2016). The gene was fused with the *green fluorescent protein* gene (*gfp*) and replaced with the IIV6 *157L* open reading frame (ORF) locus. Generated recombinant IIV6 produced enhanced insecticidal activity against *Galleria mellonella* (Linnaeus, 1758) (Lepidoptera: Pyralidae). In this study, wild type IIV6 (wt-CIV) and its two recombinants (rCIV-Δ157L-gfp and rCIV-Δ157L/gfp-AaIT) were tested on *Helicoverpa armigera* (Hübner, 1805) (Lepidoptera: Noctuidae), *Spodoptera littoralis* (Boisduval, 1883) (Lepidoptera: Noctuidae), *Lymantria dispar* (Linnaeus, 1758) (Lepidoptera: Erebidae), *Euproctis chrysorrhoea* (Linnaeus, 1758) (Lepidoptera: Erebidae) and *Tenebrio molitor* (Linnaeus, 1758) (Coleoptera: Tenebrionidae) larvae by injection in the laboratory to determine concentration-mortality responses for the different hosts.

Materials and Methods

Cell line, viruses and insects

Spodoptera frugiperda 9 (Sf9) cells (obtained from Laboratory of Virology, Wageningen University, The Netherlands) were maintained in Sf-900 II SFM (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 5% fetal bovine serum (FBS, Sigma-Aldrich, St. Louis, MO, USA) at 28°C as monolayer in flasks.

Wild type IIV6 (wt-CIV) and its two recombinants (rCIV-Δ157L-gfp and rCIV-Δ157L/gfp-AaIT) previously constructed by Ozgen et al. (2014) and Nalcacioglu et al. (2016) were provided from Karadeniz Technical University, Microbiology Laboratory. Virus titers were determined by using the 50% tissue culture infectious dose ($TCID_{50}$) (Hierholzer & Killington, 1996).

Lab-reared cultures of *H. armigera, S. littoralis, T. molitor* and field collected *L. dispar* and *E. chrysorrhoea* larvae were used in bioassays. *Helicoverpa armigera* and *S. littoralis* larvae 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 & Boppré, 1986). *Tenebrio molitor* larvae were maintained on artificial medium (400 g wheat bran, 100 g wheat flour, 100 g corn flour, small pieces of carrot) (Kim et al., 2017). *Lymantria dispar* and *E. chrysorrhoea* larvae were fed with oak leaves which are collected from the nature. The leaves were firstly cleaned with 0.05% sodium hypochlorite solution for 2 min, then rinsed three times in distilled water and dried on filter papers (Gencer et al., 2018). All insect larvae were incubated at 28°C, 60-70% RH and 16:8 h L:D photoperiod.

Bioassays

For each insect species and each virus concentration, bioassays were performed by using 30 larvae in triplicate. wt-CIV, rCIV-Δ157L-gfp and rCIV-Δ157L/gfp-AaIT stock concentrations were calculated as 1.27×10^8, 1.70×10^8 and 3.85×10^8 $TCID_{50}$/ml, respectively. Five different dilutions (1×10^7, 1×10^6, 1×10^5, 1×10^4 and 1×10^3 $TCID_{50}$/ml) were prepared from each virus. All insect larvae were kept on ice until their movements slowed. Then, 5 µl of each dilution were injected to insect larvae with a plastic syringe and a 0.30 mm x 8 mm gauge needle. Injections for lepidopteran larvae were made from the right side of the abdomen, about a quarter above the anus. Coleopteran larvae were injected between the epidermis and cuticle of an abdominal sternite. The control group of insects were injected with only water. The larvae were individually kept in 40 ml plastic boxes with 16:8 light/dark periods at 28°C and 60% RH. Starting from the first day following injections, mortality, physical and behavioral changes of the larvae were observed daily until 21 d post infection (Kalha et al., 2014).

Mortality data were corrected using Abbott's formula (Abbott, 1925), LC_{50} and LT_{50} values were calculated by probit analysis using MS Excel (Finney, 1952).

Confirmation of dead larvae

To prove that insect death was a result of iridovirus infection, total DNA isolation was performed from dead larvae by using a DNA isolation kit (DNeasy Blood & Tissue Kit, Qiagen, Hilden, Germany) according to the manufacturer's instructions. The presence of iridoviruses was confirmed by polymerase chain reaction (PCR) using primers for *mcp* (*major capsid protein*) gene of IIV6 (forward primer: 5'-ATGTCTATTTCTTCGTCAAATGTAAC-3' and reverse primer: 5'-TTGGAAATTAATTCTCATTTCATTGTAT-3'). PCR amplification was performed in 0.2-ml PCR tubes, with 25 µl volumes containing 10-60 ng of DNA, 0.2 mM of each primer and each dNTP, 0.5 unit of Taq DNA polymerase and 2.5 µl of 10X reaction buffer (Promega) and 1.5 mM of $MgCl_2$. PCR conditions were adjusted as follows: one cycle for 3 min at 95°C was followed by 35 cycles of 30 s at 95°C, 30 s at 50°C and 60 s at 72°C. The amplification was completed with a final extension step of 10 min at 72°C. PCR products were visualized on 0.1% agarose gel with ethidium bromide.

Results

The mortalities of all insects infected with wt-CIV, rCIV-Δ157L-gfp and rCIV-Δ157L/gfp-AaIT viruses are shown in Figure 4. The highest mortalities of rCIV-Δ157L/gfp-AaIT were 100% and 98% of *E. chrysorrhoea* and *L. dispar*, respectively, for 10^7 TCID$_{50}$/ml concentration (Figure 4). *Euproctis chrysorrhoea* and *L. dispar* larvae had the highest mortalities as a result of virus injections, but they did not produce any iridescent color. A few *H. armigera* larvae infected with wt-CIV developed iridescent color that can be observed at patent iridovirus infections (Figure 1). The mortality of *H. armigera* was over 80% with rCIV-Δ157L/gfp-AaIT infections except for 10^3 and 10^4 TCID$_{50}$/ml concentrations (Figure 4). Even though the mortality was similar between wt-CIV and rCIV-Δ157L-gfp infections, the mortality of recombinant virus (rCIV-Δ157L/gfp-AaIT) infections were higher than the other viruses tested.

Figure 1. Patent infection of invertebrate iridescent virus 6 (IIV6) on *Helicoverpa armigera* larvae: a) uninfected larvae; b) infected larvae.

Spodoptera littoralis larvae were the least affected of the species used in this study. However, those infected larvae were not able to emerge properly from the pupae. The emerged *S. littoralis* adults, infected previously with wt-CIV, all had physical defects and were not able to fly (Figure 2). Malformed pupae appeared from the larvae infected with rCIV-Δ157L/gfp-AaIT and could not develop to adults properly. Mortalities reached a maximum of 60% with the recombinant iridoviruses infections on *S. littoralis* larvae (Figure 4). On the other hand, the mortality of *S. littoralis* larvae infected with wt-CIV remained below 40%.
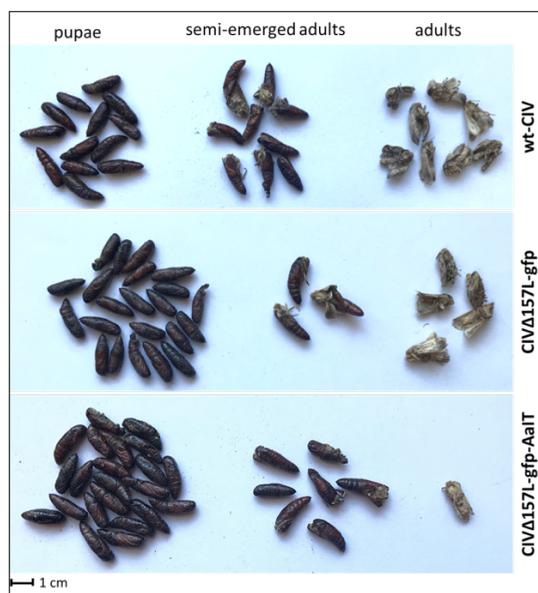

Figure 2. Defects in *Spodoptera littoralis* pupae and adults as a result of iridovirus infection.

Viruses expressing toxin genes caused paralysis of all larvae except *S. littoralis* at different levels. However, in *T. molitor* larvae, paralysis was clearly seen 3 d after virus infections with 10^6 and 10^7 TCID$_{50}$/ml concentrations. Paralyzed larvae could not move their bodies, except their legs. Additionally, these insects died a few days after they became paralyzed. The color of the dead *T. molitor* larvae infected with each type of iridoviruses used in this study appeared to be darkened (Figure 3).
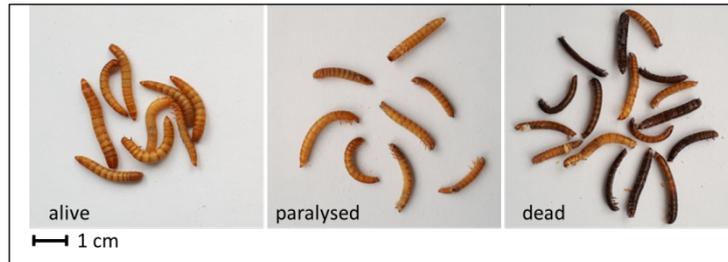

Figure 3. Effects of recombinant iridovirus expressing toxin gene on *Tenebrio molitor* larvae.

The highest concentrations of wt-CIV, rCIV-Δ157L-gfp and rCIV-Δ157L/gfp-AaIT caused 57, 76 and 83% mortality of *T. molitor* larvae, respectively (Figure 4).
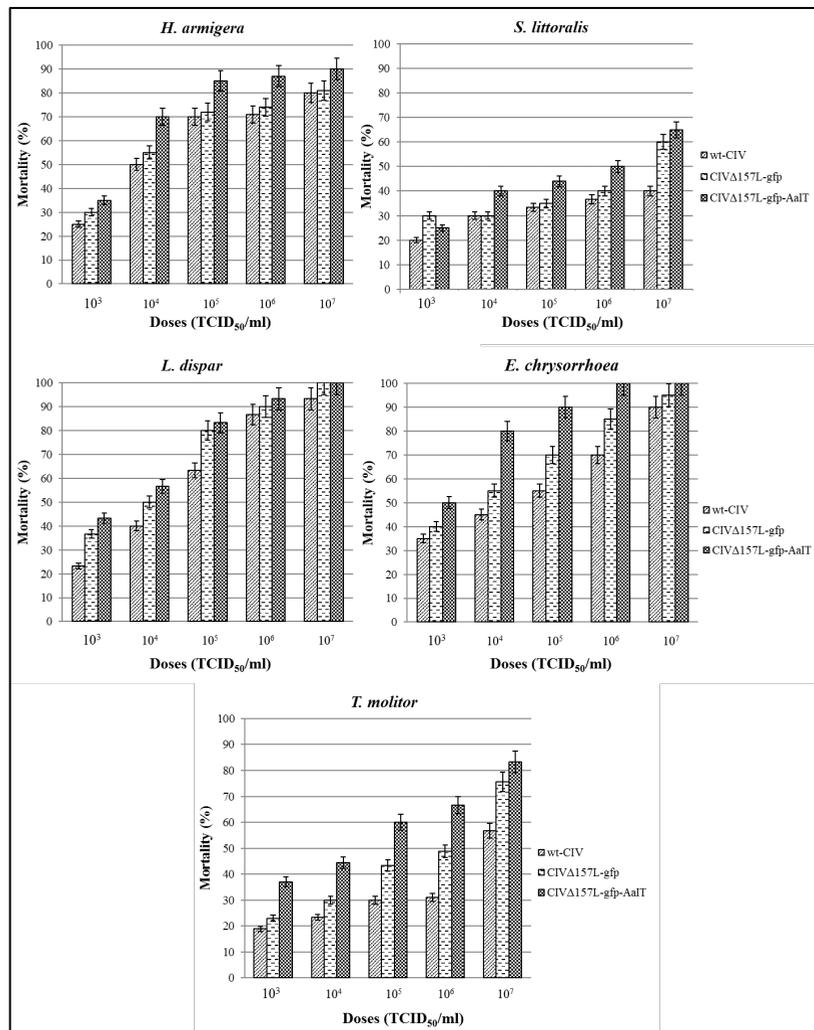

Figure 4. Mortality of insect larvae resulting from iridovirus infections. Bars show standard errors.

Insect deaths as a result of virus infections were confirmed by PCR (Figure 5). Control groups of each insect successfully completed metamorphosis process during the bioassay period.
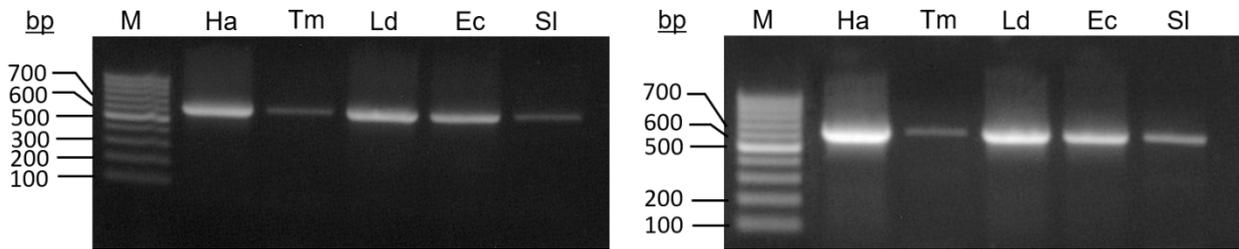

Figure 5. Verification that insect larvae have died due to virus infection. PCR fragments (630 bp) amplified from DNA isolated from wt-CIV (a) and rCIV-Δ157L/gfp-AaIT (b) infected insect larvae with *mcp*-specific primers. Ha, *Helicoverpa armigera;* Tm, *Tenebrio molitor;* Ld, *Lymantria dispar;* Ec, *Euproctis chrysorrhoea;* Sl, *Spodoptera littoralis*; and M, marker (100 bp).

The LC_{50} values for wt-CIV infections were calculated as 0.1×10^6, 0.2×10^6, 0.2×10^6, 0.9×10^7 and 3.3×10^9 $TCID_{50}$/ml on *E. chrysorrhoea*, *H. armigera*, *L. dispar*, *T. molitor* and *S. littoralis* larvae, respectively. The three lowest LC_{50} values of rCIV-Δ157L-gfp were determined as 0.4×10^5, 0.5×10^5 and 0.9×10^5 $TCID_{50}$/ml on *E. chrysorrhoea*, *L. dispar* and *H. armigera,* respectively. The LC_{50} values of the rCIV-Δ157L/gfp-AaIT virus were lower than that of the other two viruses. The best LC_{50} values were demonstrated on *E. chrysorrhoea* and *H. armigera* larvae as follows; 0.7×10^4 and 0.1×10^5 $TCID_{50}$/ml respectively. LT_{50} values were calculated for the highest virus concentrations. The shortest days, necessary for the death of the 50% of the larvae, were obtained with of rCIV-Δ157L-gfp. All LC_{50} and LT_{50} values are shown in Table 1 for each insect and virus.

Table 1. Lethal concentrations (LC_{50}) and lethal times (LT_{50}) with wt-CIV, rCIVΔ157L-gfp and rCIVΔ157L-gfp-AaIT virus infections of five common pests. LT_{50} values were calculated for 10^7 $TCID_{50}$/ml virus concentrations

	Viruses	LC_{50} ($TCID_{50}$/ml)	Slope±SE	X^2	LT_{50}(day) (95% CL)
Helicoverpa armigera	wt-CIV	0.2×10^6 (0.01-2.1)	0.359±0.522	0.574	0.2×10^6 (0.01-2.1)
	rCIVΔ157L-gfp	0.9×10^5 (0.07-12)	0.332±0.565	0.666	0.9×10^5 (0.07-12)
	rCIVΔ157L-gfp-AaIT	0.15×10^5 (0.01-1.53)	0.394±0.514	0.527	0.15×10^5 (0.01-1.53)
Spodoptera littoralis	wt-CIV	3.36×10^9 (0.07-1605)	0.136±1.367	0.896	3.36×10^9 (0.07-1605)
	rCIVΔ157L-gfp	3.7×10^7 (0.04-336.6)	0.183±0.996	0.648	3.7×10^7 (0.04-336.6)
	rCIVΔ157L-gfp-AaIT	0.3×10^7 (0.01-12)	0.237±0.765	0.883	0.3×10^7 (0.01-12)
Lymantria dispar	wt-CIV	0.2×10^6 (0.4-1)	0.582±0.348	0.980	0.2×10^6 (0.4-1)
	rCIVΔ157L-gfp	0.5×10^5 (0.9-2.7)	0.571±0.369	0.787	0.5×10^5 (0.9-2.7)
	rCIVΔ157L-gfp-AaIT	0.2×10^5 (0.5-1.5)	0.581±0.377	0.820	0.2×10^5 (0.5-1.5)
Euproctis chrysorrhoea	wt-CIV	0.1×10^6 (0.2-1.6)	0.398±0.476	0.839	0.1×10^6 (0.2-1.6)
	rCIVΔ157L-gfp	0.4×10^5 (0.7-3.4)	0.471±0.428	0.982	0.4×10^5 (0.7-3.4)
	rCIVΔ157L-gfp-AaIT	0.7×10^4 (0.1-4.4)	0.641± 0.384	0.734	0.7×10^4 (0.1-4.4)
Tenebrio molitor	wt-CIV	0.9×10^7 (0.07-11.4)	0.335±0.559	0.335	0.9×10^7 (0.07-11.4)
	rCIVΔ157L-gfp	0.8×10^6 (0.1-4.97)	0.484±0.397	0.475	0.8×10^6 (0.1-4.97)
	rCIVΔ157L-gfp-AaIT	0.7×10^5 (0.08-7.7)	0.380±0.504	0.472	0.7×10^5 (0.08-7.7)

Discussion

Invertebrate iridoviruses (IIVs) infect agriculturally and medically important insect species. However, these viruses have attracted little interest due to the often-low prevalence of lethal infections in host populations (Williams, 2008).

Insect viruses that do not produce high mortality on the target insect pest are often thought of as ineffective biocontrol agents. Enhancement of virus infectivity, through genetic engineering, is a method to enhance their effectiveness as biocontrol agents. A recombinant iridovirus harboring an insect specific neurotoxin has been constructed previously. This recombinant iridovirus showed increased speed of kill in *G. mellonella* larvae (Nalcacioglu et al., 2016). In this study, we examined the infectivity of wild type and recombinant iridoviruses (rCIV-Δ157L-gfp and rCIV-Δ157L/gfp-AaIT) on five different species of insect larvae. IIV6 infection was done by injection because IIVs are poorly infectious when applied orally but are highly infectious by injection (Williams et al., 2005).

In a study performed by Jakob et al. (2002) peroral infection of IIV6 was investigated on a few Lepidoptera larvae including *G. mellonella*, *Spodoptera exigua* (Hubner, 1808) (Lepidoptera: Noctuidae), *S. littoralis, H. armigera* and *Agrotis segetum* (Denis & Schiffermüller, 1775) (Lepidoptera: Noctuidae). Their results showed that only *G. mellonella* became orally infected with the virus. In the same study, *L. dispar* larvae was tested for peroral infection of cricket iridovirus (CrIV) which was considered to be a variant and/or a novel strain of IIV6. However, no CrIV infection was observed in *L. dispar* larvae. Invertebrate iridescent virus 29 infection was reported in a laboratory colony of *T. molitor* larvae previously (Kelly et al., 1979). *E. chrysorrhoea* and *L. dispar* have been tested first time for IIV6 infection in the present study. These insects are remarkably sensitive to IIV6 according to the current biotest results. Bioassay results on *H. armigera* larvae showed that IIV6 was highly effective on this insect. Iridescent color changes were clearly detected in heavily infected *H. armigera* larvae.

The lowest mortality was recorded with *S. littoralis* larvae in this study. Mortality results were not statistically significant, but infected *S. littoralis* larvae entered the pupal stage in a shorter time than uninfected ones. This result is opposite for the other infected insect larvae that entered to the pupal stage later than those that were not infected. The pupae of the infected *S. littoralis* did not development to healthy adults. Developmental stages were observed in three different styles; non-emerging pupae, semi-emerged adults and developed into flightless adults with deformed wings.

An insect-selective neurotoxic polypeptide derived from scorpion venom (AaIT), has recently been used to engineer different organisms such as viruses, fungi, bacteria and plants against insect pests (Deng et al., 2019). Many types of different baculoviruses including *AaIT* gene have been constructed to enhance the insecticidal activity against lepidopteran larvae (Treacy & All, 1996; Yao et al., 1996; Elazar et al., 2001). A recombinant baculovirus containing a scorpion toxin gene (AcMNPV contains AaIT) produced a significant decrease in the time to kill (LT$_{50}$ 88 h) *Heliothis virescens* (Fabricius, 1777*)* (Lepidoptera: Noctuidae) larvae compared to wild type AcMNPV (LT$_{50}$ 125 h) (McCutchen et al., 1991). Similarly, a recombinant baculovirus Bombyx mori nuclear polyhedrosis virus (BmNPV) carrying a synthetic *AaIT* gene has been assayed in *Bombyx mori* (Linnaeus, 1758) (Lepidoptera: Bombycidae) larvae. Those results indicate that baculoviruses, carrying toxin genes, can be used to reduce insect feeding damage and can result with increased mortality (Maeda et al., 1991). Although iridoviruses kill insect larvae later than baculoviruses, the results of this study have shown that recombinant iridovirus containing the *neurotoxin* gene caused death in a shorter time than the wild type virus.

rCIV-Δ157L-gfp was highly insecticidal activity on all used insect larvae compared to the wild type IIV6. This result may be relevant with deleted ORF 157L in its structure. ORF 157L shows homology to the iap (inhibitor of apoptosis) gene of a baculovirus (Razvi & Welsh, 1995). Although it is shown that 157L

does not directly inhibit apoptosis (İnce et al., 2008), it may have an indirect function at apoptosis inhibition of the virus. Deletion of this gene can cause insect cells to go into apoptosis and eventually insect death. This may be the reason for high insecticidal activity of rCIV-Δ157L-gfp.

In conclusion, considering all insects used in this study, lethal effects were determined on *H. armigera, L. dispar* and *E. chrysorrhoea* larvae when they were injected with IIV6. *Spodoptera littoralis* was the most resistant species to IIV6 infection. *T. molitor* larvae survived longer, even if they were paralyzed. We conclude that the paralyzed beetle larvae survived longer than lepidopteran larvae without access to food.

In this study, the lethal effects of wild type and recombinant iridoviruses on various hosts were investigated by injection and these viruses were found to be lethal to Coleoptera and Lepidoptera orders. Virus infection of insects in nature through hemolymph can occur by rare mechanisms. The virus may infect insect larvae through wounds or parasites and parasitoids may introduce these viruses to the susceptible hosts (Williams et al., 2005). However, since these mechanisms do not occur commonly in the nature, another way need to be found for an effective infection of insects with iridoviruses. Encapsulation may be a good choice for iridoviruses. Encapsulation of the virus by a protective substance and infecting the insects via the oral route will be our next study on iridoviruses.

References

Abbott, W. S., 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 18: 265-267.

Bergomaz, R. & M. Boppré, 1986. A simple instant diet for rearing Arctiidae and other moths. Journal of the Lepidopterists' Society, 40 (3): 131-137.

Chinchar, V. G., P. Hick, I. A. Ince, J. K. Jancovich, R. Marschang, Q. Qin, K. Subramaniam, T. B. Waltzek, R. Whittington, T. Williams & Q. Y. Zhang, 2017. ICTV virus taxonomy profile: Iridoviridae. Journal of General Virology, 98 (5): 890-891.

Deng, S. Q., J. T. Chen, W. W. Li, M. Chen & H. J. Peng, 2019. Application of the scorpion neurotoxin AaIT against insect pests. International Journal of Molecular Sciences, 20 (14): 3467.

Elazar, M., R. Levi & E. Zlotkin, 2001. Targeting of an expressed neurotoxin by its recombinant baculovirus. Journal of Experimental Biology, 204 (15): 2637-2645.

Finney, D. J., 1952. Probit Analysis: A Statistical Treatment of the Sigmoid Response Curve. Cambridge University Press, Cambridge, England, 256 pp.

Fukuda, T., 1971. Per os transmission of Chilo iridescent virus to mosquitoes. Journal of Invertebrate Pathology, 18 (1): 152-153.

Gencer, D., Z. Bayramoglu, R. Nalcacioglu, R. G. Kleespies, Z. Demirbag & I. Demir, 2018. Characterisation of three Alphabaculovirus isolates from the gypsy moth, *Lymantria dispar dispar* (Lepidoptera: Erebidae), in Turkey. Biocontrol Science and Technology, 28 (2): 107-121.

Henderson, C. W., C. L. Johnson, S. A. Lodhi & S. L. Bilimoria, 2001. Replication of Chilo iridescent virus in the cotton boll weevil, *Anthonomus grandis*, and development of an infectivity assay. Archives of Virology, 146 (4): 767-775.

Hierholzer, J. C. & R. A. Killington, 1996. "Virus Isolation and Quantitation, 25-46" In: Virology Methods Manual (Eds. B. W. Mahy & H. I. Kangro), Virology Methods Manual Academic Press, London, 374 pp.

Hunter, W. B., S. L. Lapointe, X. H. Sinisterra, D. S. Achor & C. J. Funk, 2003. Iridovirus in the root weevil *Diaprepes abbreviatus*. Journal of Insect Science, 3 (9): 1-6.

İnce, İ. A., M. Westenberg, J. M. Vlak, Z. Demirbağ, R. Nalçacıoğlu & M. M. van Oers, 2008. Open reading frame 193R of Chilo iridescent virus encodes a functional inhibitor of apoptosis (IAP). Virology, 376 (1): 124-131.

Jakob, N. J., R. G. Kleespies, C. A. Tidona, K. Müller, H. R. Gelderblom & G. Darai, 2002. Comparative analysis of the genome and host range characteristics of two insect iridoviruses: Chilo iridescent virus and a cricket iridovirus isolate. Journal of General Virology, 83 (2): 463-470.

Jenkins, D., W. Hunter & R. Goenaga, 2011. Effects of Invertebrate Iridescent Virus 6 in *Phyllophaga vandinei* and its potential as a biocontrol delivery system. Journal of Insect Science, 11 (44): 1-10.

Jensen, D. D., T. Hukuhara & Y. Tanada, 1972. Lethality of Chilo iridescent virus to *Colladonus montanus* leafhoppers. Journal of Invertebrate Pathology, 19 (2): 276-278.

Kalha, C. S., P. P. Singh, S. S. Kang, M. S. Hunjan, V. Gupta & R. Sharma, 2014. ''Entomopathogenic Viruses and Bacteria for Insect-Pest Control, 225-244''. In: Integrated Pest Management: Current Concepts and Ecological Perspective (Ed. D. P. Abrol). Academic Press, San Diego, 576 pp.

Kelly, D. C., M. D. Ayres & T. Lescott, 1979. A small iridescent virus (type 29) isolated from *Tenebrio molitor*: a comparison of its proteins and antigens with six other iridescent viruses. Journal of General Virology, 42 (1): 95-105.

Kim, S. Y., H. G. Kim, H. J. Yoon, K. Y. Lee & N. J. Kim, 2017. Nutritional analysis of alternative feed ingredients and their effects on the larval growth of *Tenebrio molitor* (Coleoptera: Tenebrionidae). Entomological Research, 47 (3): 194-202.

Maeda, S., S. L. Volrath, T. N. Hanzlik, S. A. Harper, K. Majima, D. W. Maddox, B. D. Hammock & E. Fowler, 1991. Insecticidal effects of an insect-specific neurotoxin expressed by a recombinant baculovirus. Virology, 184 (2): 777-780.

Marina, C. F., J. I. J. E. Arredondo-Jimenez, J. E. Ibarra, I. Fernandez-Salas & T. Williams, 2003. Effects of an optical brightener and an abrasive on iridescent virus infection and development of *Aedes aegypti*. Entomologia Experimentalis et Applicata, 109 (2): 155-161.

McCutchen, B. F., P. V. Choudary, R. Crenshaw, D. Maddox, S. G. Kamita, N. Palekar, S. Volrath, E. Fowler, B. D. Hammock & S. Maeda, 1991. Development of a recombinant baculovirus expressing an insect-selective neurotoxin: Potential for pest control. Bio/Technology, 9 (9): 848-852.

Mitsuhashi, J., 1967. Infection of leafhopper and its tissues cultivated in vitro with Chilo iridescent virus. Journal of Invertebrate Pathology, 9 (3): 432-434.

Nalcacioglu, R., H. Muratoglu, A. Yesilyurt, M. M. van Oers, J. M. Vlak & Z. Demirbag, 2016. Enhanced insecticidal activity of Chilo iridescent virus expressing an insect specific neurotoxin. Journal of Invertebrate Pathology, 138: 104-111.

Ohba, M., 1975. Studies on the parthogenesis of Chilo iridescent virus 3. Multiplication of CIV in the silkworm *Bombyx mori* L. and field insects. Scientific Bulletin of the Faculty of Agriculture Kyushu University, 30: 71-81.

Ozgen, A., H. Muratoglu, Z. Demirbag, J. M. Vlak, M. M. van Oers & R. Nalcacioglu, 2014. Construction and characterization of a recombinant invertebrate iridovirus. Virus Research, 189: 286-292.

Razvi, E. S. & R. M. Welsh, 1995. Apoptosis in viral infections. Advanced Virus Research, 45: 1-60.

Treacy, M. F. & J. N. All, 1996. Impact of insect-specific AaHIT gene insertion on inherent bioactivity of baculoviruses against tobacco budworm, *Heliothis virescens*, and cabbage looper, *Trichoplusia ni*. Beltwide Cotton Conferences, 2: 911-917.

Williams, T., 2008. Natural invertebrate hosts of iridovirases (iridoviridae). Neotropical Entomology, 37 (6): 615-632.

Williams, T., V. Barbosa-Solomieu & V. G. Chinchar, 2005. A decade of advances in iridovirus research. Advances in Virus Research, 65: 173-248.

Yao, B., Y. Pang, Y. Fan, R. Zhao, Y. Yang & T. Wang, 1996. Construction of an insecticidal baculovirus expressing insect-specific neurotoxin AaIT. Science China Life Sciences, 39 (2): 199-206.