Eroglu and Demırbag
Egyptian Journal of Biological Pest Control (2022) 32:53
https://doi.org/10.1186/s41938-022-00552-4

Egyptian Journal of Biological Pest Control

RESEARCH Open Access

An environmentally safe and tolerant microbial insecticide utilizing *Helicoverpa armigera* single nucleopolyhedrovirus (HearNPV-TR)

Gozde Busra Eroglu[1] and Zihni Demırbag[2*]

Abstract

Background: Baculoviruses are significant biocontrol agents of pests in agriculture; however, ultraviolet light B (UV-B) and high temperature inactivate them in the environment within a short time.

Results: In this study, formulations of *Helicoverpa armigera* nucleopolyhedrovirus Turkey isolate (HearNPV-TR) were prepared. Crude virus, the oil-based formulations of the virus, and a commercial *H. armigera* NPV product were exposed to UV-B light and various temperatures (28, 35, and 42 °C) at different periods (0, 1, 3, 5 h.). While the UV-B application decreased the virulence of crude virus up to 32% after 5 h. exposure, it decreased the virulence of oil-based formulation by (15%). Similarly, the virulence of the crude virus by 70% at 42 °C, while was reduced by 10% in the formulations at the same temperature. Therefore, 5% oil formulation was enough to keep the virulence of the virus underexposed to UV-B and high temperatures. LC_{95} values were calculated as 1×10^8 OBs/ml^{-1} in concentration–response tests, and mortality rates were 90 and 92% in pot assays for commercial product and 5% oil formulation, respectively.

Conclusions: The results showed that 5% oil formulation of HearNPV-TR was an important biocontrol preparation to protect virus virulence under suppressive environmental conditions.

Keywords: Baculovirus, *Helicoverpa armigera*, Microbial control, Oil-based formulation

Background

Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae) larvae are polyphagous pests in agricultural products that cause economic losses to economic crops worldwide (Sivasupramaniam et al. 2007). Chemical insecticides have been used to control *H. armigera*; however, their uses are no longer favored due to the negative effects on the environment and human health, as well as insects' resistance build-up (Whalon et al. 2018). As an alternative to chemical pesticides for the control of *H. armigera*, baculoviruses have been preferred due to the narrow host range and being environmentally friendly (Wang et al. 2005). To date, about 1000 baculoviruses have been isolated from pests and more than 60 commercial products have been developed (Beas-Catena et al. 2014).

Although baculoviruses are important bio-pesticide material, they are inactivated shortly due to the high temperature and the UV light in the natural environment (Priyadharshini 2009). It is indicated that ultraviolet B (280–320 nm wavelength) in the sunlight is one of the important factors influencing the infectivity of baculovirus (Ignoffo et al. 1974). Therefore, it has been shown

*Correspondence: zihni@ktu.edu.tr

[2] Department of Biology, Faculty of Science, Karadeniz Technical University, 61080 Trabzon, Turkey
Full list of author information is available at the end of the article

Springer Open

© The Author(s) 2022. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

that some protective substances added to the crude virus increased the protection of the virus against suppressive environmental conditions (Shapiro et al. 2012). The clay-based materials contained in the solid formulations, like kaolin, decrease the infectivity of the inclusion body because it increases the pH. In this case, the formulation shortens the persistence of the virus and reduces its virulence. On the other hand, it has been shown that the oil-based formulations are more stable on the foliage against environmental factors, such as rain and wind, and have high shelf life due to low pH levels (Batista et al. 2001).

In this study, oil-based formulations of the *H. armigera* single nucleopolyhedrovirus (HearNPV-TR) isolated from Turkey were prepared to make the crude virus tolerant to UV-B and temperature. It was determined that the highest tolerance from formulations exposed to UV-B and heat at different times contained 5% oil. Concentration–response tests and pot assay were performed against *H. armigera* larvae. According to the results obtained, the tolerance of the crude virus to natural environmental conditions was retained by using an oil-based formulation.

Methods

Virus source, production, and purification

The HearNPV-TR was isolated in earlier studies (Eroglu et al. 2019) and obtained from the Department of Biology at Karadeniz Technical University, Trabzon, Turkey. Besides, whole-genome analysis of the HearNPV-TR isolate was determined (Eroglu et al. 2020). For virus production, *H. armigera* larvae were collected from a tomato crop in the vicinity of Gümüşhane, Turkey, between July and August 2018. Larvae were normalized at laboratory conditions for a generation before they were used in the bioassays. They were placed individually into plastic cups (25 ml) containing nearly100 mg of artificial diet (Nagarkatti and Prakash 1974). Approximately 100 3rd instar larvae were infected with the droplet method for virus production (Hughes et al. 1986). The infected larvae were incubated at 26 °C and 60% RH. One week after the infection, dead larvae were collected and transferred to a falcon tube and homogenized in dH_2O at a ratio of 1: 2. The solution was filtered through cheesecloth and centrifuged at 5000 × g for 30 min. Pellet, suspended with dH_2O was loaded on 30% sucrose and centrifuged at 5000 × g for 30 min. The pellet was re-suspended with dH_2O. The concentration of the stock solution was determined to be 1×10^{10} OBs/ml^{-1} on the Thoma's hemocytometer.

Oil-based formulation of HearNPV-TR

The oil-based formulations were prepared by mixing the 25 ml of the HearNPV-TR (1×10^{10} OBs/ml^{-1}) solution with 5, 7.5, and 10 ml of sunflower oil, and 1 ml of emulsifier (E323 soy lecithin). After that, 10 ml feeding stimulant (E422 glycerine), 1 ml of thickener (E415 xanthan gum), 0.5 ml of spreader sticker (Silwet L-77), 0.2 ml of optical brightener (calcofluor white M2R), and 1 ml of a physiological stress factor for larvae (E284 boric acid) were added and brought to 100 ml with sterile dH_2O. The mixture was homogenized in a vortex, and the pH of the formulation was measured. This formulation was transferred into a glass bottle, sheltered from light, and stored at − 20 °C.

Exposure to ultraviolet B and heat assays

The UV-B lamp of 15 watts, 306 nm (Fotodyne Inc., Germany) was used for exposure to UV light in laboratory conditions. The crude HearNPV-TR, commercial HearNPV (*Helicoverpa armigera* NPV) product, 3 oil-based formulations (5, 7.5, and 10%) whose final concentrations were 1×10^9 OBs/ml^{-1} and control group (only dH_2O) were exposed to 300 mm above the test dishes UV-B irradiation in 24 well plates for 0, 1, 3, and 5 h. Three replicates were prepared for each specimen. The infectivity of the crude virus, commercial product, and oil formulation after being exposed to UV-B were then applied against 30 3rd instar *H. armigera* larvae using the leaf disk bioassay method (Akhanaev et al. 2017). For this application, 10 µl of each solution was inoculated onto fresh 4 mm^2 diameter lettuce disks cut with a cork borer. Then, leaf disks were placed on the 24 well plates. Third instar *H. armigera* larvae were placed on each leaf disk and continued incubation at 26 °C. The crude HearNPV-TR, commercial product, 3 oil-based formulations, and control group were incubated in 24 well plates for 0, 1, 3, and 5 h. at 28, 35, 42 °C at 60% RH. Temperature values were selected according to the summer temperatures of the region where *H. armigera* is common. The specimens exposed to the temperature were inoculated to the larvae in the same bioassay conditions.

Concentration–response tests

The 5% oil-containing formulation was selected for the concentration–response test as it had a sufficient tolerance against UV-B and high temperature. In order to compare the infectivity of commercial products, 5% oil formulation, and control group, they were used in the leaf disk method. The concentrations of viral solutions were calculated by a Thoma's hemocytometer, and eight different concentrations (10^3 to 10^{10} OBs/ml^{-1}) were prepared. The control group was treated with dH_2O only. Thirty larvae of *H. armigera* were used for each test, and the experiments were performed in three replicates. Infected larvae and control groups were reared on fresh lettuce leaves. Larvae were incubated at 26 °C and 60% RH. All

groups were examined for a 2-week period. Mortality rates were calculated by Abbott's formula (Abbott 1925). The lethal concentration (LC_{50} and LC_{95}) was determined by SPSS 24.0 software.

Pot assay

Lettuce seedlings in ($80 \times 29 \times 20$ cm) plastic oblong pots containing a mixture of sterile soil and peat (3: 2) were grown for 20 days before use. The experiment was carried out in a growth chamber under a 16 h light 8 h dark at 28 °C per day. After 20 days, 20 leaf lettuce plants were used for inoculation. The pots were covered with cheesecloth. The spreader sticker (0.5 ml, Silwet L-77) contained in the formulation was added to the control group to ensure that the droplets were evenly distributed to the leaf surface. All solutions were used according to LC_{95} values (1×10^8 OBs/ml^{-1}), which spread on leaf surfaces using a handheld sprayer bottle. Thirty larvae of 3rd instar *H. armigera* were placed in each pot.

Results

Exposure to ultraviolet B and heat assays

The crude HearNPV-TR, oil formulations of the virus, and commercial products were exposed to UV-B light at different periods (0, 1, 3and 5 h.). After then, 3rd instar *H. armigera* larvae were infected with the solutions, and observed for 14 days. The mortality rates of larvae are indicated in Fig. 1a–d. Before UV-B irradiation, the mortality rate of the crude virus was detected as (98%) on larvae while oil formulations and the commercial product

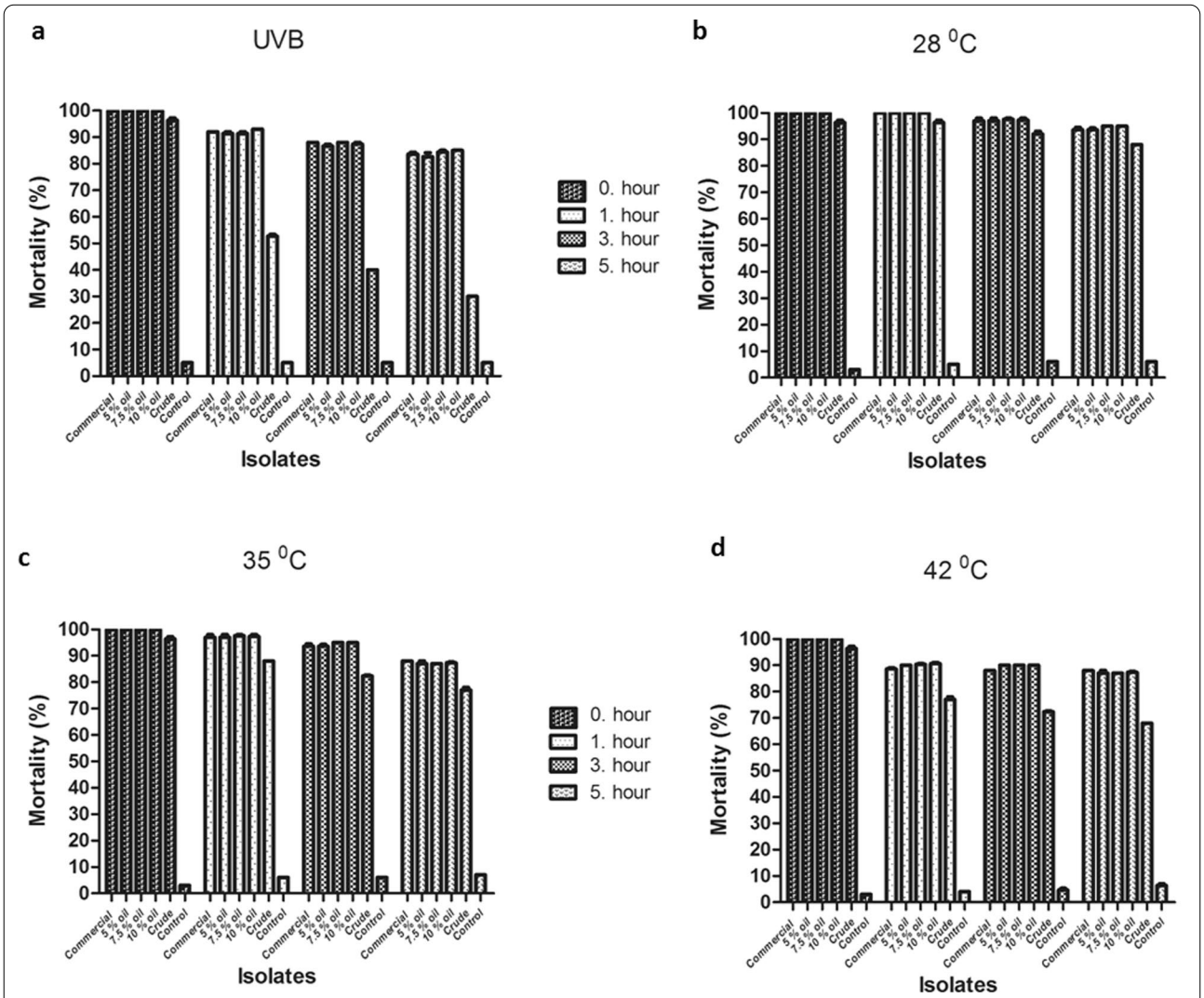

Fig. 1 Mortality rates after UV-B and temperature application of crude virus, oil contain formulations, and commercial product against 3rd instar Helicoverpa armigera larvae. **a** UV-B, **b** 28, **c** 35, and **d** 42 °C

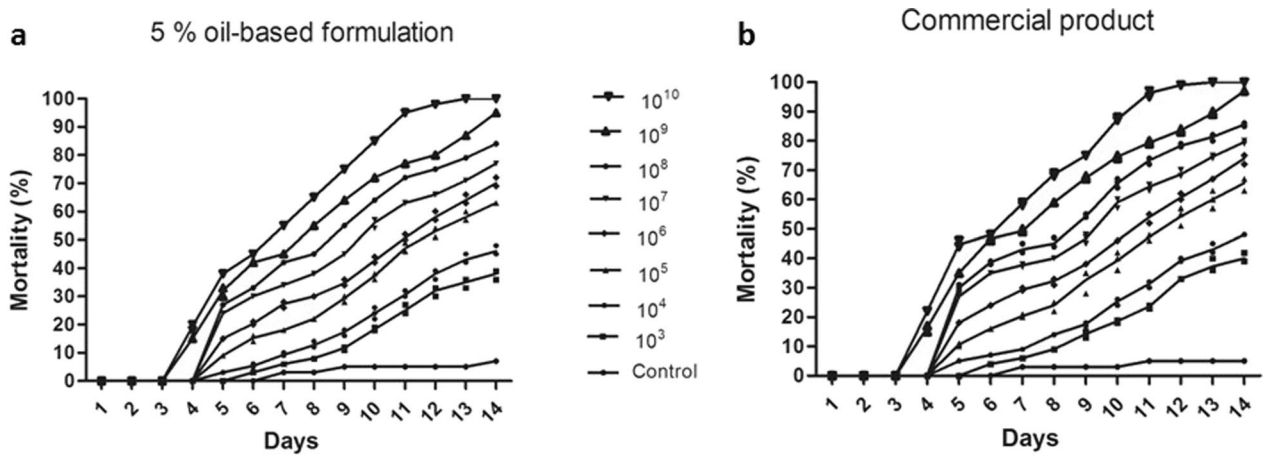

Fig. 2 Concentration response values of control group, 5% oil formulation and commercial product on 3rd instar Helicoverpa armigera larvae

showed (100%) mortality against 3rd instar *H. armigera* larvae. After 1, 3, and 5 h. of exposure to UV-B irradiation, mortality rates of the crude virus on larvae were 52, 39, and 32%, respectively, while mortality values of the oil formulations and commercial products were above (85%) on the larvae even after 5 h. exposure. So, it was determined that the virulence effect of the crude HearNPV-TR decreased when exposed to UV-B light. However, the oil formulations protected the crude HearNPV-TR from the negative effects of UV-B light significantly (Fig. 1a).

The crude virus, oil formulations, and commercial products were also exposed to 3 different temperatures (28, 35, and 42 °C) for 0, 1, 3, and 5 h. The temperatures of 28 and 35 °C did not cause an important change in the virulence effect of crude virus and formulations (Fig. 1b, c). However, the virulence of the crude HearNPV-TR remarkably decreased at 42 °C. After exposing the crude virus to 42 °C for 1, 3, and 5 h, mortality rates on the larvae were calculated as 73, 70, and 68%, respectively. Furthermore, oil formulations and commercial products exposed to 42 °C showed (90%) mortality on the larvae even after 5 h (Fig. 1d). Results of UV-B and heat assays showed that 5% oil formulation was sufficient to protect the virus against environmental conditions. So, 5% oil formulation was selected for concentration–response tests and pot assays.

Concentration–response tests

Mortality results of 5% oil formulation and commercial product resulted in 100% protection for the highest concentration (1×10^{10} OBs/ml^{-1}) on 3rd instar larvae within 14 days (Fig. 2). The LC_{50} and LC_{95} values of 5% oil formulation and commercial product were determined to be 1.9×10^{4}, 1.5×10^{4} and 1.6×10^{8}, 1.1×10^{8} OBs/ml^{-1}, respectively (Table 1).

Table 1 Mortality values of HearNPV products against 3rd instar larvae of *Helicoverpa armigera*

Biotest	Mortality rate	LC_{50}	LC_{95}	χ^2	df
Commercial product	100%	1.5×10^{4}	1.1×10^{8}	2.0	2
5% oil formulation	100%	1.9×10^{4}	1.6×10^{8}	2.0	2

χ^2 Chi square, *df* degree of freedom

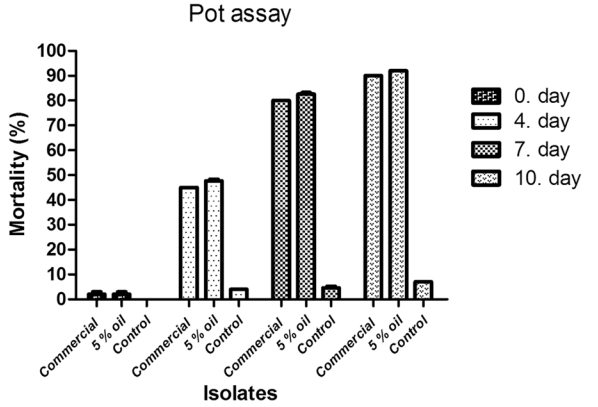

Fig. 3 Mortality rates of control group, 5% oil formulation and commercial product in lettuce seedlings against 3rd instar Helicoverpa armigera larvae

Pot assay

The LC_{95} (1×10^{8} OBs/ml^{-1}) concentration of the products was used for the pot assay. The 5% oil formulation, commercial product, and control group were exposed to natural sunlight on 3rd instar *H. armigera* larvae. Mortality values of 5% oil formulation and the commercial

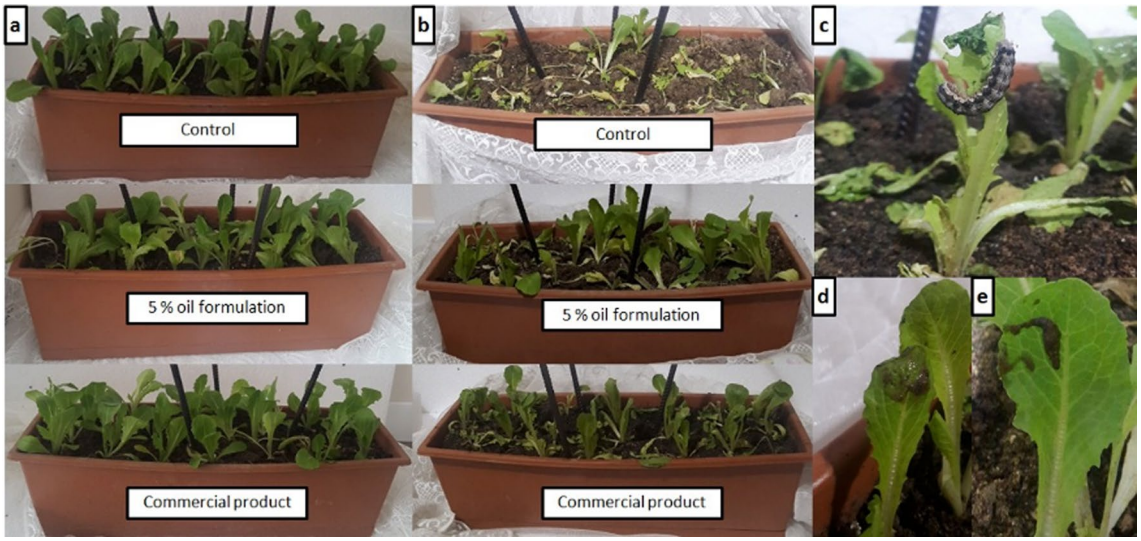

Fig. 4 Morphological effects of control group, 5% oil formulation and commercial product on 3rd instar Helicoverpa armigera larvae in lettuce seedlings. **a** Before application for all test groups, **b** after application, **c** Foliage consumption of H. armigera larvae on untreated lettuce seedlings, **d**, **e**. A cadaver of H. armigera larvae died after application of the **d** 5% oil formulation, and **e** commercial product

product showed 92 and 90% protection, respectively, for 10 days (Fig. 3). The protection levels of the formulations and control group are shown in Fig. 4. In the pots, where the 5% oil formulation and commercial product were applied, the larvae began to die from 4th day and the lettuce seedlings were not damaged. However, in the control group, larvae were fed with lettuce seedlings, and consumption all seedlings were at the end of the 10th day.

Discussion

Up to now, the oil-based formulation has not been produced in the formulations against *Heliothis* species produced from other baculoviruses are proved highly tolerant to environmental factors (such as UV, heat, wind, and rain) (Shapiro, et al. 2012).

The most significant feature that should be in a formulation is long-term stability (Airenne et al. 2013). One of the factors, the pH value, significantly affects the shelf life of the baculovirus formulations. The pH of the solution prior to the addition of NPV should be 5–7. Because OBs structures dissolve in alkaline substrates, it causes virulence to decrease in a short time (Batista et al. 2001). Generally, cotton seed oil and corn oil were used in the oil-based baculovirus formulations (Shapiro et al. 2012). The sunflower oil was chosen to be used in this study because of the low cost. As well, some natural additives other than oil made the formulation more effective. In previous studies, the use of various feeding stimulants such as glycerol was found to be beneficial in the level of the effect of the virus. They were reported that glycerol facilitated consumption by insects as well as accelerated the death rate because it enhances the taste of the virus (Narendrakumar et al. 2017). Baculoviruses may be suspended with a thickening agent (xanthan gum) in liquid formulations; however, surfactants must also be included to aid in the fluidity (Ríos-Velasco et al. 2012). Besides, surfactant substances such as Silwet L-77 allow the formulation to spread evenly across the entire leaf surface (Camacho et al. 2015). The optical brighteners, especially calcofluor, tinopal, leucophor, and blankophor are both protect baculoviruses from UV and damage to the peritrophic membrane of insects. Thus, these substances are added to the formulations to accelerate baculovirus infection (Ibargutxi et al. 2008). In addition, boric acid used as a source of physiological stress in larvae increased the virulence (Bhutia et al. 2012).

An oil-based formulation prepared from the crude virus which would be relatively more resistant under abiotic conditions such as ultraviolet light, rain, wind, and temperature was purposed. Therefore, some natural ingredients such as glycerol, xanthan gum, Silwet L-77, calcofluor white M2R, and boric acid were added to the baculovirus origin and sunflower oil-based formulation.

The UV-B and temperature tolerance of 3 different oil formulations (5, 7.5, and 10%) against 3rd instar *H. armigera* larvae were determined. Besides, these data were compared to crude HearNPV-TR and commercial products. Thus, it was determined that the 5% oil-containing formulation provided an adequate tolerance to UV-B and high temperature.

To protect the OBs from environmental conditions, so far, many different products have been formulated.

Prabhu and Mahalingam (2017) have prepared an additional formulation of some substances (starch, tinopal, tween 80) and nucleopolyhedrovirus (1×10^9 OBs/ml^{-1}) isolated from *Diaphania pulverulentalis*. The prepared formulation initially (0. min) had a 98% mortality rate but decreased to 66% after 1 h of UV in laboratory conditions. Batista et al. (2001) prepared 2 different formulations as emulsifiable oil (EO) and wettable powder (WP) of Anticarsia gemmatalis NPV. The crude virus, WP, and EO were exposed to UV in the laboratory for 5 min. The mortality rates of crude virus, WP, EO which were not exposed to UV-B were initially 92, 99, and 98%, whereas those exposed to UV decrease to 19, 37, and 37% in 5 min. In this study, the formulation containing 5% sunflower oil showed enough protection even after 5 h. UV-B exposure (84%), while the virulence of crude HearNPV-TR reduced to 31%. In the temperature assays, the virulence of the crude HearNPV-TR decreased to 68% at the highest temperature (42 °C) and the longest time (5 h.), whereas the virulence of the 5% oil formulation remained almost the same (85%). Thus, it was determined that the sunflower oil-based formulation made the crude virus tolerant to UV-B and high temperature.

Eroglu et al. (2019) applied different concentrations of crude HearNPV-TR between 1×10^3 and 1×10^8 OBs/ml^{-1} against 2nd instar *H. armigera* larvae. The mortality rate was calculated to be (98%) for the maximum concentration (1×10^8 OBs/ml^{-1}) and the LC_{50} value was determined to be 1.5×10^3 OBs/ml^{-1} after 14 d. In this study, 8 different concentrations (1×10^3 -1×10^{10} OBs/ml^{-1}) of 5% oil-based formulation and commercial products were used against 3rd instar *H. armigera* larvae. For the highest concentration, the mortality values were (100%) (formulation) and (100%) (Commercial product). According to the mortality rates in this study, high virulence was obtained at lower concentration than the concentrations used in the literature against *H. armigera* larvae.

In the pot assays, Elamathi et al. (2012) applied 10^9 OBs/ml^{-1} concentrations of various H. armigera NPV formulations on marigold seedlings against *H. armigera*. After 96 h. the mortality rate was (86%) for 4 g formulation. Gupta et al. (2010) performed with 3×10^{12} OBs/ml^{-1} concentration of 3 different *H. armigera* NPV India isolates (Samba, Udheywalla, Chenani) containing tinopal (0.1%) and jaggery (1%) on tomato seedlings. The mortality rates of 3rd instar *H. armigera* larvae were determined as 9% (Samba), 86% (Udheywalla), and 81% (Chenani) after 9 days. In this study oil-based formulation and commercial products were applied with 1×10^8 OBs/ml^{-1} concentration on lettuce seedlings against 3rd instar *H. armigera*. The mortality rates were calculated as (92%) (Oil formulation) and (90%) (Commercial product) for 10 d. As a result, it was observed that the oil-containing formulation had almost the same virulence as a commercially available NPV product in the market.

Conclusions

Among entomopathogenics, viruses, baculoviruses have been used for the control of pest insects. Earlier studies have shown that the crude virus is required to be formulated so that it would not be inactivated due to sunlight UV-B radiation and high temperature in its nature applications. In this study, a sunflower oil-based formulation of HearNPV-TR with UV-B and high-temperature tolerance against *H. armigera* was produced. The results showed that this formulation was a promising viral control agent also it is ecofriendly and lower cost than chemical products.

Abbreviations

UV-B: Ultraviolet light B; NPV: Nucleopolyhedrovirus; Hear: *Helicoverpa armigera*; h: Hour.

Acknowledgements

This study was financially supported by Karadeniz Technical University (Project No.: BAP01 FBK2019-8123).

Author contributions

The authors contributed equally. All authors read and approved the final manuscript.

Funding

This study was financially supported by Karadeniz Technical University (Project No.: BAP01 FBK2019-8123).

Availability of data and materials

All datasets are presented in the main manuscript.

Declarations

Ethics approval and consent to participate

An ethics certificate is not required in the study.

Consent for publication.

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Department of Molecular Biology and Genetics, Faculty of Science, Erzurum Technical University, Erzurum, Turkey. [2]Department of Biology, Faculty of Science, Karadeniz Technical University, 61080 Trabzon, Turkey.

Received: 26 January 2022 Accepted: 9 May 2022
Published online: 13 May 2022

References

Abbott WS (1925) A method of computing the effectiveness of an insecticide. J Econ Entomol 18:265–267

Airenne KJ, Hu YC, Kost TA, Smith RH, Kotin RM, Ono C, Matsuura Y, Wang S, Yla-Herttuala S (2013) Baculovirus: an insect-derived vector for diverse

gene transfer applications. Mol Ther 21:739–749. https://doi.org/10.1038/mt.2012.286
Akhanaev YB, Belousova IA, Ershov NI, Nakai M, Martemyanov VV, Glupov VV (2017) Comparison of tolerance to sunlight between spatially distant and genetically different strains of Lymantria dispar nucleopolyhedrovirus. PLoS ONE 20:1–13. https://doi.org/10.1371/journal.pone.0189992
Batista A, Alves S, Augusto PR (2001) Stability and persistence of two formulations containing Anticarsia gemmatalis nuclear polyhedrovirus (AgMNPV). Neotrop Entomol 30:411–416. https://doi.org/10.1590/S1519-566X2001000300012
Beas-Catena A, Sánchez-Mirón A, García-Camacho F, Contreras-Gómez A, Molina-Grima E (2014) Baculovirus bio-pesticides: An overview. J Anim Plant Sci 24:362–373
Bhutia KC, Chakravarthy AK, Doddabasappa B, Narabenchi GB, Lingaraj VK (2012) Evaluation and production of improved formulation of nucleopolyhedrosis virus of *Spodoptera litura*. Bull Insectol 65:247–256
Camacho JE, Behle RW, Villamizar LF, Gomez MI (2015) Effect of spray dryer processing parameters on the insecticidal activity of two encapsulated formulations of baculovirus. Biocontrol Sci Tech 8:911–927. https://doi.org/10.1080/09583157.2015.1020761
Elamathi E, Cholan JR, Vijayakumar N, Ramamourti A (2012) Formulation and optimisation of various nuclear polyhedrosis virus isolates and assessment of their insecticidal activity against *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) larvae. Arch Phytopathol Plant Prot 45:750–776. https://doi.org/10.1080/03235408.2011.595908
Eroglu GB, Nalcacioglu R, Demirbag Z (2019) A new helicoverpa armigera nucleopolyhedrovirus isolate from *Heliothis peltigera* (Denis & Schiffermuller) (Lepidoptera: Noctuidae) in Turkey. Turk J Biol 43:340–348. https://doi.org/10.3906/biy-1902-64
Eroglu GB, Inan C, Nalcacioglu R, Demirbag Z (2020) Genome sequence analysis of a Helicoverpa armigera single nucleopolyhedrovirus (HearNPV-TR) isolated from *Heliothis peltigera* in Turkey. PLoS ONE 15(6):e0234635. https://doi.org/10.1371/journal.pone.0234635
Gupta RK, Raina JC, Arora RK, Bali K (2010) Selection and field effectiveness of nucleopolyhedrovirus isolates against *Helicoverpa armigera* (Hubner). Int J Virol 6:164–178. https://doi.org/10.3923/ijv.2007.45.59
Hughes PR, Van Beek NAM, Wood HA (1986) A modified droplet feeding method for rapid assay of *Bacillus thuringiensis* and baculoviruses in noctuid larvae. J Invertebr Pathol 83:187–192
Ibargutxi MA, Muñoz D, Bernal A, Ruiz de Escudero I, Caballero P (2008) Effects of stilbene optical brighteners on the insecticidal activity of *Bacillus thuringiensis* and a single nucleopolyhedrovirus on *Helicoverpa armigera*. Biol Control 47:322–327. https://doi.org/10.1016/j.biocontrol.2008.08.019
Ignoffo CM, Hostetter DL, Pinnell RE (1974) Stability of *Bacillus thuringiensis* and Baculovirus *Heliothis* on soybean foliage. Environ Entomol 3:117–119
Nagarkatti S, Prakash A (1974) Rearing of *Heliothis armigera* (Hubner) on an artificial diet. Techonol Bull 17:169–173
Narendrakumar G, Ritayan D, Sarath Sasi G, Preethi TV (2017) Effect of Baculovirus with adjuvants against *Helicoverpa armigera* (HBN) on Cotton and evaluation of its effect in Different Instar. Res J Pharm Technol 10:1–3. https://doi.org/10.5958/0974-360X.2017.00369.9
Prabhu S, Mahalingam CA (2017) Effect of sunlight and UV light against DpNPV (Nuclear Polyhedrosis Virus) formulation on larval mortality of mulberry leaf webber, diaphania pulverulentalis Hampson. Int J Curr Microbiol Appl Sci 6:1897–1905. https://doi.org/10.20546/IJCMAS.2017.603.215
Priyadharshini P (2009) Molecular characterization of NPV of Diaphania pulverulentalis (Hampson) and establishment of phylogenetic relationship with other lepidoptera and baculoviruses (unpublished doctoral dissertation). University of Agricultural Sciences, Bangalore
Ríos-Velasco C, Gallegos-Morales G, Berlanga-Reyes D, Cambero-Campos J, Romo-Chacón A (2012) Mortality and production of occlusion bodies in *Spodoptera frugiperda* Larvae (Lepidoptera: Noctuidae) treated with nucleopolyhedrovirus. Florida Entomol 95:752–757. https://doi.org/10.1653/024.095.0326
Shapiro M, El Salamouny SA, Jackson DM, Shepard BM (2012) Field evaluation of a kudzu/cottonseed oil formulation on the persistence of the beet armyworm nucleopolyhedrovirus. J Entomol Sci 47:197–207. https://doi.org/10.18474/0749-8004-47.3.197
Sivasupramaniam S, Head GP, English L, Li YJ, Vaughn TT (2007) A global approach to resistance monitoring. J Invertebr Pathol 95:224–226. https://doi.org/10.1016/j.jip.2007.03.013
Wang D, An SH, Guo ZJ, Hu HJ, Zhang CX (2005) Characterization of Helicoverpa armigera nucleopolyhedrovirus orf33 that encodes a novel budded virion derived protein, BV-e31. Adv Virol 150:1505–1515. https://doi.org/10.1007/s00705-005-0534-9
Whalon ME, Mota-Sanchez D, Hollingworth RM, Duynslager L (2018) Arthropod pesticide

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Submit your manuscript to a SpringerOpen journal and benefit from:

- ► Convenient online submission
- ► Rigorous peer review
- ► Open access: articles freely available online
- ► High visibility within the field
- ► Retaining the copyright to your article

Submit your next manuscript at ► springeropen.com